

Allergy against chironomids (non biting midges) in adult atopic patients

NILS E. ERIKSSON, CARSTEN SCHOU

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SUMMARY. *The aim of this investigation was to evaluate the prevalence of atopic sensitization to chironomids (CHI) in patients with asthma and/or rhinitis (A/R), and to study concomitant sensitization to CHI and other allergens. Skin prick tests were performed with 3 different CHI extracts as well as with common inhalant allergens in 600 consecutive patients, 495 of which had A/R. Allergen specific IgE antibodies in the sera against CHI, shell fish and cockroaches were analyzed with Magic Lite.*

59 (12%) of the patients with A/R had a positive skin test with CHI. Positive skin tests with house dust mites and a storage mite were more common in CHI allergic patients than in other atopic patients. Nasal or conjunctival provocation tests, performed on 23 of the patients with positive skin test with CHI, were clearly positive in 7 cases (30%), questionable in 8 (35%) and negative in 8 cases (35%).

Magic Lite, performed on sera from 50 of the patients with positive skin test against CHI, was positive with CHI in 39 cases (78%), with crayfish in 33 (66%), shrimp 20 (40%), cockroach 21 (40%) and with crab in 3 cases (6%).

It is concluded that sensitization against CHI is common in patients with A/R. The clinical relevance of the positive test results is, however, unknown. Concomitant sensitization with CHI, crustaceans and cockroach is common.

Key words: *allergy, chironomids, crustaceans, IgE antibodies, insects, skin prick test.*

Nils E. Eriksson, Lung- and Allergy Clinic, Dept of Medicine, Läns-sjukhuset, S-30185 Halmstad, Sweden.

Carsten Schou, Allergologisk Laboratorium, Postbox 408, DK-2970 Hørsholm, Denmark.

INTRODUCTION

Chironomid larvae (CHI), non-biting midges, are used as aquarium fish food, called «red mosquito larvae» (RML). Allergy to CHI is found,

however, not only among patients handling fish food. Widespread allergy against CHI (adults as well as larvae) has been reported from Sudan (Gad El Rab and Kay 1988) and from Japan (Kino *et al.*, 1987). According to an earlier

study, 14% of our atopic patients have an unquestionably positive ($>2+$) skin prick test (SPT) with an extract produced from chironomid larvae (Eriksson *et al.*, 1989). Another study indicated that the sensitization rate with CHI was higher among farmers than among our urban patients (Eriksson *et al.*, 1991). The clinical relevance of these positive test results is unknown.

The aim of the present study was to estimate the prevalence of CHI allergy in adult patients with asthma and rhinitis and to study concomitant sensitization to CHI and other allergens, especially arthropods.

PATIENTS AND METHODS

A) Evaluation of CHI extracts with SPT on CHI sensitized patients

20 adult individuals (mean age 34 years) with a previously known allergy against CHI were skin prick tested in duplicate with the following allergen extracts: (1) *Chironomus thummi thummi* larvae (CTT) extract from Allergologisk Laboratorium Copenhagen (ALK), in the following named CTT-ALK, employing 3 concentrations: 0.02, 0.2 and 2 gl^{-1} . (2) Our earlier used extract from CHI larvae (fish food, «red mosquito larvae», RML-LB) produced by Dr Lars Belin, Göteborg, with a concentration of 1/100 (w/v) (Eriksson *et al.*, 1989) (3) A crude CHI larvae extract received from Dr X Baur, München, Germany (RML-XB), with a concentration of 1 gl^{-1} (w/v) (Baur *et al.*, 1982). (4) A purified *Chironomus thummi* hemoglobin extract (*Chi t I*), received from Dr X Baur, with a concentration of 0.1 gl^{-1} (w/v) (Baur *et al.*, 1982).

As controls, twenty healthy persons were tested with the same extracts.

B) Studies on 600 consecutive patients

SPT's were performed on 600 consecutive adult patients (mean age 37, range 11-80 years) at the Lung- and Allergy Clinic with three CHI extracts (CTT-ALK 2 gl^{-1} , RML-LB 1/100 and *Chi t I* 0.1 gl^{-1}) in addition to 11 common inhalant allergens (Fig. 1).

