Non-Hodgkin's lymphoma in the course of asthma – incidence or predisposition?

Współistnienie chłoniaka nieziarniczego z astmą – przypadek czy predyspozycja?

Małgorzata A. Czarny-Działak[1], Damian Strzemecki[2], Wiesław Kryczka[3], Martyna Głuszek-Osuch[4,5,6], Anna Słuszniak[6], Tomasz Kryczka[7,8]

[1]Department of Family Medicine and Geriatrics, Institute of Medical Sciences, Faculty of Medicine and Health Science, Jan Kochanowski University, Kielce, Poland
Head of the Department: Andrzej Jaroszyński MD, PhD, Prof. JKU
[2]Department of Experimental Pharmacology Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland
Head of the Department: Prof. Paweł Grieb MD, PhD
[3]Faculty of Medicine and Health Science, Jan Kochanowski University, Kielce, Poland
Head of the Department: Marcin Pasiarski MD, PhD
[4]Laboratory of Psychology and Medical Pedagogy, Faculty of Medicine and Health Sciences, Institute of Public Health, Jan Kochanowski University, Kielce, Poland
Head of the Laboratory: Aldona Kopik PhD
[5]Clinic of Internal Disease and Gastroenterology, Central Clinical Hospital Ministries of Internal Affairs and Administration Mental Health Clinic, Central Clinical Hospital Ministries of Internal Affairs and Administration, Warsaw, Poland
Head of the Clinic: Prof. Grażyna Rydzewska MD, PhD
[6]Department of Medical Biology, Cancer Marker Department, Świętokrzyskie Oncology Center, Kielce, Poland
Head of the Department: Anna Słuszniak PhD
[7]Department of Experimental Pharmacology, Mossakowski Medical Research Centre Polish Academy of Sciences, Warsaw, Poland
Head of the Department: Prof. Paweł Grieb MD, PhD
[8]Department of Medical Biology, Medical University of Warsaw, Warsaw, Poland
Head of the Department: Gabriela Oledzka PhD

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Asthma is a chronic inflammatory disease of the respiratory tract. Inflammation is induced by mediators released by the plurality of cells of the immune system. In this process, the main role is played by cytokines such as interleukin-25 (IL-25) [1–7] and mast cells that, by the excretion of a number of cytokines, induce bronchospasm [6, 7]. The diagnosis of asthma is still based on clinical symptoms and spirometry test. Approx. 80% of patients with asthma develop rhinitis that is the ordinary inflammation of the upper respiratory tract [2, 3, 6]. This confirms the involvement of the entire respiratory tract in the mucosal inflammatory process. Non-Hodgkin's lymphoma (NHL) is a cancer of the lymphoid system. The most common symptoms include swollen lymph nodes and/or the extralymphatic presence of a tumour, and generalised symptoms like fever, night sweats, or weight loss [8] and the enlargement of the spleen or liver, abdominal pain, or dyspeptic symptoms. A significant splenomegaly is usually related to anaemia, thrombocytopaenia, or leukopaenia. Most patients with NHL reveal impaired immunity from the beginning of the disease, and treatment (especially chemotherapy and immunotherapy) intensifies this condition. The impairment of the humoral and cellular immunity in NHL patients predispose to increased morbidity for atypical infections [8–10]. Tumor necrosis factor α (TNF-α), tumour necrosis factor is a homotrimer formed as an integral membrane protein, which, under specific metalloproteinase TACE (TNF-α converting enzyme), is released from the cell. It is produced by many cells: monocytes, macrophages, and lymphocytes. The strongest factor stimulating its release is the lipopolysaccharide component of the bacterial membrane. Practically all nucleated cells contain the receptors for TNF-α on their surface, hence its differential effects [4]. Interleukin-25 belongs to the family of cytokines of the IL-17 group showing the pleiotropic mechanism of action via receptor IL-17RB. Its other (older) name is IL-17E. Interleukin-25 is secreted by Th2 helper cells and mast cells; it inhibits production of IL-1 and IL-23 [4] but induces the release of other cytokines like IL-4, IL-5, IL-13, and eotaxin [4]. This stimulates eosinophils and production of IgE antibodies associated with the development of allergy. Interleukin-25 facilitates the process of diapedesis of neutrophils, which are an important factor in the development of severe asthma. It is already known that after the use of a specific antibody against intercellular adhesion molecule 1 (ICAM-1) the transfer of neutrophils to the respiratory tract is decreased. Interleukin-25 also

