SEVERE ASTHMA FORUM 2

Monitoring and Treatable Traits
in Severe Asthma

The Severe Asthma Forum book series intends to publish scientific monographs based on papers at the annual scientific conference Severe Asthma Forum - SAF, South-eastern meeting (Slovenia, Croatia, Serbia). The monographs will be published during the annual SAF conference, and they will bring the latest research and reviews in the field of diagnosis and treatment of asthma.

2.0

SEVERE ASTHMA FORUM

2

Monitoring and Treatable Traits
in Severe Asthma

Edited by Sabina Škrgat

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Predgovor | Preface

Sabina Škrgat[1,2]

Pričujoča monografija je nastajala samozavestno. Ker je bila pred njo že naša prva, ki je orala ledino. In je našla mesto v rokah specializantov pnevmologije in zdravnikov, ki so v njej videli priložnost za nadgradnjo in preverjanje svojega znanja. In to me je neznansko veselilo. Veselile so me malo pomečkane monografije v rokah mladih zdravnikov s podčrtanim in barvno označenim tekstom nekaterih poglavij.

Astma forumi (SAF), ki so sledili s svojimi temami, so tudi temelj za nastanek sedaj druge monografije. Na tem mestu se posebej zahvaljujem profesorjem Sanji Popović-Grle, Mitji Košniku in Zorici Lazić za opravljeno recenzentsko vlogo. Besedila smo povezali v zgodbo *Monitoring and Treatable Traits in Severe Asthma* in si tako dopustili opredelitev do disfunkcionalnega dihanja, fenotipov astme in nekaterih astmi pridruženih bolezni ter spremljanja bolnikov z astmo. Nekatera poglavja so zastavljena tako, kot v klinični praksi v dobrih centrih za hudo astmo tudi v resnici postopamo – multidisciplinarno.

Ker obravnava bolnika s hudo astmo ni zgolj zdravljenje, je kanček umetnosti.

Srečno.

Our first monograph already confidently found a place in the hands of pneumology residents and doctors, who saw an opportunity to upgrade and test their severe asthma knowledge. And that made me incredibly happy. I was delighted to see monographs in hands of young doctors with an underlined and colour-coded text of some chapters in the process of learning.

Severe Asthma Forums (SAFs), which followed with their topics, are also the cornerstone for now the second Monograph. At this point, I would especially like to thank professors Sanja Popović-Grle, Mitja Košnik and Zorica Lazić for their role of peer review. Chapters have been linked to the story of *Monitoring and Treatable Traits in Severe Asthma* and thus allowed us to define dysfunctional breathing, asthma phenotypes, some asthma comorbidities and monitoring of patients with severe asthma. Some chapters are written according to our clinical work in good severe asthma centres- with typical multidisciplinary approach.

Because treating a patient with severe asthma is not just a therapy, it is also a bit of art.

Good luck!

1 University Medical Centre Ljubljana, Ljubljana, Slovenia

2 Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Dysfunctional Breathing

Dysfunctional Breathing – View of Otorhinolaryngologist

Maja Šereg Bahar [1, 2]

Abstract

Background. Dysfunctional breathing – vocal cord dysfunction (VCD) or paradoxical vocal fold movement (PVFM) is inappropriate vocal fold movement. Adduction of the vocal folds appears during inspiration, resulting in dyspnea and inspiratory and sometimes expiratory stridor, and acute upper airway obstruction. It is a functional disorder, an important mimicker of asthma, leading to unnecessary morbidity and high medical utilization, unnecessary drug use, and high-dose corticosteroid use. The gold standard test for diagnosis of VCD is direct visualization of the vocal folds by laryngoscopy while a patient has symptoms or is combined with special maneuvers that trigger symptoms.

Methods. The recent papers on vocal cord dysfunction were reviewed.

Results. VCD is an important differential diagnosis of refractory asthma, that is widely unrecognized. But concomitant vocal cord dysfunction and asthma are seen in a high degree of patients, up to 50%. VCD is a benign and self-limiting disorder and there are no long-term sequelae. Correct diagnosis is important due to proper treatment. The cornerstone of the VCD treatment is speech therapy like respiratory retraining, learning breathing techniques, and different maneuvers that enable quick release of symptoms. Psychotherapy and hypnosis are important modes of treatment as well. Medications and botulinum toxin are used rarely.

Conclusions. We should suspect VCD in patients with asthma-like symptoms that do not respond to conventional asthma therapy or are induced by stress and exercise. A team of different specialists is necessary to find the correct diagnosis and proper treatment.

Keywords: dyspnea, vocal cords dysfunction, speech, and language therapy, maneuvers

Introduction

More than 70 terms have been used to describe the abnormal movement of the true vocal cords. The two most encountered terms in medical literature are paradoxical vocal fold motion (PVFM) and vocal cord dysfunction (VCD)[9]. PVFM/VCD is a condition characterized by abnormal adduction of the vocal folds during inspiration, leading to episodic dyspnea, wheezing, and stridor[23]. VCD is an intermittent extrathoracic airway obstruction presenting mainly during inspiration leading to dyspnea of varying intensity[21].

Epidemiology

The overall incidence of VCD in the general population is not well defined, because of the lack of uniformity in definitions and diagnostic criteria for VCD. The incidence is underappreciated in clinical practice. Prevalence has been reported to range from 2.5% of patients presenting to an asthma clinic to

up to 22% of patients with recurrent emergency department visits for dyspnea[8]. Patients with exertional dyspnea are reported to have VCD in 12%, and patients with exercise-induced asthma in 9%. Patients with "refractory asthma" have VCD in 10% alone, 30% of them have both VCD and asthma[36]. VCD affects mainly children, young adults, and athletes, with female predominance (65%: 35%). Family history was not proven[18].

