

IMMUNOPATHOGENESIS OF ASTHMA

YKL-40 Protein is a Marker of Asthma

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Background. Gaining asthma control is still a challenge in a large number of patients. It could be facilitated by using biomarkers indicating the grade of inflammation and correlating with clinical picture. Chitinases and chitinase-like proteins play a role in Th2-type inflammation. Thus, they may be useful in diagnosing and monitoring of asthma. **Objectives.** The aim of the study was to investigate the relevance of YKL-40 as a good biomarker of asthma, its control, and severity. **Methods.** Level of YKL-40 was determined by means of immunoassay in sera of 59 asthmatics (39 women, 20 men, aged 23–76 years) and 29 healthy controls (18 women, 11 men, aged 20–80 years). Asthma severity and control were assessed according to GINA guidelines. Differences between groups were compared with the use of Mann–Whitney's *U*-test. Correlations between variables were assessed with Pearson's test. **Results.** Symptoms of asthma were found to be controlled in 12 (20%), partly controlled in 17 (29%), and uncontrolled in 30 (51%) patients. YKL-40 levels were significantly higher, on average, in asthmatics compared to control group (median levels: 125.3 U and 84.1 U, respectively, $p < .001$). YKL-40 correlated with the number of blood eosinophils ($r = 0.376$, $p = 0.05$). However, no relations have been found between YKL-40 level and asthma severity, control, or total serum IgE ($r = -0.05$, $p = .05$). **Conclusion.** YKL-40 seems to be a good marker of asthma. However, its level may not correlate with clinical outcome.

Keywords asthma control, chitinases, chitinase-like protein, eosinophilia

INTRODUCTION

Asthma is a chronic inflammatory disorder affecting 300 million people worldwide. Deteriorated quality of life, poor control of symptoms, and exacerbations remain significant problems even despite progress in terms of pharmacotherapy. In most of the populations, total control of symptoms is achieved only in 30–50% of cases (1, 2). The outcomes might have been improved by earlier diagnosis and better monitoring. Thus, new biomarkers are still being searched to achieve these goals and, as a result of better management, ameliorate patients' quality of life. In recent years, several questions have been raised on the role of chitinase and chitinase-like proteins (CLPs) in chronic bronchial inflammation.

Chitinases are ubiquitous in lower life forms, for example, crabs, insects, and spiders where they degrade chitin—essential part of cell walls and exoskeleton. They are also found in hosts fighting infection provoked by chitin-containing organisms. Contrary to chitinases, CLPs bind chitin but do not have degrading activity. Mammalian chitinases and CLPs (acidic mammalian chitinase—AMC, chitotriosidase, oviductin, human cartilage glycoprotein—HcGP/YKL-40) are produced by macrophages in response to parasitic or fungal infection. YKL-40 levels were also found elevated in rheumatoid arthritis, myocardial infarction, diabetes, Crohn's disease, and various cancers (3–6).

Reaction to parasitic infection is in some aspects similar to mechanisms of allergic inflammation. As a

result it has been hypothesized that CLP may play a significant role in allergic inflammation. YKL-40 levels have been found higher in asthmatics compared to healthy populations (7). Moreover, its concentration was elevated in bronchoalveolar lavage fluid after allergen challenge (8). However, possible correlations with clinical outcomes still need to be investigated. In Polish asthmatic population, no studies on the role of CLP in asthma have been conducted so far.

The aim of this study was to investigate the role of YKL-40 as a possible marker of asthma, its severity, and control.

PATIENTS AND METHODS

Eighty-eight adult inhabitants of the Pomerania region (northern Poland) were recruited over a period from November 2007 to March 2009.

The study group comprised 59 patients, including 39 females and 20 males aged 23–76 years with chronic asthma, confirmed by reversibility test, and treated for at least 6 months. Patients were recruited from the In- and Outpatient Departments of Allergology, Medical University of Gdansk, Poland. Exclusion criteria were episodic asthma, concomitant chronic respiratory disease (COPD, interstitial disease, etc.), immunological disorders, cancer, current immunosuppression, hepatic cirrhosis, and renal insufficiency. Pregnant women were not eligible for the study.