increases the lifespan of eosinophils, the key cells in the inflammatory process in asthma [5]. This interleukin participates in chronic inflammation process, particularly in the gastrointestinal tract, and induces inflammation in lungs and respiratory tracts [1]. It is already known that this cytokine controls type 2 immune response, and we know that the advantage of Th2 over Th1 is characteristic for allergic diseases [1]. Allergic rhinitis often leads to asthma. Inflammation of the lower respiratory tract is accompanied by inflammation of the upper respiratory tract [2, 3, 6, 11]. This increases allergies and participation of pro-inflammatory cytokines. However, we must remember that IL-25 has a dual mechanism of action: it prevents the development of devastating inflammation by the inhibition of the development of chronic inflammatory diseases, and on the other hand it stimulates the immune response promoting the development of chronic inflammatory diseases like asthma or other allergies [12, 13]. Both asthma and cancer diseases seem to be influenced by genetic and environmental factors. There is a theory that both diseases are the result of a slight malfunction of the immune system of the patient. Due to this, if the patient develops the autoimmune disease, sometime later distant types of diseases, like cancer, are suddenly revealed. The special cases are Hodgkin's and non-Hodgkin's lymphomas, which more frequently are found in atopic patients [14]. However, a clear relationship between these haematological malignancies and asthma has not been revealed so far. Here we report a case of asthma combined with non-Hodgkin's lymphoma.

The aim of the study was to describe of the case of a 35-year-old patient with a long history of asthma, chronic sinusitis, and non-Hodgkin B cellular lymphoma.

A 35-year-old woman with long-term chronic sinusitis and severe asthma was diagnosed with B cellular lymphoma (from the lymphoplasmacytic line) in 2015 and then treated with chemotherapy – also during the study. Previously she developed leukocytoclastic vasculitis in the course of mixed crioglobulinemia in 2014, and she went through partial nephrectomy due to bright-cell kidney cancer. She was also successfully treated on infiltrative lung tuberculosis some years earlier. Monoclonal gammopathy was diagnosed at that time. The patient, since she was 25 years old, has been diagnosed with gastroesophageal reflux disease and fatty liver. She was often hospitalised at the allergology, pulmonology, dermatology, infectious diseases, rheumatology, and haematology departments. Significant progress of severity of asthma was noted within the previous 3 years. Due to frequent infections of the respiratory tract as well as urinary tract or skin infections (last year she suffered from erysipelas) she was often admitted to the hospital.

Peripheral blood was collected by standard protocol. Briefly, blood samples were allowed to separate and clot at room temperature for 30 min, followed by centrifugation at 1000 g for 20 min. The serum samples were then aliquoted and stored at –80°C.

The concentrations of 9 cytokines were analysed in duplicate using a Luminex-based platform (Bio-Plex 200; Bio-Rad, Hercules, CA, USA) and the 9-plex assay for measurement of IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13, granulocyte macrophage colony-stimulating factor (GM-CSF), and interferon γ (INF-γ), and TNF-α was used for analysis (Bio-Plex Pro Human Cytokine Th1/Th2 Panel; Bio-Rad Laboratories, CA, USA) with the standard protocol.

The concentration of IL-25 was measured with ELISA method using ready-to-use kit SEB 694Hu (Producer: USCN Life Science Inc. Houston, USA). The measurement range was from 15.6 to 1000 ng/ml. By the step-by step dilutions of the initial standard of IL-25 (10,000 pg/ml) the following standards 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml, and 15.6 pg/ml were obtained and used for instrument calibration and standard curve processing. The blood samples were taken into tubes with gel – with no anticoagulant, which were left for 30 min at room temperature. Next, after the end of the coagulation process, the samples were swirled for 20 min at 2500 rpm (1000× g). Then serum was separated into three tubes, and samples were stored at –70°C until collection of the appropriate number of respondents. Concentration of interleukin was determined by enzyme-linked immunosorbent assay ELISA, with the use of reagent kit SEB 694 Hu. The actual measuring range of the kit varied from 15.6 to 1000 ng/ml. After the initial dilution of the standard 10,000 pg/ml, standards 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 31.2 pg/ml, and 15.6 pg/ml were obtained. In the kit 96-well plates coated with IL-25-specific antibodies conjugated with biotin were utilised. After adding standards and patients' serum, the well plates were incubated for 2 h at 37°C. Content was extracted and 100 µl of reagent A added. Next, the well plates were incubated for 1 h at 37°C. After triple rinsing of wells, 100 µl of reagent B was added and incubated for another 30 min at 37°C. Content was extracted, and the well plates were rinsed five times. 90 µl of substrate solution (TMB) was added and the well plates were incubated for 20 min at 37°C (protecting them from direct sunlight). Next, 50 µl of sulphuric acid (stop solution) was added in order to complete the reaction. The well plates were read at 450 nm on an ELISA reader with the possibility of automatic calculation of concentration. The concentration of IL-25 in samples was determined by comparing the optical density of the sample with the standard curve. In the whole study 80 people participated: 20 volunteers and 60 patients suffering from asthma – 20 with chronic mild asthma, 20 with moderate, and 20 with severe. The grouping criteria to a certain degree of severity were clinical signs, cat-