Vocal cord anatomy and function

The larynx is a valve separating the trachea from the upper aero-digestive tract. The glottis consists of the true and false vocal cords and an opening between them, the rima glottidis. The principal muscle for vocal cord abduction is the posterior cricoarytenoid (PCA). Adduction is performed mainly by the lateral cricoarytenoid muscle (LCA). During normal inspiration, the glottic opening is controlled through the medullary respiratory center, via the vagus nerve, which leads to contraction of the PCA muscle and therefore to vocal cord abduction. During normal expiration there is a decrease in the tonic activity of the PCA muscle and contraction of the LCA muscle, resulting in a 10-40% narrowing of the rima glottides, allowing air movement to and out of the lungs[18]. The larynx has the function of protection the lower airway, which is strictly reflective. The other functions of the larynx are respiration and phonation, which are regulated partially by involuntary brainstem reflexes and may be initiated voluntarily. Pulmonary protection is mediated by the glottic closure and cough reflexes, to protect the lower airway from noxious inhaled stimuli and aspiration of foreign material during respiration. The cough reflex is initiated by an adverse stimulus triggering one of the many sensory receptors of the larynx[1,9]. In VCD there is a brief inappropriate adduction of the vocal folds during inspiration. This may manifest with audible inspiratory sounds[32].

Pathogenesis of VCD

The etiology of VCD is complex and multifactorial. VCD is a functional disorder. In the pathogenesis of VCD, there are several pathogenetic mechanisms. The essential pathophysiology is that of a hyper-functional laryngeal reflex to protect the lower airways[21]. The sensitivity of the laryngeal sensory receptors is increased and the response of the glottic closure and cough reflex to several triggers is heightened. It is analogous to bronchial or nasal hyper-responsiveness. Direct stimulation of the sensory nerve endings in the upper or lower respiratory tract and hyperventilation may also lead to glottic narrowing due to underlying laryngeal hyper-responsiveness. Another possible etiology of VCD is neurological, where autonomic neurophysiologic balance is altered. Central brain regions such as the medulla, midbrain, and prefrontal cortex are polysynaptic linked with the larynx and the balance can be altered[37]. The hypothesis is, that there is an initial inflammatory insult, which causes laryngeal hyper-responsiveness and/or altered autonomic balance, which may be short or persistent. Subsequent stimuli (psychological stresses or cold air and irritants) induce local presynaptic reflexes causing airway narrowing[18]. While the etiology of this disease is still unclear, many support the theory that VCD has a psychiatric basis[6,9,25,36].

Specific triggers of VCD

Specific triggers are not always identified, because VCD episodes quickly begin and end. VCD triggers are classified into three groups: irritants, psychological and emotional, and exertional[6]. Initially, one patient has a single trigger, then develop multiple triggers, that were previously benign. Self-reported triggers are upper respiratory tract infection, occupational exposures, talking, laughing, singing, acid reflux, cough, different foods, physical exertion, exercise, postnasal drip, weather changes, emotional stressors, odors, strong

scents and other airborne triggers, chlorine in swimmers and others[9].

The role of psychogenic factors – triggers

Initial reports of VCD emphasized the dominant underlying psychological disorders. It is still thought that psychological stimuli can trigger VCD, including anxiety disorder, stress, somatoform disorders, depression, psychiatric illness, social stress in competitive sports, prior history of sexual abuse, conversional profile, and others. Psychological stimuli can trigger VCD and are considered major precipitating factors for VCD[18]. Not all patients have an underlying psychiatric illness. And anxiety can be also the result of chronic respiratory illness, not the cause[9,17,22].

Irritant triggers

Irritant triggers can be extrinsic including chemical, olfactory, and even visual. Irritants are environmental and occupational irritant exposure to smoke, gasses, vapors, dust, airborne pollutants, and odors. Triggers can be intrinsic such as gastroesophageal reflux, sinusitis, postnasal drip, pharyngitis, and laryngitis. They lead to chronic inflammation and hyper-responsiveness. Reflex adduction of vocal cords might be protective and is responsible for the development of VCD[1,9,18].

The role of gastroesophageal reflux disease – GORD

There is much speculation in different studies. In some, there is a high proportion of GORD in patients with VCD (95%), and in others low proportion of patients with GORD. In a group of elite athletes with exercise-induced laryngeal obstruction (EILO), only 2.3% had GORD[38]. VCD is triggered by acid reflux in some patients. Laryngospasm is induced by hydrochloric acid in the esophagus by sensitization of subglottic chemoreceptors through a vagally mediated mechanism. Reflux events cause vocal cord adduction and apneas. La-

ryngeal irritation associated with GORD may also contribute to bronchial constriction. It is a vagally mediated reflex[18].

Exercise as a trigger

EILO – exercise-induced laryngeal obstruction is caused by maximal exercise or athletic competitions, it can be also seen during routine exercise, but is related to exercise intensity. EILO symptoms resolve quickly on exercise cessation[16]. Most patients are highly competitive, elite athletes and military personnel, who are required to exercise regularly. EILO is common, affecting 5-7% of adolescents and up to a quarter of athletes presenting with "exertional asthma-type". EILO has a female preponderance and a peak age of onset in the teenage years. It has been speculated that the laryngeal growth difference between genders seen in the peri-pubertal age group might explain this observation[38]. Patients develop wheezing during exercise. The differential diagnosis is asthma, but methacholine challenge testing is negative, as is the bronchoprovocation testing[9]. Some patients can have both asthma and VCD. 35 – 56% of patients with VCD have coexistent asthma. EILO represents a maladaptive response to exercise. Increased work breathing might contribute to exercise limitation. Endurance training induces large and significant adaptations within the cardiovascular, musculoskeletal, and hematological systems. But the structural and functional properties of lungs and airways do not change in response to repetitive physical activity. In elite athletes, the pulmonary system may become a limiting factor to exercise. As a consequence, of this respiratory paradox, the highly trained athlete may develop intrathoracic and extrathoracic obstruction, expiratory flow limitation, respiratory muscle fatigue, and exercise-induced hypoxemia. All of these maladaptations may influence performance[29]. Increased and abnormal ventilation through the narrowest part of the airway causes the collapse of laryngeal