The control group comprised 29 healthy individuals, including 18 females and 11 males aged 20–80 years. They were participants of epidemiological studies conducted in the Department of Allergology, randomly chosen from local population. Exclusion criteria were

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similar to the asthma group but, additionally, they included any chronic respiratory disorders and current or past symptoms of allergy.

STUDY PROTOCOL

Having given informed consent, all the subjects underwent clinical assessment and spirometry (Lungtest 1000, MES, Cracow, Poland). Asthma severity and level of control were assessed according to GINA 2006 guidelines. In order to determine atopic status, skin-prick tests were performed with common allergens: grass, trees, weeds, cat, dog, *Dermatophagoides farinae*, *Dermatophagoides ptenonyssinus*, and molds (*Allergopharma*). Atopy was defined as at least one positive response.

Five milliliters of venous blood was collected from every participant of the study. Blood was centrifuged and serum stored at a temperature of -20°C . YKL-40 was detected with commercially available ELISA kits (Quidel, San Diego, CA, USA; catalog no. 8020) in accordance with manufacturer's instructions.

All data were analyzed using Statistica 8.0 statistical software. Demographic data were analyzed with χ^2 test or *t*-test. Concentration of YKL-40 was compared in asthmatics and controls as well as in groups of asthmatics of different severity, level of control, and atopic status. For these analyses, Mann-Whitney's *U*-test was applied. Correlations between YKL-40, IgE (25 patients), and blood eosinophilia (59 patients) were assessed with Pearson's test. Level of significance was defined as $p \leq .05$.

Study protocol was approved by Local Bioethics Committee at Medical University of Gdańsk.

RESULTS

Characteristics of study groups have been presented in Table 1. Symptoms of asthma were found to be controlled in 12 (20%), partly controlled in 17 (29%), and uncontrolled in 30 (51%) patients. In 15 (25.3%) patients, asthma was assessed as mild, in 24 (40%) as moderate, and in 20 (34.7%) as severe.

At least one positive result of skin prick test was found in 31 asthmatics (52%) and 3 controls (10%). Sensitizations to house dust mites, grass, and weeds were the most common.

TABLE 1.—Characteristics of the study groups.

	Asthma group	Control group	<i>P</i> -value
Age (years)	45 $\pm$ 12.3	41 $\pm$ 15.6	.10
FEV ₁ /FEV ₁ predicted	83.1 $\pm$ 21.2	101.19 $\pm$ 10.7	<.001
Number of eosinophils ($10^9/\text{L}$)	0.256 $\pm$ 0.176	0.127 $\pm$ 0.122	.038
Positive skin-prick tests	31 (52%)	3 (10%)	.001
Total serum IgE (IU/mL)	194.3 $\pm$ 130	—	

Note: Data are presented as mean values $\pm$ SD.

Mean serum YKL-40 level was 137.9 U/L, median 112.9 U/L. There was no correlation with age or gender. It was significantly higher, on average, in asthmatics (median 125.3 U; interquartile range 94.16–155.14) compared to control group (median 84.1 U; interquartile range 50.94–124.0; $p < .001$) (Figure 1). YKL-40 level correlated with blood eosinophilia ($r = 0.376$, $p = .05$) (Figure 2). However, no relations have been found between its level and asthma severity, control, or atopic status (total serum IgE, positive skin prick tests) (Figures 3–5).

DISCUSSION

Chitinases are evolutionarily conserved hydrolases. By degrading chitin, they play an important role in controlling homeostasis in lower life forms. They are also produced by hosts fighting infections caused by chitin-containing organisms. Although chitin is not synthesized by mammals, chitinases have been described in several species, including humans. Their role is still not fully understood. However, their expression at inflammation sites suggests that they play a role in antiparasitic defense and repair mechanisms.

In the study of Zhu et al. (9) AMC was found to be upregulated in ovalbumin mouse model and its expression was induced by IL-13. Inhibition of AMC activity prevented from airway hyperresponsiveness. In another animal model, oral administration of chitin downregulated Th2 inflammation (10). Thus, it may be hypothesized that chitinases induce Th2 inflammation. This type of reaction originally evolved to deal with parasitic infections, but is also typical for allergy and asthma. As a consequence chitinases may be regarded as a bridge linking the two phenomena.