Table 1. Classification of the asthma severity based on clinical symptoms according to GINA 2007 criteria

Clinical symptoms	Mild chronic asthma	Medium chronic asthma	Severe chronic asthma
Daytime symptoms	More often than once a week, less frequent than once a day	Occurs every day	Occurs every day
Nocturnal symptoms	More often than twice a month	More often than once a week	Frequent
Exacerbations	Can disturb sleep and make daytime activity more difficult	Can disturb sleep and make daytime activity more difficult	Frequent
FEV_1 or PEF	More or equal to 80% of proper value	60–80% of proper value	Less or equal to 60% of proper value
FEV_1 or PEF variability	< 20–30%	> 30%	> 30%
Other	–	The everyday necessity of inhalation with short-acting β_2-adrenergic receptor agonists	Restricted physical activity

FEV_1 – first-second expulsion volume, PEF – peak expulsion flux.

Table 2. The disease control criteria according to GINA 2014

Criteria	Daily symptoms	Restriction of the living activity	Night-time symptoms, awakenings	The need of interim treatment	Lung activity	Exacerbations
Controlled asthma	Not occurring (less than or equal to twice a week)	Not occurring	Not occurring	Never (less than or equal to twice a week)	Correct	Not occurring

Table 3. Levels of interleukin

Variable	IL-2	IL-4	IL-5	IL-10	IL-12p70	IL-13	GM-CSF	INF-γ	TNF-α
Patient	NE	NE	NE	NE	0.7	NE	NE	NE	2.82
Mother	NE	NE	NE	NE	4.45	NE	NE	NE	149.52
Brother	NE	NE	1.16	1.04	3.83	0.65	NE	NE	18.76

NE – not elevated.

egorised according to the Global Initiative for Asthma (GINA) (Tables 1 and 2).

Blood for analysis of IL-25 was sampled from every patient, using the ELISA method. Laboratory tests were carried out at the Holy Cross Oncology Center in the Institute of Cancer Markers. We received permission from the Bioethics Committee to carry out the tests.

The concentration of IL-25 in the samples was defined by comparing the optical density of the examined sample to the standard curve. In the blood sample of our patient in the first examination (Producer: USCN Life Science Inc.) using this method the reaction was not observed. According to own observations the concentration of IL-25 of "our" patient should be in the lower range of the calibration curve. However, in the next ELISA measurement (Producer: Sun Red, Shanghai, China) the concentration of this cytokine was within normal range – 606.06 ng/l (the higher concentration of this cytokine was located in the range above or equal to 2585.9 ng/l).

According to the clinical symptoms and spirometry test, the patient was diagnosed with chronic severe asthma. The severity of asthma was assessed according to GINA criteria (Global Initiative for Asthma).

The patient's asthma was under control during the visit.

The patient must meet all criteria to display controlled asthma. In the case of the patient the IL-25 was measured twice: with ELISA test, and the cytokine profile was evaluated H1/Th2. Although in the ELISA test no elevated levels of IL-25 were discovered, Bio-Plex provided interesting results: surprisingly low level of TNF-α (2.82 ng/ml) in the patient compared to other family members with IL-25 high levels in her mother and brother – 149.52 ng/ml and 18.79 ng/ml, respectively. The results of other cytokines remained in normal range. Interestingly, the mother of the patient had a long history of chronic asthma (for many years, inhaled glucocorticosteroids in high doses), while her brother had never developed any symptoms of such disease (Table 3).

The question arises whether this low level of TNF-α had an influence on the development of other diseases in the patient, while the high level of TNF-α in the family members seemed to be a protecting factor. We already know, from the example of mice with decreased ability of producing TNF-α, that they are approximately 1000 times more susceptible to fatal infections with *Escherichia coli* bacteria, whereas in the medical history of our patient we noticed severe infection with *Mycobacterium tuberculosis* with the following progression of infiltrative tuberculosis. It is also known that some non-Hodgkin lymphomas, i.e. MALT, can develop during chronic bacterial infection. Maybe the impaired immune system of our patient led to the infection that predisposed the development of lymphoma [4, 8]. Tumor necrosis factor α can influence the immune system through other cytokines like IFN-γ released from lymphocytes or interleukin 1 and 6 (IL-1, IL-6) or a factor stimulating the creation of macrophage and granulocyte colonies (GM-CSF), G-CSF, M-CSF, PDGF, PAF, and others [4]. Then, the decreased level of TNF-α could be responsible for the decreased nonspecific immune response that triggered frequent infections of the respiratory system, urinal tracts, and skin.