structures. In pediatric patients, it is hypothesized that increased laryngeal diameter due to growth might spontaneously improve their exercise capacity[26]. Asthma is common in endurance sports athletes, likewise the prevalence of EILO is high. Cross-country skiers and biathletes have a very high prevalence of asthma and EILO. Coexisting EILO and asthma seem to be common in skiers, especially females. It is mainly believed to be due to repeated and prolonged inhalation of cold dry air, therefore, leading to osmotic changes and epithelial damage in the airways[19]. Laryngopharyngeal reflux, allergy, infections, irritants, temperature, the humidity of the air in the surroundings, and psychological aspect are also the etiological factors of EILO[15].

Other possible etiologies for VCD
There are some other factors as extubation after general anesthesia. Central neurological disorders like Arnold Chiari malformation, Parkinson's syndromes, ALS (amyotrophic lateral sclerosis), and others may be associated with VCD[18].

Clinical features of VCD
Clinical presentation can be very variable, ranging from no symptoms to mild dyspnea, and acute onset respiratory distress, which can mimic an asthma attack[9]. VCD episodes frequently begin and end abruptly. Patients are not hypoxic and have a normal level of consciousness. If the patient is with altered mental status or hypoxemia, more serious causes should be considered. Symptoms are periodic: shortness of breath, asthma-like symptoms during exercise, and intense emotion, which does not respond to asthma drugs. Other symptoms are air hunger, dyspnea, choking sensation, chest pain, stridor, voice changes, difficulty in speaking and swallowing, globus sensation, intermittent aphonia, dysphonia, chronic cough, throat clearing, panic, and anxiety which worsen respiratory symptoms[9]. The period from symptom onset to diagnosis

of VCD is greater than 4 years[8]. Patients with asthma may also have comorbidities, such as VCD, which are associated with worse asthma outcomes, increased symptoms, more exacerbations, and poorer quality of life[24]. Improved VCD control can reduce asthma medication use[18,23].

Important differential diagnosis od VCD
Patients with VCD are often misdiagnosed as having refractory asthma, which can lead to unnecessary morbidity and high medical utilization, unnecessary drug use and high dose corticosteroid use, emergency room visits, hospitalizations, and even intubation. They found that 42% of all VCD subjects had been previously misdiagnosed with asthma for an average of 9 years[9,34]. The differential list for suspected VCD is broad and includes any disorder with episodic dyspnea, cough, and wheezing. There are many mimickers of VCD, with asthma at the top of the list. Other conditions are psychogenic disorders, anaphylaxis, aspiration of foreign body, angioedema, chronic obstructive pulmonary disease, croup, epiglottitis, extrinsic airway compression, laryngomalacia, laryngospasm, laryngeal tumor, laryngeal dystonia, exercise-induced bronchospasm, vocal cord paresis, laryngeal and tracheal stenosis, and others[9,18,35].

Diagnosis of VCD
Diagnosis is made by careful history, physical examination – laryngoscopy, and spirometry or pulmonary function testing[9]. Imaging has no role in the evaluation of VCD[32]. A careful history is very important. We should be suspicious in a patient with asthma-like symptoms unresponsive to bronchodilators or corticosteroids, absence of nocturnal symptoms, and more difficulty with inspiration than expiration. Asthma inhalers can even trigger or exacerbate symptoms[1]. Symptoms in VCD patients are precipitated by stress, emotional factors, or anxiety. The patient has no spu-

tum[18]. We should be suspicious in a patient with exercise-induced asthma-like symptoms, or an athlete with choking sensation during exercise and irritant-induced asthma-like symptoms. But we must be aware that VCD with asthma is possible, and we should be also aware of exercise-induced bronchospasm[32].

Assessment of symptoms

Relevant issues should be discussed with patients. We can use standardized questionnaires, for example, 12-item VCDQ (vocal cord dysfunction questionnaire), which is a valid tool for symptom monitoring and tracking improvement in scores after speech therapy. It also gives insight into which symptoms are important to patients and could guide future therapy refinements[11]. Another one is the Pittsburgh VCD index, which helps distinguish VCD from asthma. This scoring system correctly diagnosed VCD in 77.8% of patients. Since many patients have coexistent VCD and asthma, further diagnostic tests should be performed, if a strong suspicion of asthma exists[33].

Physical examination

The physical examination in patients with VCD is normal when they are not experiencing an acute attack. During symptoms, we can identify high-pitched wheezing, stridor, tachypnea, hoarseness, dysphonia, cough, and respiratory distress. Arterial hypoxemia is usually lacking. The patient has a normal oxygen saturation. Only in a few patients with VCD, we can identify the presence of hypoxemia. Laryngoscopy is the gold standard for the diagnosis of VCD. Direct visualization of the vocal folds via flexible, trans-nasal fiber-optic laryngoscopy should be done while a patient has symptoms. Complete adduction of the vocal folds during inspiration and a formation of a small posterior glottal chink during exhalation is seen[18]. In asymptomatic patients, we can provoke symptoms by deep breathing, cold air, phonation, forced expira-

tion, and exercise[7,21]. Patients with VCD often show inappropriate vocal fold movement during inspiration or expiration when laryngoscopy is performed immediately following a bronchoprovocation challenge with methacholine. Therefore, laryngoscopy should be ideally performed after a bronchoprovocation challenge with methacholine. We should avoid benzodiazepines and lidocaine before the examination. Negative laryngoscopy in an asymptomatic patient does not rule out VCD[5,18].