YKL-40 is a CLP synthesized by human macrophages, monocytes, neutrophils, synoviocytes, and epithelial cells (11, 12). Its role in the pathogenesis of asthma and allergy remains unclear. It has been demonstrated that its level is higher in asthmatics and correlates with severity of the disease (7). Moreover, YKL-40 has been found to facilitate allergen sensitization and IgE production (13).

Some significant correlations have also been revealed in the genetics of CLPs. YKL-40 is encoded by the chitinase-3-like 1 gene (*CHI3L1*). Single nucleotide polymorphisms in *CHI3L1* promoter have been found to be associated with elevated YKL-40 levels as well as atopy, asthma, and bronchial hyperreactivity (14, 15).

Our study shows that in Polish population, serum levels of YKL-40 are significantly higher in patients with asthma compared to healthy controls. The data are in line with previous findings of Chupp et al. (7). The highest levels (440–664 U/L) were revealed in patients with uncontrolled and partly controlled asthma, including three participants with aspirin asthma.

Contrary to the study by Chupp et al., we have not found any statistically significant correlation with the clinical course of asthma. Some trends that did not reach significance (e.g., possible correlation between

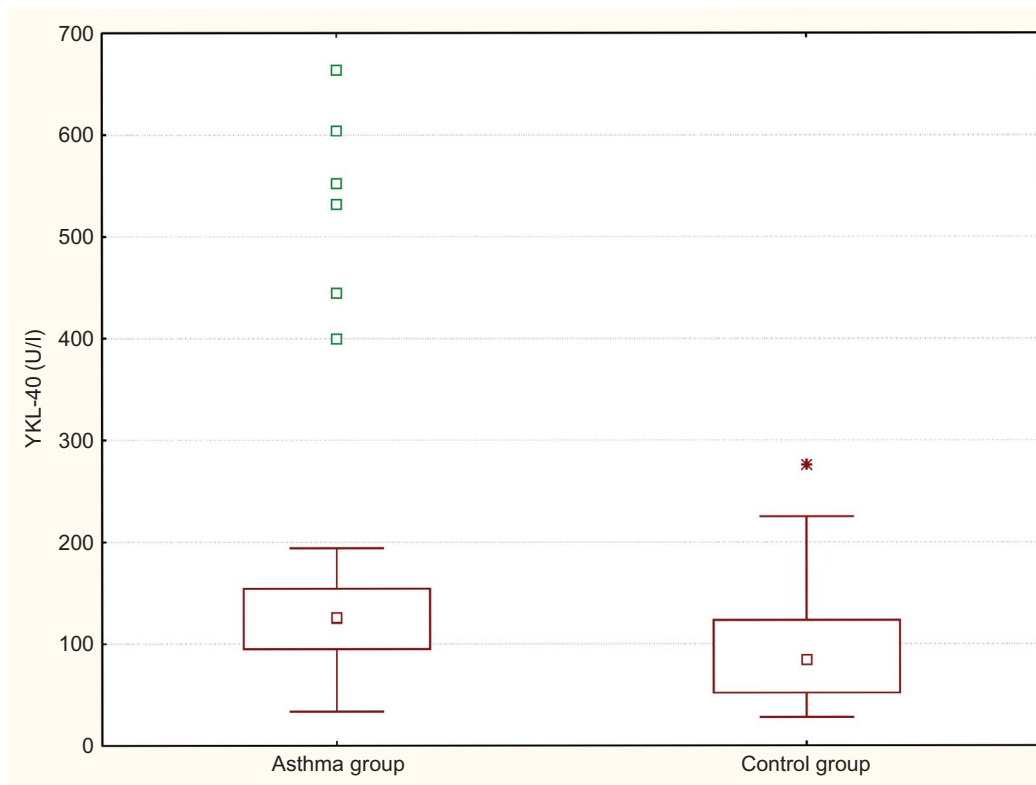


FIGURE 1.—YKL-40 concentration in relation to the presence of asthma ($n = 88$, $p < .001$). Data are presented as median (central square), 25th and 75th percentiles (top and bottom of boxes), 10th and 90th percentiles (top and bottom of bars), and outliers (stars, squares).