Recent reports indicate that IL-25 can activate eosinophil proteins during allergic inflammation [12] and that cytokine is the key one participating in the Th2-type response in asthma [1, 13]. It also induces, acting in parallel with other cytokines, the inflammation process in the respiratory tract – which is similar to the one in asthma [2, 5]. Especially pivotal is participation of this cytokine in controlling the initiation and the course of Th2 response, leading to dominance of the Th2 response in the immune reaction. We know that, from the immunological point of view, the prevalence of Th2 over Th1 is typical for allergic diseases [1]. This interleukin also facilitates the process of penetration of neutrophils from the arteries to the inflammatory changed tissues affected by the disease, which is a key element in the development of severe asthma. The literature data above suggest that the concentration of this interleukin should increase while the disease is exacerbating. The results of our analyses do not confirm the above data. Although the mechanism of action of IL-25 is well-described in animals, in humans it needs to be further explored [5]. It was also highlighted that some kinds of cancer appear more often in patients with allergy than in those with any other diseases [14]. Interestingly, it was also shown that both asthma and HD were associated with T helper 2 dominant lymphocyte (Th2) response [15], and the dominance of Th2 over Th1 is characteristic for allergy or atopic diseases [1]. It means that the allergens may stimulate the immune system through an IgE-mediated pathway, which is also associated with risk for Hodgkin's and non-Hodgkin's lymphoma. In this regard, IL-25, which is mostly released by the Th2 lymphocytes, and is an important component of the chronic inflammatory processes or atopic responses of the immune system via induction of other cytokines, so it should be at high concentration levels [4]. To our surprise, the level of IL-25 was below the detection threshold, which suggests that the accompanying disease, non-Hodgkin's lymphoma, could down regulate the secretion of this interleukin in lymphocytes. However, it is hard to prove this possibility as well as to explain the importance of this phenomenon for the patient. On the other hand, our patient experienced significant progression of asthma, according to medical history of the last few years. As we know, the deviation in asthma toward a shift in the T-lymphocyte response from Th1- to Th2-dominated activity might inhibit the immunological protection against cancer. This shift in the immune response could also exacerbate the effects of carcinogens acting synergistically with the asthma. In addition, impaired mucociliary system and pulmonary function in asthma patients lead to insufficient removal of the molecules from the respiratory tracts. Then, some of such molecules remaining in the lungs, if chemically highly reactive, could have the potential to cause multiple mutations leading to development of cancer. In addition, glucocorticoids that are taken for asthma may facilitate development of cancer due to the cellular and humoral immunosuppression [16]. Interestingly, the low concentration values were also shown for TNF-α, which is known to be associated with the changes in populations of cancer cells [4]. However, comparing the obtained level of TNF-α of the patient with the ones of her close relatives, we found increased levels of this cytokine in both mother and brother (data not published, obtained earlier): 149.52 pg/ml and 18.76 pg/ml, respectively. Interestingly, the medical history of the mother showed a long history of severe asthma but with no record of other systemic diseases, while the brother of the patient had never complained of asthma symptoms. The study implies that the systemic two diseases relate to the imbalance of the immune system. There can exist the cross-promotion, and the symptoms overlap between the two diseases. Then the question arises whether the lowering of some cytokines in blood serum in the course of severe asthma might indicate the possibility of development of cancer. An example of such a phenomenon is the blood serum level of cholesterol that decreases at the very beginning of development of cancer [17]. Further research should explore this issue.

The level of TNF-α factor can be an indicator of the immune system condition and reactivity to bacterial infections. A lack of change in the concentration levels of IL-25 in blood plasma of asthma patients could be due to the good control of the disease. Obtaining the Th1/Th2 cytokine profile in blood plasma might be an important diagnostic tool for a number of dis-

eases, especially infective, inflammatory, and neoplasmatic ones. However, it requires further research. Especially that the study was conducted only once. The role of these cytokines and TNF-α in the development of cancer and allergic diseases requires further research by the authors.

Conflict of interest

The authors declare no conflict of interest.

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Address for correspondence:

Małgorzata A. Czarny-Działak MD, PhD
Department of Family Medicine and Geriatrics
Institute of Medical Sciences
Faculty of Medicine and Health Science
Jan Kochanowski University
ul. Malachitowa 32 A, 25-705 Kielce, Poland
Phone: +48 602 570 179
E-mail: drmczarny@interia.pl