Allergen specific IgE antibodies against CTT, crustaceans (crab, crayfish and shrimp) and cockroach were determined with Magic Lite (ML) on the sera of patients with positive SPT against CHI. Furthermore, Radiø Allergo Sorbent Test (RAST) was performed on selected sera with CTT-ALK and with two of the CHI species known to cause allergy in Japan, *Chironomus yoshimatsui* and *Tokunagayusurika akamusi*.

In vivo methods

SPT was performed as described earlier (Eriksson *et al.*, 1989). A wheal reaction of the same size as that of a positive histamine reference (histamine HCl 10 gl^{-1}) was recorded as three plus (3+).

Nasal or conjunctival provocation tests (PTs) were performed with CTT-ALK 0.2 gl^{-1} or 2 gl^{-1} on those CHI-SPT positive patients in the second part of the study, who volunteered for the provocation test. The tests were performed as described elsewhere (Eriksson *et al.*, 1989). The patients were asked to send in a written report of symptoms appearing 2, 8 and 24 hours after the testing.

In vitro methods

Magic Lite. Specific IgE to CCT, crustaceans and German cockroach was tested by Magic Lite chemiluminescence test (ML) essentially as described elsewhere (Sturgess *et al.* 1986, Woodhead and Weeks 1989, Weeks *et al.* 1983). Results were expressed as Allergy Classes (AC) 0-5. Non-specific binding was evaluated by performing ML with CTT on the sera of 5 non-CHI sensitized atopic individuals with a high grade grass pollen allergy.

RAST. Cyanogen bromide-activated paper discs were coupled at 1 mg CTT extract per ml (40 µl/disc). For each disc, 50 µl of serum was used. IgE binding was revealed with iodinated anti-IgE (18.000 cpm/disc; Pharmacia, Sweden). Results were expressed as percentages of total binding, a procedure regarded to be acceptable for the limited purposes of this study.

Allergen extracts

The CCT-ALK extract was produced as follows: 90 g of larvae from *Chironomus thummi thummi* were crushed in a mortar and stirred over night in 0,125 M ammonium bicarbonate. The material was centrifuged 40 min. at 15.000 x G and filtered through Whatman ø 1 filter paper. The extract was dialysed 3x12 hours against 100 volumes 0,125 M ammonium bicarbonate. After freeze-drying the extract was redissolved and the dialysing steps were repeated. By a final freeze-drying the yield was 1.11 g. For SPT the extract was redissolved in Coca's solution and sterilized through 0.22 µm filter. Sterile glycerol (50%) was added and the extract was ampouled under sterile conditions. For provocation the extract was redissolved in sterile water and filtered through 0.22 µm filter before freeze-drying in sterile ampoules. All operations were performed at 5° C. The extract was reconstituted with sterile saline immediately before use.

The RML-LB, RML-XB and *Chi t I* extracts are described above. The crab, crayfish, shrimp and German cockroach extracts used in ML were the commercially available ML extracts (ALK, Hörsholm, Denmark) and the common inhalant allergens used in the SPT were the commercially available Soluprick extracts from the same manufacturer (Fig. 1).

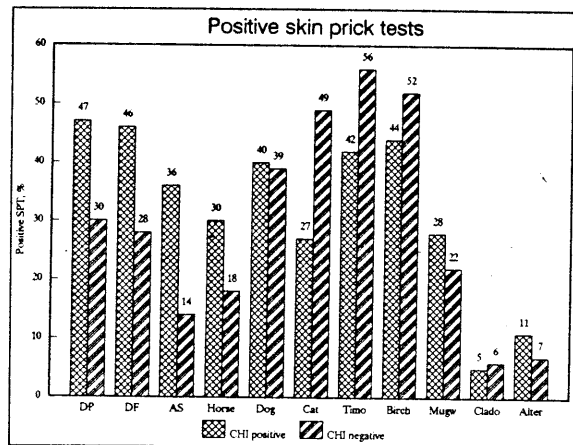


Figure 1. Comparison between SPT results of 59 CHI-sensitized (CHI positive) and 196 other atopic patients (CHI negative) with A/R. Soluprick 10 HEP extracts were used with *Dermatophagoides pteronyssinus* (DP), *Dermatophagoides farinae* (DF), dog, horse, cat, timothy (Timo), mugwort (Mugw) and birch, Soluprick 1/20 w/v extracts with *Alternaria* (Alter) and *Cladosporium* (Clado) and Soluprick «Diephuis» 1000 NE/ml with *Acarus siro* (AS). The differences between the two groups are statistically significant with AS ($P < 0.001$), DP ($P < 0.05$), DF ($P < 0.05$) and cat ($P < 0.01$, in the opposite direction).