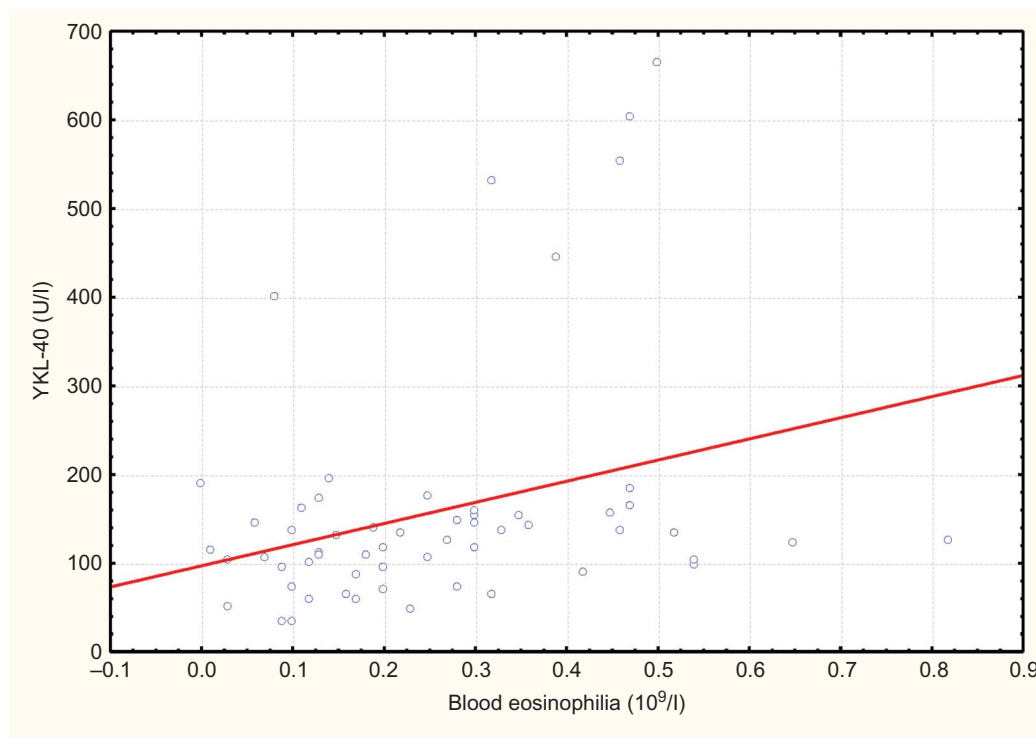


FIGURE 2.—YKL-40 levels in relation to eosinophilia in asthma group ($n = 59$, $r = 0.309$, $p = .05$).

YKL-40 levels and asthma control; Figure 4) should be investigated in larger samples.

Although YKL-40 level correlated with blood eosinophilia ($r = 0.376$, $p = .05$), no significant relation has been found between its level and total IgE

($r = -0.05$, $p = .05$). This is inconsistent with previous studies by Chupp et al. and Tang et al. (16). In the latter, serum YKL-40 level was higher in patients with asthma exacerbation compared to group with stable asthma and correlated with both blood eosinophilia and

