Gene expression analysis in predicting the effectiveness of insect venom immunotherapy

Marek Niedoszytko, MD, PhD,[a,b] Marcel Bruinenberg, PhD,[c,d] Jan de Monchy, MD, PhD,[b] Cisca Wijmenga, PhD,[c] Mathieu Platteel, BSc,[c] Ewa Jassem, MD, PhD,[a] and Joanne N. G. Oude Elberink, MD, PhD[b] *Gdansk, Poland, and Groningen, The Netherlands*

Background: Venom immunotherapy (VIT) enables longtime prevention of insect venom allergy in the majority of patients. However, in some, the risk of a resystemic reaction increases after completion of treatment. No reliable factors predicting individual lack of efficacy of VIT are currently available.
Objective: To determine the use of gene expression profiles to predict the long-term effect of VIT.
Methods: Whole genome gene expression analysis was performed on RNA samples from 46 patients treated with VIT divided into 3 groups: (1) patients who achieved and maintained long-term protection after VIT, (2) patients in whom insect venom allergy relapsed, and (3) patients still in the maintenance phase of VIT.
Results: Among the 48.071 transcripts analyzed, 1401 showed a >2 fold difference in gene expression ($P < .05$); 658 genes (47%) were upregulated and 743 (53%) downregulated. Forty-three transcripts still show significant differences in expression after correction for multiple testing; 12 of 43 genes (28%) were upregulated and 31 of 43 genes (72%) downregulated. A naive Bayes prediction model demonstrated a gene expression pattern characteristic of effective VIT that was present in all patients with successful VIT but absent in all subjects with failure of VIT. The same gene expression profile was present in 88% of patients in the maintenance phase of VIT.
Conclusion: Gene expression profiling might be a useful tool to assess the long-term effectiveness of VIT. The analysis of differently expressed genes confirms the involvement of immunologic pathways described previously but also indicates novel factors that might be relevant for allergen tolerance.
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Key words: *Insect venom allergy, venom immunotherapy, gene expression, microarray assessment, prediction of treatment efficacy*

Abbreviations used
CLDN1: Claudin 1
IVA: Insect venom allergy
MAPK: Mitogen-activated protein kinase
PRLR: Prolactin receptor
SLC16A4: Solute carrier family 16
SNX33: sh3 and px domain containing 3
STAT: Signal transducer and activator of transcription
TWIST2: Transcription factor twist homolog 2
VIT: Venom immunotherapy

Insect venom allergy (defined as at least 1 systemic IgE mediated reaction in a lifetime after an insect sting) is present in approximately 1% to 3% of general population.[1]

Venom immunotherapy (VIT) with bee, yellow jacket, or *Polistes* venom is the treatment of choice in patients with insect venom allergy (IVA). At reaching maintenance dose, the risk of a systemic reaction to a subsequent sting is reduced from 70% (ie, before the start of VIT) to 3% to 15%.[2] To reach long-term protection, the maintenance phase has to be continued for at least 3 years in patients with mild systemic reactions and at least 5 years in patients with severe systemic reactions.[3] This procedure probably enables lifelong prevention of anaphylactic reactions in the majority of patients.[3]

However, in some patients, the risk of a systemic reaction to a re-sting reappears and increases after stopping the treatment. Currently there is no certain way to predict the individual efficacy of VIT except for deliberate sting challenges, but it is known that a number of factors are associated with a worse outcome of immunotherapy. First is the duration of treatment. The risk of a resystemic reaction after 2 years of VIT is higher than in patients who stopped after 3 to 5 years (30% vs 3%).[1,2,4] Second, it is known that patients with side effects during treatment are more prone to a lower degree of protection.[1,2] Hence, prolongation of VIT may reduce the risk for resystemic reaction in these patients.[1,2] Third, the amount of allergen routinely administered might not be sufficient to stimulate full protection in all individuals. It has been shown that continuation of VIT with higher dose (eg, 200 ug) is able to reduce this risk.[5] Fourth, it was demonstrated that the risk at a systemic reaction after completing the treatment is related to the culprit insect. In patients with yellow jacket venom allergy, the long-term effectiveness of therapy is assessed to be 85% to 95%, whereas in patients allergic to bee venom, this is 75% to 85%.[1] Fifth, coexistence of mastocytosis and even elevated serum tryptase level might increase the risk of inefficacy of VIT.[6,7] The current guidelines of European Academy of Allergy and Clinical Immunology indicate that patients with negative skin tests and undetectable specific IgE to insect venom have a diminished risk of relapse after stopping VIT.[2-4]

From [a]the Department of Allergology, Medical University of Gdansk; and [b]the Department of Allergology, [c]the Department of Genetics, and [d]Lifelines, University Medical Center Groningen, University of Groningen.

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Reprint requests: Marek Niedoszytko, MD, PhD, Department of Allergology, Medical University of Gdansk, Debinki 7 80-952 Gdansk, Poland. E-mail: mnied@amg.gda.pl.
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Finally, it is known that less severe sting reactions are associated with better protection after completing the treatment.[4]

Overall, this means that 10% to 20% of subjects remain vulnerable to the culprit insect venom in spite of completing the treatment.[1-3,6,7]

The aim of this study was to determine whether gene expression profiles may predict the efficacy or inefficacy of VIT. We determined whole genome gene expression profiles of patients who successfully completed treatment and compared their gene expression profiles with patients who had repeated systemic sting reactions in spite of VIT. On the basis of these results, we built a naive Bayes prediction model that subsequently was evaluated in a group of patients still on a maintenance dose of VIT.[8,9]

METHODS

Patients

A total of 46 patients treated with VIT were included. All patients experienced 1 or more severe systemic reactions before starting VIT. Inclusion criteria were the diagnosis of IVA on the basis of medical history (grade III or IV systemic reaction according to Mueller[10] before VIT) and positive skin tests or specific immunoglobulin E. Exclusion criteria were lack of consent, pregnancy, severe chronic or/and malignant disease, or mastocytosis. Patients started immunotherapy at the day ward, reaching 1/10 of the maintenance dose, and continued in the outpatient clinic with 1 injection weekly, increasing the amount of venom during approximately 6 weeks. Subsequently all patients received a maintenance dose of 100 μg every 6 weeks for 3 to 5 years. The study was approved by the Medical Ethical Committee of the University Medical Center Groningen (METc 2008/340).