Continuous laryngoscopy during exercise – CLE test

Video recorded trans nasal flexible laryngoscopy and larynx examination are performed during exercise from the rest to the peak exercise – continuous laryngoscopy exercise test (CLE test). Any form of physical exercise can be used, running, or cycling on stationary bicycles, which provoke symptoms[15]. A flexible laryngoscope is attached to the head via a helmet. The tip of the scope is introduced through the nose into the larynx, allowing visualization of the supraglottic and glottic structures in real time throughout the exercise. During testing cardiopulmonary data is collected, as the patient exercise to peak in an attempt to reproduce EILO symptoms. EILO-related findings on laryngoscopy include vocal fold narrowing, supraglottis narrowing, obstruction, and/or collapse of supraglottic structures. CLE is the test of choice for EILO[32].

Other tests

Pulmonary function tests, methacholine challenge testing, spirometry, and flow volume loops are also done in VCD and EILO patients[1].

Treatment

Correct diagnosis is essential for proper treatment. The patient should be reassured that the condition is benign and self-limited. The treatment approach is multidisciplinary. Pri-

mary care physician, pulmonologist, allergist, ENT doctor, gastroenterologist, neurologist, psychiatrist and psychologist, speech pathologist and athletic trainer participate in the treatment. Effective long-term therapy requires psychosocial support, speech therapy, and biofeedback[9].

Speech therapy

Speech therapy is the cornerstone of the treatment[18]. It is the most common long-term treatment of VCD. Patients are educated about the pathophysiology of VCD and are educated about the suppression of laryngeal abusive behaviors (cough and throat clearing). Patients are trained on how to control the laryngeal area and maintain an adequately open airway during respiration. Patient are allowed to view their laryngoscopy, to understand and accept the disease. Visual feedback allows the patient to modify their breathing, visual feedback enables a reduction in symptoms and the use of medication. Breathing techniques are learned by the speech and language therapist (SLT), and VCD symptoms, and triggers are assessed. Patient education is a crucial component of the treatment. The therapist offers supportive counseling. Respiratory retraining is practiced by the SLT. Desensitization is attempted to be achieved for specific irritants. Voice therapy and different breathing techniques are practiced – quick release techniques and different maneuvers. Studies have shown that speech therapy can achieve symptom control and eliminate emergency department visits in 90% of patients with VCD[8,14,23]. Non-pulmonary-related shortness of breath treated with respiratory retraining can effectively eliminate dyspnea. The patient should perform respiratory retraining exercises three to four times daily for four weeks, and daily exercises for two additional months[13].

Treatment of VCD – maneuvers

Different maneuvers can be used to achieve a quick release of symptoms. It is necessary to repeat them often, to ensure that a patient can respond automatically when acutely symptomatic (5 repetitions 20 times per day). Phonation of soft "s" sound while exhaling, is successful to divert attention from inhalation, and give auditory feedback on air movement. Another maneuver is panting, which activates the PCA muscle, a laryngeal abductor. Coughing and sniffing are also releasing maneuvers. Sniffing reduces air turbulence and shifts the narrowest part of the breath from the larynx to the nose. Common breathing techniques include jaw trust, nasal inspiration with pursed-lip exhalation, and breathing through a large-diameter straw or cut endotracheal tube. These techniques are designed to interrupt the irregular respiratory pattern or spasm and allow familiar neurologic signals to reengage and relax the vocal folds[8,18,30].

EILOBI breathing techniques

In EILO adduction of the vocal folds and/or inspiratory prolapse of the supraglottic structures during high-intensity exercise appears. Although respiratory retraining is a primary therapy of EILO, many patients report symptom persistence despite the adequate performance of traditional techniques. EILOBI (EILO biphasic) inspiratory breathing techniques are novel breathing techniques for EILO therapy[20]. Patients are encouraged to train in biphasic inspiratory breathing: from high inspiratory resistance then rapidly changed to low resistance breathing. High resistance inspiratory phase (tongue variant - inhaling through the nose, tooth variant - inhaling through the teeth placed firmly against the lower lip, lip variant - inhaling through the pursed lips) is followed by low resistance inspiratory phase – inhalation through a wide-open mouth. Thus, optimizing the glottic aperture with maneuvers that can be performed during high-intensity exercise is achieved[8,30].

Inspiratory muscle training devices

Treatment of EILO in athletes and other patients with VCD with inspiratory muscle training devices is possible. It is a conservative treatment tool to achieve better control of the vocal folds. Patients can use resistive flow-dependent devices. The inspiratory valve increases resistance to inspiration and decreases the inspiratory rate of airflow, consequently, there is less turbulence and less stimulation of the vocal folds[4]. Another one is the use of continuous positive airway pressure – CPAP to relieve acute symptoms of VCD. CPAP relieves dyspnea by slowing the expiratory flow, thereby increasing lung volume, which in turn results in a more open glottis. CPAP is also reducing the effort needed for inspiration by establishing a favorable pressure gradient for inhalation[18].

Psychotherapy

Psychotherapy remains an important mode of treatment in patients with VCD. There are many forms of psychotherapy used in VCD, which include relaxation therapy to alleviate the distress associated with symptoms, identification of stressors, development of new coping strategies for dealing with stressors, family therapy, and behavioral cognitive therapy[18]. Hypnosis and self-hypnosis induce relaxation. Biofeedback may be used in conjunction with psychotherapy for treating patients with VCD[2,3,12,17,25].