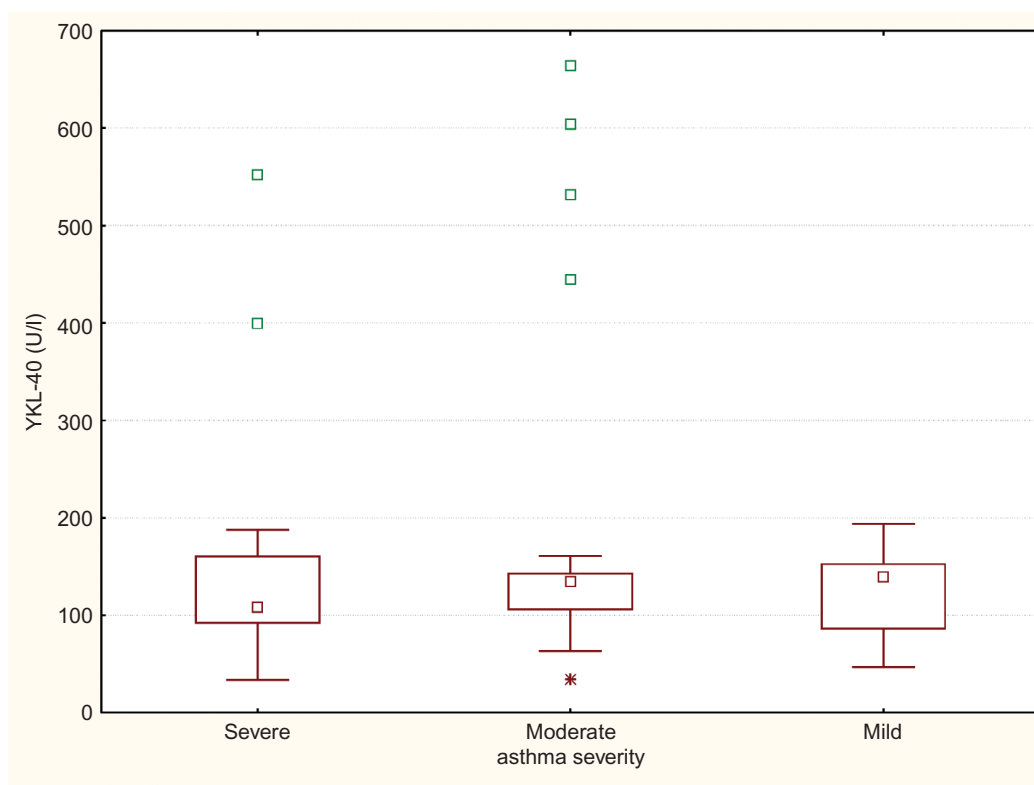


FIGURE 3.—YKL-40 levels in relation to severity of asthma ($n = 59$, $p = .12$). Data are presented as median (central square), 25th and 75th percentiles (top and bottom of boxes), 10th and 90th percentiles (top and bottom of bars), and outliers (stars, squares).

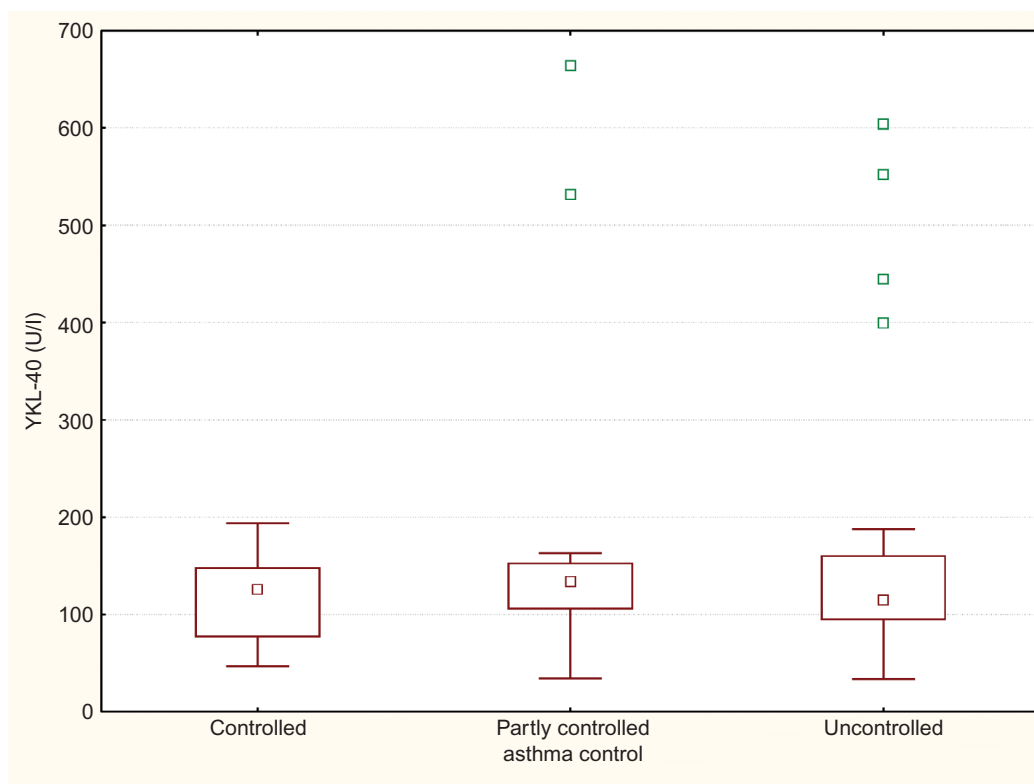


FIGURE 4.—YKL-40 levels in relation to asthma control ($n = 59$, $p = 0.07$). Data are presented as median (central square), 25th and 75th percentiles (top and bottom of boxes), 10th and 90th percentiles (top and bottom of bars), and outliers (stars, squares).