Statistics

Differences between groups were tested with Fischer's exact test.

RESULTS

A) Evaluation of CHI extracts with SPT

SPT performed with CHI extracts on 20 healthy individuals was negative in all except one person. ML showed that his serum contained IgE antibodies in low concentration against CTT-ALK (AC=1). On the 20 CHI sensitized patients, the RML-LB extract gave the strongest SPT reactions, on an average a 3+ reaction. The mean diameters and mean number of pluses with the test extracts are shown in Tab. I. The CTT-ALK extract with a concentration of 2 gl⁻¹ was chosen for use in the second part of the study.

B) Studies on 600 consecutive patients

SPT results. Of the 600 consecutive patients, 280 (47%) had one or more positive (>0) SPT, 266 (44%) with common inhalant allergens and 61 (10%) with CHI extracts. Of the 496 consecutive patients who had A/R, 254 (51%) had positive SPT with one or more allergens, 240 (48%) with common inhalant allergens and 59 (12%) positive SPT with one or more of the CHI extracts. Of the 59 patients having positive SPT with CHI extracts, 14 had isolated positive SPT with CHI and 45 positive SPT with common inhalant allergens as well. Of the 254 atopic patients (i.e., patients with positive SPT results), 23% had positive SPT with CHI extracts. The quantitative SPT results with the different CHI allergen extracts are shown in Tab. II.

Comparison between CHI-sensitized and other atopic patients with asthma/rhinitis. Positive SPT with the mite allergens were more common in CHI sensitized patients than in patients not sensitized to CHI, whereas the opposite result was found with cat allergen (Fig. 1). Asthma was more common (39%) in the CHI sensitized group than in other atopics (20%) ($p < 0.05$). The ages of patients with CHI sensitivity (mean 34 years) did not significantly differ from that of atopic patients without CHI sensitivity (mean 32 years). Fourteen per cent of the patients in both groups had an aquarium in their home. The proportion of urban/rural residences did not differ between the groups.

Provocation tests were performed on 23 patients (12 nasal and 11 conjunctival provocation tests). Of these 7 (30%) were clearly positive, giving moderate or severe symptoms, whereas in 8 cases (35%) only slight symptoms, of doubtful clinical relevance, were elicited with CTT-ALK 2 gl^{-1} . Eight provocation tests (35%) were negative. Three of the patients reported moderate symptoms and 9 patients reported slight symptoms 8 hours after the visit at the clinic. No isolated late reaction was reported. Some anamnestic data as well as test results of the patients subjected to provocation tests are seen in Tab. III. Only two patients reported that they earlier had noticed symptoms when exposed to «red mosquito larvae» used as aquarium fish food.

Table I. Skin prick test results with different allergen extracts in 20 patients with a known allergy against chironomids. (Mean diameters and number of pluses, standard deviations in brackets).

Allergen	Diameter (mm)	No of pluses
CTT-ALK 0.02 g/l	2.4 (2.2)	1.0 (1.0)
CTT-ALK 0.2 g/l	3.7 (2.9)	1.5 (1.3)
CTT-ALK 2 g/l	5.2 (3.5)	2.1 (1.5)
RML-LB	7.4 (2.9)	3.0 (1.2)
RML-XB	1.4 (1.9)	0.6 (0.9)
Chi tI	0.6 (1.2)	0.3 (0.7)
Histamine 10 g/l	7.1 (0.8)	3+ (definition)

Table II. SPT results with CHI allergens in 59 patients having positive SPT to one or more of the CHI allergens.