The following 3 groups of patients were included (Table I):

Group 1 included patients who did not experience a systemic reaction in spite of being stung at least 3 times with the relevant insect after stopping VIT (n = 17). There were 9 (53%) men and 8 (47%) women, with a mean age of 53 years (range, 28-70) in this group.

Group 2 included patients who experienced at least 2 systemic reactions after field re-stings with the relevant insect (n = 12). There were 4 (33%) men and 8 (67%) women, with a mean age of 56 years (range, 42-75) in this group. The severity of the reaction to the re-sting was assessed as grade III in 80% (before VIT, 58%) and grade IV in 20% (before VIT, 42%) of patients according to the Mueller[10] scale. The restart of venom immunotherapy was offered to all patients from this group.

Group 3 included patients who were still in the maintenance phase of VIT (3-5 years) and had not been stung since the start of the therapy (n = 17). There were 6 (35%) men and 11 (65%) women, with a mean age of 55 years (range, 21-75) in this group.

TABLE I. Demographic and clinical patient data

	Long-term protection Group 1	Failure of treatment Group 2	Maintenance phase of VIT Group 3
No. of subjects	17	12	17
Age (y), (range)	53 (28-70)	56 (42-75)	54 (21-75)
Sex male/female (%)	50/50	36/64	31/69
Years of VIT, no. (SD)	3.15 (0.6)	3.3 (0.7)	4 (0.8)
Yellow jacket/bee allergy (%)	94/6	84/16	100/0
Mueller class III/IV (%)	64/36	58/42	0/100
sIgE yellow jacket (kU/L), mean (SD)	5.7 (7)	9.5 (19)	4.2 (4.7)
sIgE honeybee (kU/L), mean (SD)	0.2 (0.5)	0.9 (1.7)	0.3 (0.5)
Tryptase (ng/mL), mean (SD)	—	—	2.2 (4.3)
Methylhistamine in urine (μm/mkrea), mean (SD)	94 (38)	101 (29)	109.6 (41)
Asthma, no. (%)	1 (7)	1 (9)	4 (25)
Hypertension, no. (%)	1 (7)	3 (27)	2 (12.5)
No. of re-stings after VIT, mean (range)	5 (2-30)	2 (1-3)	—
Reaction to re-sting Mueller class III/IV (%)	0	80/20	—
Time interval between end of VIT and re-sting (y), (range)	3.5 (2-12)	4.2 (2-8)	—

Collection of blood samples

From all patients, RNA was isolated from the whole blood by using the PAXgene Blood RNA Tubes (Qiagen, Valencia, Calif). All tubes were immediately frozen and stored at –20°C until RNA isolation (maximum period, 2 months). RNA was isolated by using the PAXgene Blood RNA Kit CE (Qiagen, Venlo, The Netherlands). All RNA samples were stored at –80°C until labeling and hybridization.

The quality and concentration of RNA were determined by using the 2100 Bioanalyzer (Agilent, Amstelveen, The Netherlands) with the Agilent RNA 6000 Nano Kit. Samples with a RNA integrity number >7.5 were used for further analysis on expression arrays.

Gene expression

For amplification and labeling of RNA the Illumina TotalPrep 96 RNA Amplification Kit was used (Applied Biosystems, Nieuwerkerk ad IJssel, The Netherlands). For each sample, we used 200 ng RNA. The Human HT-12_V3_expression arrays (Illumina, San Diego, Calif) were processed according to the manufacturer's protocol. Slides were scanned immediately by using an Illumina BeadStation iScan (Illumina).

Image and data analysis

First line check, background correction and quantile normalization of the data were performed with Genomestudio Gene Expression Analysis module v 1.0.6 Statistics. Entities containing at least 75% of samples with a signal intensity value above the 20the percentile in 100% of the samples in at least 2 groups were included for the further analysis.

Data analysis was performed by using the GeneSpring package version 8.0.0 (Agilent Technologies, Santa Clara, Calif). Genes for which expression was significantly different between compared groups were chosen based on a $\log_2$ fold change >2 in gene expression, t test P value <.05 and Benjamin-Hochberg false discovery rates <.01.[11,12] The naive Bayes prediction model was used to build a prediction model assessing the effectiveness of VIT.[8,9] The naive Bayesian classifier is a mathematical process computing the probability of classifying the patient from group 3 as a treatment success or treatment failure based on the results of gene expression.[8,9] The selection of genes and their influence on classification in a particular group is based on results obtained in groups 1 and 2. The naive Bayesian classifier assumes that the impact of single gene expression is unrelated to other genes in the prediction model. The method does not take into account the interactions of the genes composing the model or gene-environmental interactions.

Functional annotation of genes was described by using the Go Process analysis and Kyoto encyclopedia of genes and genomes pathways[13-15] with the Genecodis functional annotation web based tool.[16,17]

Clinical data for this study were analyzed with Statistica 8.0 (StatSoft, Tulsa, Okla).

RESULTS

Whole genome gene expression analysis was performed on RNA samples isolated from all blood cells in whole blood of 46 patients with IVA treated with VIT. From all 48.804 probes present on the array, 48.071 transcripts had sufficient data for further analysis.