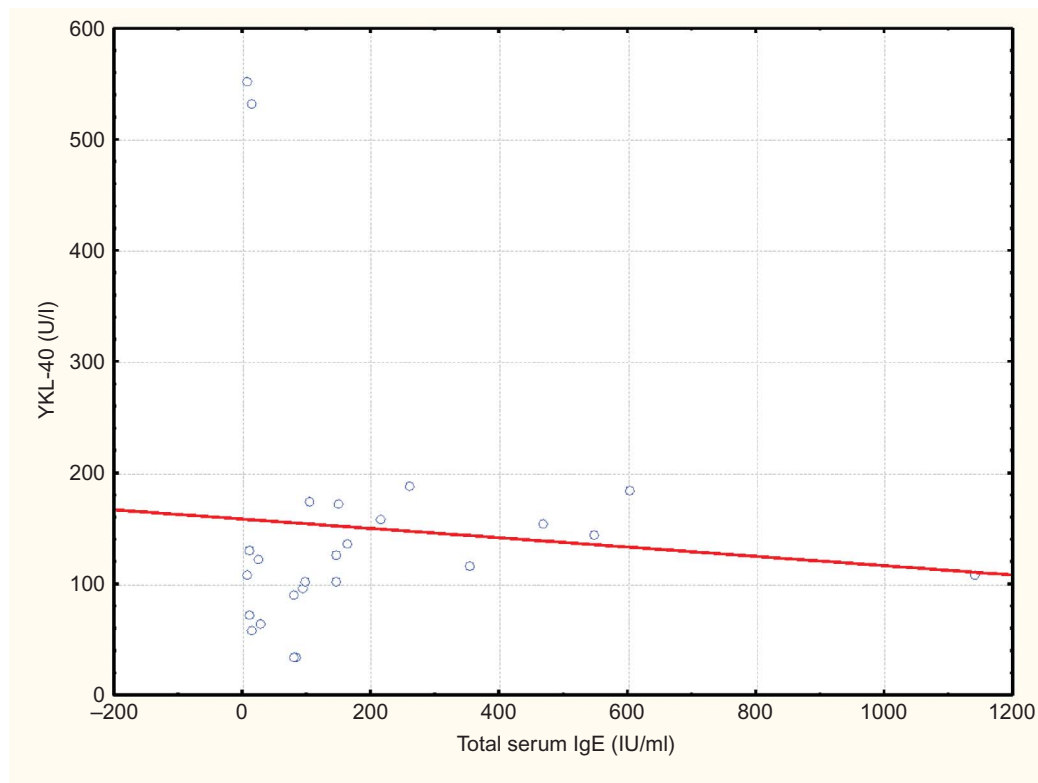


FIGURE 5.—YKL-40 levels in relation to total serum IgE ($n = 25$, $r = -0.05$, $p = .05$).

IgE. However, in none of these studies patients were stratified according to GINA classification based on clinical symptoms.

Although our population was similar to the groups included into the studies by Chupp et al. and Tang et al. in terms of age, further comparison is impossible due to the lack of detailed characteristics of their clinical picture. Asthma is a heterogenic disease with several phenotypes reflecting varied inflammatory patterns. It still remains unclear whether chitinases are upregulated in general asthmatic population or exclusively in some of the phenotypes. The latter scenario would explain contradictions in results of the cited studies.

CONCLUSIONS

CLP YKL-40 seems to be a good marker of asthma. Its level correlates with blood eosinophilia ($r = 0.376$, $p = .05$). However, such relations were not found for clinical outcome or total serum IgE ($r = -0.05$, $p = .05$). Further studies are necessary to confirm its role in several phenotypes of asthma.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the writing and content of this paper.

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