Surgical treatment

Surgical treatment – supraglottoplasty is used only in refractory cases. It gives good results in selected patients. The most commonly used method is to cut the aryepiglottic folds closer to the epiglottis and to remove the mucosa and cuneiform cartilage from the aryepiglottic fold with the help of a laser. The use of a suture that pulls the epiglottis towards the root of the tongue and lateralization of one vocal fold with the suture are also described[27,39]. EILO surgery appears to be a safe and effective option for individuals with moderate to severe supraglottic-type EILO who have failed initial conservative treatment[10].

Botulinum toxin

Botulinum toxin laryngeal injection is rarely used in VCD treatment. Chemical denervation is achieved and paralysis, the vocal folds are in the open position. It is useful in laryngeal dystonia. It is used only in severe cases of refractory VCD, that do not respond to conventional therapy, and in patients with refractory dyspnea symptoms following appropriate medical therapy and respiratory retraining protocols[28,36].

Treatment of VCD with medications – pharmacotherapy

In persistently symptomatic patients, mild sedatives may facilitate VCD management. Benzodiazepines are effective in terminating acute symptoms and relieving anxiety. Before giving this medication, we should confirm normal oxygen saturation and exclude hypercapnia[18]. When breathing techniques are unsuccessful, helium-oxygen inhalation and noninvasive positive-pressure ventilation may be successful in resolving VCD. Heliox is the mixture of oxygen (20%) and helium (80%), it is less dense than air and reduces the work of breathing. Inhalation of Heliox reduces turbulence in the airway and eliminates respiratory noise[7,18]. In some centers, they use inhalation of anticholinergic drugs. Neuromuscular treatment such as Gabapentin is successful in some patients. GORD treatment is reasonable if GORD is proven, the success of such treatment is very good in those patients in whom reflux has been demonstrated[21,31].

Prognosis

VCD is a benign and self-limiting disorder. The majority of patients respond to speech therapy. There are no long sequelae[18,32].

Conclusions

VCD is an important differential diagnosis of asthma, that is widely unrecognized. If misdiagnosed as asthma, VCD can lead to high medical utilization, unnecessary high-dose steroid use, and other dangerous consequences. We should suspect VCD in patients with asthma-like symptoms that do not respond to conventional asthma therapy or are induced by stress and exercise. The gold standard test for VCD is direct visualization of the vocal cords by laryngoscopy. The cornerstone of VCD treatment is speech therapy. VCD should be included in the differential diagnosis for patients reporting episodic dyspnea or respiratory distress. Patients with asthma may also have comorbidities such as VCD. Identifying and treating VCD should be included in the management of patients with asthma.

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Dysfunctional Breathing – View of Pulmonologist

Ivan Kopitović[1,2], Milica Mirić[1]

Abstract

Dysfunctional breathing (DB) is a respiratory disorder defined by non-regular breathing patterns. It is a breathing condition where long term changes in breathing pattern result in symptoms (main is dyspnoea) but also non-respiratory symptoms, with no prominent cardio-respiratory or neuro-psychiatric disease (sometimes secondary to them). It has been identified in different age groups and also among asthma patients. Although DB has been investigated for long time, it is poorly understood because of lack in clinical trials and validated outcome measures specific to this population. DB irregularity is often missed, because of the similarity of its associated symptoms (dyspnoea, tachycardia, and dizziness) to those of other common cardiorespiratory diseases such as heart failure, asthma and chronic obstructive pulmonary disease (COPD). The high rates of misdiagnosis of DB suggest that health care professionals do not fully understand this condition and may therefore fail to provide patients with an appropriate treatment. A holistic assessment is the most appropriate way to improve understanding and diagnostic accuracy.

Keywords: dysfunctional breathing, hyperventilation, pulmonary function tests

Abbreviations
ACQ - Asthma Control Questionnaire
AQLQ - Asthma Quality of Life Questionnaire
BMI - Body Mass Index
BHT - Breath Holding Time
BPAT - Brompton Breathing Pattern Assessment Tool
CART - capnography-assisted respiratory therapy
COPD - Chronic Obstructive Pulmonary Disease
CPET - Cardiopulmonary Exercise Testing
DB - Dysfunctional breathing
ET-CO2 - end-tidal carbon dioxide measurement
HVPT - Hyperventilation Provocation Test
HVS - Hyperventilation syndrome
MARM - Manual Assessment of Respiratory Motion
NQ - Nijmegen Questionnaire
SEBQ - Self-Evaluation of Breathing Questionnaire

Introduction

Dysfunctional breathing (DB) is a chronic or recurrent alteration of normal breathing pattern, recognized as an important differential diagnosis for individuals with "unexplained" dyspnoea.[1] Hidden in the complex management of many respiratory diseases, such as asthma, COPD and "long-COVID", DB may exacerbate these diseases/disorders, reduce symptom control and increase medication and healthcare service use.[2–6] Recently, more evidence-based classification, diagnostic criteria and treatment modalities of DB have been developed.[2]

Dysfunctional breathing in the modern era?

Eighty-five years ago a group of authors remarked: "Patients presenting well known pattern of symptoms haunt the offices of physicians and specialists in every field of medical practice. They are often shunted from one physician to another, and the sins of commission inflicted upon them fill many black pages in our book of achievement."[7]

In 1975, in the respiratory physiology department of Papworth Hospital, Cambridge, England, the specialists dubbed this phenomenon simply as the "multiple doctor" or the "fat folder syndrome".[8] In the late 1960's, one of the newly reported major side effects of recently introduced oral contraceptives was venous thromboembolism. Since then, as many physicians encountered young ladies taking birth-control pills that reported syncopal attacks and other possible manifestations of pulmonary embolism, dysfunctional breathing (most known as "hyperventilation syndrome" (HVS) has become more and more recognized in different clinical settings.