Allergen	Number of pluses					Positive No	% of all tested atopic patients (n=254)
	Neg	1+	2+	3+	$> 3+$		
CTT-ALK	10	13	22	11	3	49	19%
RML-LB	4	14	25	12	4	55	23%
Chi tI	49	2	3	5	0	10	4%

Comparison between CHI-sensitized and other atopic patients with asthma/rhinitis. Positive SPT with the mite allergens were more common in CHI sensitized patients than in patients not sensitized to CHI, whereas the opposite result was found with cat allergen (Fig. 1). Asthma was more common (39%) in the CHI sensitized group than in other atopics (20%) ($p < 0.05$). The ages of patients with CHI sensitivity (mean 34 years) did not significantly differ from that of atopic patients without CHI sensitivity (mean 32 years). Fourteen per cent of the patients in both groups had an aquarium in their home. The proportion of urban/rural residences did not differ between the groups.

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Table III. Some anamnestic data and test results of patients subjected to provocation tests. (1) +=have contact with aquarium earlier; ++=have contact with aquarium presently. (2) 0=no symptoms, 1=slight symptoms, 2=moderate symptoms, 3=severe symptoms. (3) have noticed symptoms on exposure to red mosquito larvae. (4) PT positive with CTT-ALK 0.2 gl^{-1} . nd=non determined.

Age and initial	Aquarium (1)		Known contact with RML	Symptoms elicited by crustaceans	Test results with CTT-ALK		
	at home	at friends' homes			SPT	ML	PT (2)
36 OB	++	—	+(3)	—	6	5	3 (4)
21 PT	++	—	+(3)	—	4	nd	3
13 MK	+	++	+	?	3	3	3
23 CS	+	—	+	—	2	1	3
30 KC	+	—	+	—	3	nd	2
26 KA	—	—	—	—	3	2	2
28 AE	+	—	—	—	2	1	2
19 TT	+	—	+	+	3	2	1
21 AH	+	—	+	+	2	2	1
55 RS	—	++	—	+	2	1	1
36 LOE	—	—	—	+	2	2	1
36 YS	—	—	—	—	2	1	1
51 BS	—	—	—	—	1	0	1
48 LS	—	++	+	+	1	0	1
33 AA	+	—	—	—	0	0	1
33 PB	—	—	—	—	2	1	0
24 SSP	—	+	—	+	2	1	0
53 OE	+	—	—	—	2	0	0
44 LK	+	—	—	+	2	1	0
29 LB	+	—	+	—	1	nd	0
17 FA	+	—	—	—	0	1	0
66 OA	—	—	—	—	0	1	0
68 RJ	—	—	—	—	0	0	0

Specific IgE antibodies in serum. The ML performed with CTT on the sera of five grass pollen allergic patients showed no unspecific binding. ML, performed on sera from 50 consecutive patients, having positive SPT with CHI, was positive in 72% with CHI, in 62% with crayfish, in 42% with cockroach, in 36% with shrimp and in 8% with crab. The ML classes of 70 sera (CHI sensitized patients from part A and part B of the study grouped together) are seen in Tab. IV.

Table IV. Results of determination of IgE antibodies with Magic Lite (ML) against CTT-ALK, crustaceans and cockroach in sera from 70 patients (from part A and part B of the study) with positive SPT with CHI.

Allergen	ML (Allergy class)						Positive ML	
	0	1	2	3	4	5	No	%
CTT-ALK	17	31	17	4	0	1	53	76
Crayfish	28	13	20	7	2	0	42	60
Shrimp	40	6	17	6	1	0	30	43
Cockroach	41	17	9	3	0	0	29	41
Crab	57	4	6	2	1	0	13	19

Of the 14 patients who had isolated positive SPT with CHI, 12 had CHI-specific IgE antibodies in their sera, according to ML. One of the two patients with negative CHI-ML had a positive ML result with crayfish.

A good correlation was found between RAST determinations of specific IgE against CTT, *Chironomus yoshimatsui* and *Tokunagayusurika akamusi* (Fig. 2).

Correlations between in-vivo and in-vitro results. When the SPT results and ML results with CTT-ALK were compared, a fairly good correlation was seen, although individuals with a positive SPT and negative ML as well as a few patients with the opposite result were seen (Tab. V).