Definition

There is no formal definition of DB. Barker and Everard reviewed the literature and suggested a new definition of DB: "an alteration in the normal biomechanical patterns of breathing that results in intermittent or chronic symptoms which may be respiratory and/or non-respiratory".[9] Various terms have been used: earlier it was mainly described as a hyperventilation syndrome, while nowadays it is more often called dysfunctional breathing, functional breathing disorder, breathing pattern disorder and behavioural or psychogenic breathlessness, etc. The later terminology results from better understanding of pathophysiological processes underlying the abnormal pattern of breathing which itself does not necessarily include hyperventilation. In this narrative review we will prefer the term "dysfunctional breathing".

Aetiology and pathophysiology

Efficient breathing results from balanced motion between the upper rib cage and the lower rib cage and the abdomen. It requires synchronized movement of diaphragm, abdominal and rib cage muscles.[10] Discoordination of muscle contractions results in sensations of dyspnoea and is often present in DB, especially in apical, thoracic dominant breathing.[11]

A simplified physiological process encountered in hyperventilation may help partly understand relationships between causes of DB and its consequences (Figure 1). Hyperventilation is an increase in ventilation that is greater than that required by metabolic needs or arterial blood gas tensions. It may be acute, episodic, and chronic. Furthermore, it has been well-described that stressful events, especially emotional upset, can elicit a habitual change in breathing pattern.[12] Indeed, changes in breathing depend on a variety of external and internal factors (i.e. cold, heat, hypoxia, pain and panic).[13] DB can be described as a habit in breathing and in some cases includes over-breathing (increase in both tidal volume and respiratory rate). Thus, it may result in decrease in carbon dioxide. Consequently, hypocapnia directly induces cerebral vasoconstriction and cerebral hypoxia, while kidneys

excrete the excess bicarbonate ion. Hydrogen ion deficiency suppresses hydrochloric acid formation by the stomach as well. Further on, in the state of alkalosis, smooth muscles of the digestive tract also constrict, while haemoglobin slowly delivers smaller amount of oxygen (Bohr effect). Hypocalcaemia, secondary to calciuria induced by alkalemia, results in poor muscle and nerve function, e.g. hyperexcitability of skeletal and visceral muscles. As in the *vicious circle,* all potential resultant symptoms (Table 1) exaggerate previously existing anxiety, which in its turn, aggravate disordered pattern of breathing. The regulation of breathing involves both voluntary (cor-

tex) and involuntary (neural, emotional, endocrine, and metabolic) control mechanisms. Numbers of hormones participate in ventilatory regulation. For example, hyperventilation with resultant hypocapnia may be present during the luteal phase of the menstrual cycle as well as in pregnancy.[14] However, respiratory complaints appear to have a stronger relationship to breathing pattern.[15] Thus, pathophysiological mechanisms underlying DB require further research on the levels of neural ventilatory control and skeletal muscle metabolic function, which would include processes in the normocapnic settings as well.[16]
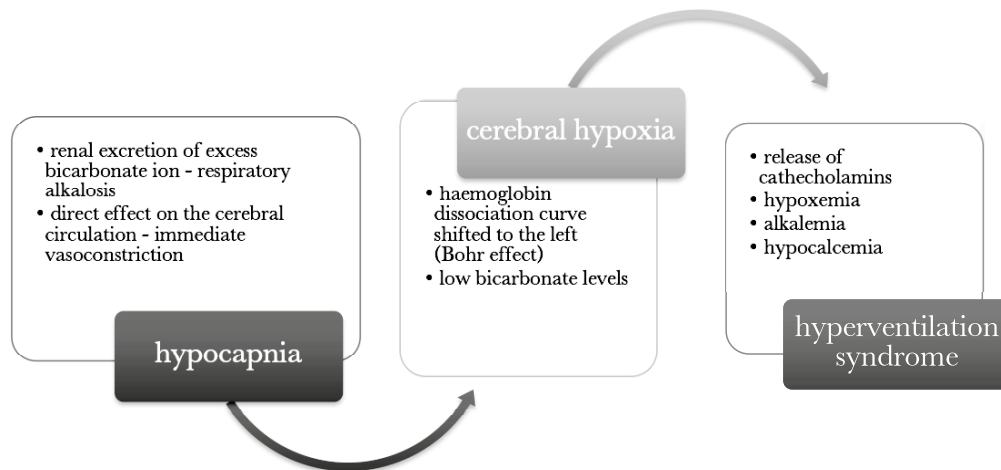

Figure 1.Pathophysiologic process in hyperventilation

Epidemiology

The prevalence of DB in general population is estimated to be approximately 8%.[8] As the disordered is ill-defined without standardized diagnostic tools, it is hard to estimate its true prevalence. Dysfunctional breathing may affect individuals across all age groups and is more often found in asthmatics who are female, with poor asthma control, frequent exacerbations and comorbid anxiety states.[17–19]

Presentation

The key respiratory symptom in dysfunctional breathing is breathlessness ("air hunger"). Non-respiratory associated symptoms may be attributable to hyperventilation (increased minute ventilation) and respiratory alkalosis such as paraesthesia (i.e. tingling), numbness, dizziness, palpitations and, rarely, tetany. Also, frequently reported are chest tightness, chest pain, deep sighing, exercise-induced dyspnoea and frequent yawning. However,

none of the symptoms are specific to dysfunctional breathing.[20]

Table 1. Symptoms and signs of disfunctional breathing

System	Most probably caused by hypocapnia in hyperventilation
Cardiovascular	Palpitations, tachycardia, precordial pain, cold hands or feet
Respiratory	Shortness of breath, chest pain, chest tightness
Gastrointestinal	Globus, dysphagia, epigastric pain, aerophagy, bloated feeling in the stomach
Neurological	Central: dizziness, disturbance of consciousness, blurred vision, Peripheral: paresthesia (tingling fingers), tetany (rarely)
Musculoskeletal	Muscle pains, tremors, tetany
Psychic	Feeling tense, anxiety
General	Fatigability, weakness, exhaustion, sleep disturbance, nightmares

In order to better illustrate respiratory complains that may be evaluated, we quote in *Table 2* the questions included in a preliminary version of Self Evaluation of Breathing Questionnaire, revised by Courtney R and Greenwood KM.[21]

Table 2. The Self-Evaluation of Breathing Questionnaire (SEBQ), revised version (21).

"I get easily breathless out of proportion to my fitness"
"I notice myself breathing shallowly"
"I get short of breath reading and talking"
"I notice myself sighing"
"I notice myself yawning"
"I feel I cannot get a deep or satisfying breath"
"I notice that I am breathing irregularly"
"My breathing feels stuck or restricted"
"My rib cage feels tight and can't expand"
"I notice that I am breathing quickly"
"I get breathless when I am anxious"
"I find myself holding my breath"
"I feel breathless in association with other physical symptoms"
"I have trouble coordinating my breathing when I am speaking"
"I can't catch my breath"
"I feel that the air is stuffy, as if not enough air in the room"
"I get breathless even when I am resting"
"My breath feels like it does not go in all the way"
"My breath feels like it does not go out all the way"
"My breathing is heavy"
"I feel that I am breathing more"
"My breathing requires work"
"My breathing requires effort"
"I find myself breathing through my mouth during the day"
"I breathe through my mouth at night while I sleep"

Classification

More than 45 years ago Lum and colleagues[8] proposed classification related to dominant pattern in breathing:

1. Rapid breathing.
2. Irregular amplitude of breaths.
3. Irregular rhythm.
4. Frequent sighs and yawns.
5. Habitual sniffing and coughing.
6. Fast breathless talking.
7. General tension in the whole body

Researchers have suggested a few alternative classifications for other patterns of DB.

Boulding and colleagues found that "tracking of respiratory flow, frequency and volumes during quiet tidal breathing, often performed before and after exercise, can give useful information to the clinician and be used for providing feedback to the patient." Further, they used those data to guide them in defining and classifying various dysfunctional breathing patterns.[2]

Classification of DB, by Boulding and colleagues:

1. *Hyperventilation syndrome* is associated with symptoms both related to respiratory alkalosis and independent of hypocapnia.
2. *Periodic deep sighing* represents frequent sighing with an irregular breathing pattern.
3. *Thoracic dominant breathing* is characterized by an absence of costal expansion and an increased reliance on upper thoracic muscles during inspiration. As a consequence, this type of breathing

results in high operating lung volumes and reduced inspiratory capacity, as in HVS. Also, it can frequently manifest in organic disease, but in the absence of disease it may be considered dysfunctional and results in dyspnoea.

4. *Forced abdominal expiration*: these patients utilize inappropriate and excessive abdominal muscle contraction to aid expiration. This type of DB results in very low lung volumes, and therefore a reduced functional residual capacity.

5. *Thoraco-abdominal asynchrony* is seen when there is delay between rib cage and abdominal contraction resulting in ineffective breathing mechanics.[2]

The most recognized model of DB is HVS, characterized by acute or chronic hyperventilation (increased minute ventilation) at rest or during exercise/stress. HVS may be part of somatic/physiological conditions, still it commonly develops secondary to psychological/behavioural factors (particularly anxiety, depression, perfectionism, and feelings of inferiority).[22–24]
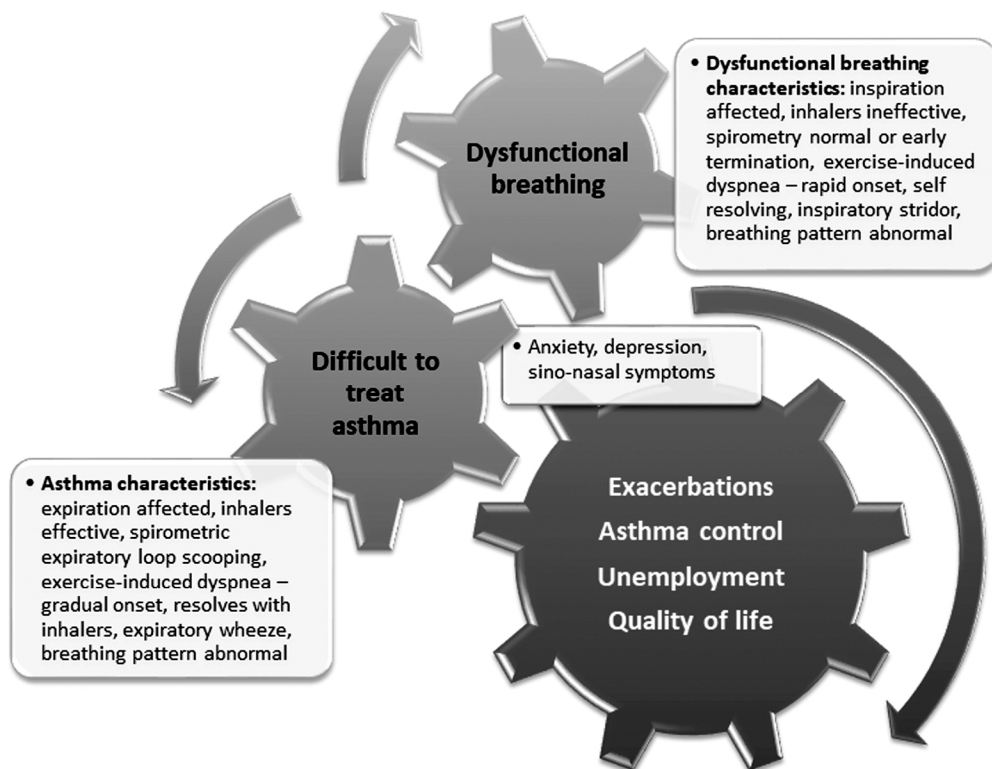
Figure 2. Differential diagnosis between DB and difficult-to-treat asthma and the detrimental effect of DB on asthma and asthma-related outcomes.[30,31]