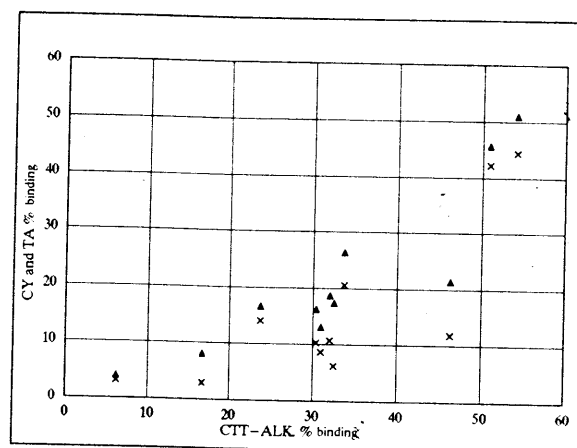


Figure 2. Correlation between RAST results with *Chironomus thummi thummi* (CTT), *Chironomus yoshimatsui* (CY) and *Tokunagayusurika akamusi* (TA) in sera of selected patients. The non-specific binding was 1.6% with CTT-ALK, 0.6% with CY and 0.8% with TA.

△ = *C. yoshimatsui*, x = *T. akamusi*.

Table V. The quantitative connection between SPT and Magic Lite (ML) analyses with CTT-ALK in 73 patients.

ML (Allergy class)	SPT result (number of pluses)					Positive SPT (%)
	0	1	2	3	>3	
>3					1	100
3				2	1	100
2		2	10	5	3	90
1		5	4	13	5	82
0		4	10	4	3	81
Positive ML (%)	73	29	85	80	100	

DISCUSSION

The finding that 12% of our adult patients with A/R (corresponding to 23% of atopic patients with A/R) have positive SPT with CHI allergens, are in agreement with an earlier study from the same clinic (Eriksson *et al.*, 1989). Allergen specific IgE antibodies against CTT, determined with ML, was found in 72% of

patients having positive SPT with CHI. This shows that at least the majority of the positive SPT results are due to real IgE sensitization. Some of the patients have been sensitized to CHI when exposed to RML used as fish food, and the sensitization of other patients might be explained by their allergy to crustaceans, cross-reacting with CHI (Eriksson *et al.*, 1989). For the majority of CHI sensitizations, however, other explanations have to be searched for. A reasonable guess is, that the patients have been sensitized when exposed to adult CHI occurring in their environment.

The results of the provocation tests, giving only 8 clearly positive reactions out of 23 tests, suggest that the CHI sensitization is of limited clinical importance.

Baur *et al.* (1982) have identified sensitization to CHI hemoglobin and have localized antigenic determinants to certain polypeptide sequences of the hemoglobins. Only 17% of our CHI sensitized patients had positive SPT with the purified hemoglobin (*Chi t I*) allergen. This finding could indicate that the sensitization of our patients more often is directed against other CHI allergens. It could also mean, however, that we used the *Chi t I* extract with a too low concentration, giving falsely negative SPT.

IgE antibodies against crustaceans and cockroach were found in many of the sera from CHI-sensitized patients. Inhibition studies, performed on the sera from some of our patients and reported elsewhere (Schou *et al.* 1991) have shown that true IgE cross-reactivity occurs between CHI, crustaceans and cockroach, thus corroborating some of the results of an earlier study (Eriksson *et al.*, 1989).

We have no explanation to the finding that positive SPT with mites (*DP*, *DF* and *Acarus siro*) were more common in the CHI sensitized patients than in other atopics. In another study, in which 50 CHI-sensitized patients were compared with 50 CHI negative patients with an

allergy against animal and/or house dust mite, SPT results with *DP* did not differ between CHI positive and CHI-negative individuals. The only significant differences found were, that 17/50 CHI-sensitized but only 1/50 of the CHI-negative patients had an aquarium in their home, and that 16/50 CHI-positive but only 7/50 CHI-negative patients reported symptoms elicited by shellfish (Eriksson, 1991).

Future evaluations should focus on the relationship between exposure and sensitization and on the possibility of cross-reactions between CHI and mites.

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