Modified from:
Denton E, Bondarenko J, Tay T, et al. Factors Associated with Dysfunctional Breathing in Patients with DifficulttoTreat Asthma. J Allergy Clin Immunol Pract. 2019;7(5):1471-1476.
Connett GJ, Thomas M. Dysfunctional Breathing in Children and Adults With Asthma. Front Pediatr. 2018;6:406.

Associated conditions

DB can occur either in the absence of organic diseases (i.e., due to psychogenic causes such as anxiety) or it may be coexisting with respiratory diseases (asthma, COPD, interstitial lung diseases), cardiovascular disease, thoracic wall abnormalities, hyperventilation in anxiety related disorders and panic disorder. Symptoms of DB can mimic asthma, which may influence the level of disease control and potentially lead to overtreatment, especially in difficult-to-treat and severe asthma phenotype, as illustrated in *Figure 2*.[17,25,26] The expected treatment success with bronchodilators and anti-inflammatory medicines may be substantially reduced due to the presence of disorder in breathing pattern. Among individuals with asthma, a positive diagnosis of DB is found in a third of women and a fifth of men.[27] Also, DB may exacerbate myofascial pain syndromes, such as temporomandibular joint disease [28], and other common conditions such as headaches and migraines.[29] DB is present in the so-called 'long COVID' syndrome as part of a long-lasting dyspnoea associated with previous SARS-CoV-2 infection persisting for months after acute infection.

Diagnostic methods

Diagnosis of DB may be established only after assessment, exclusion or adequate treatment of other possible conditions. A gold standard diagnostic method is yet to be established. Several questionnaires and functional tests have been used with less or more success. Tools should help multidimensional evaluation of breathing as it comprises three main functions: gas exchange (lung function), change in posture and movement of the trunk (biomechanical function)[9] and a "sense of self" (mental function).[32,33] We offer a brief algorithm in assessment of DB (*Figure 3*).

The Nijmegen Questionnaire (NQ) was introduced and validated in individuals with exercise induced hyperventilation syndrome, where it shows sensitivity of 91% and specificity of 95%. The questionnaire consists of 16 items of which seven are linked with respiratory symptoms, four assess excessive ventilation and five relate to central nervous system symptoms. Questions are answered in a few minutes on a five-point scale ranging from 'never' (0 points) to 'very often' (4 points).[34] The score ranges from 0 to 64, with cut-off value of 23 and more points that best indicates HVS. However, elevated score is not diagnostic of a specific syndrome. The questionnaire has been increasingly used as an outcome measure in various clinical and research settings for physiotherapists and other specialists. The cut-off value to detect DB and distinguish it from other abnormalities depends on the context in which the NQ is used, i.e. in poorly managed asthma, COPD, panic disorder and anxiety, where its specificity may be lower.[6] Actually, the score measures "functional respiratory complaints" meaning that it refers to ventilation, dyspnoea and breathing movement in relationship with stress and anxiety. Van Dixhoon and group of authors[34] that developed the questionnaire comment that "it detects transdiagnostic and probably nonmedical abnormality", "reflects a subjective aspect of DB" and that "early detection of these tension related complaints would prevent unnecessary visits to medical specialists and treatment". The NQ is not copyrighted (free to use) but depends heavily on patients' understanding of questions and adequate self-assessment.

The Self-Evaluation of Breathing Questionnaire (SEBQ) includes 25 questions of which 23 refer to breathing or forms of breath. Thus, it is complementary to NQ as it evaluates more respiratory symptoms related to the manual assessment measure.[35] In a study by Courtney R and Greenwood KM, SEBQ demonstrated both very high test–retest reliability and internal consistency in a group of adults from the general population.[21] However, as they concluded, "whilst SEBQ may bring a greater sensitivity than alterna-

DB suspected
- anamnesis
- physical exam

Dg and treat other causes
- chest X-ray, lung function tests, arterial blood gasses, ECG, blood glucose, CBC, kidney and liver function tests, consult otorhinolaryngologist and psychologist
- most common differential dg: asthma, COPD, respiratory failure, heart failure, neuromuscular diseases, panic disorder, anxiety, upper airway disorder

Persistent symptoms of DB
- assess functional respiratory complaints with observation-based tools and questionnaires, i.e. NQ, BPAT, MARM etc.

CPET
- erratic ventilation; hyperventilation with frequent sighing present at rest/during exercise; hypocapnia
- normal PaO2, alveolar-arterial pressure gradient of oxygen, dead space volume/tidal volume and arterial-ETCO2 pressure gradient at end-exercise

exercise-induced dyspnea
- asthma or exercise-induced bronchoconstriction
- deconditioned - abnormal or normal physiological limit
- exercise-induced arrhythmia, intracardiac shunting, exercise-induced anaphylaxis, etc.

DB
- dyspnea independent from bronchospasm and unresponsive to beta-agonists
- functional (disordered breathing pattern or paradoxal vocal fold motion disorder) or structural (phrenic nerve palsy, repaired diaphragmatic hernia, bronchomalacia, laryngomalacia, subglotic stenosis, etc.)
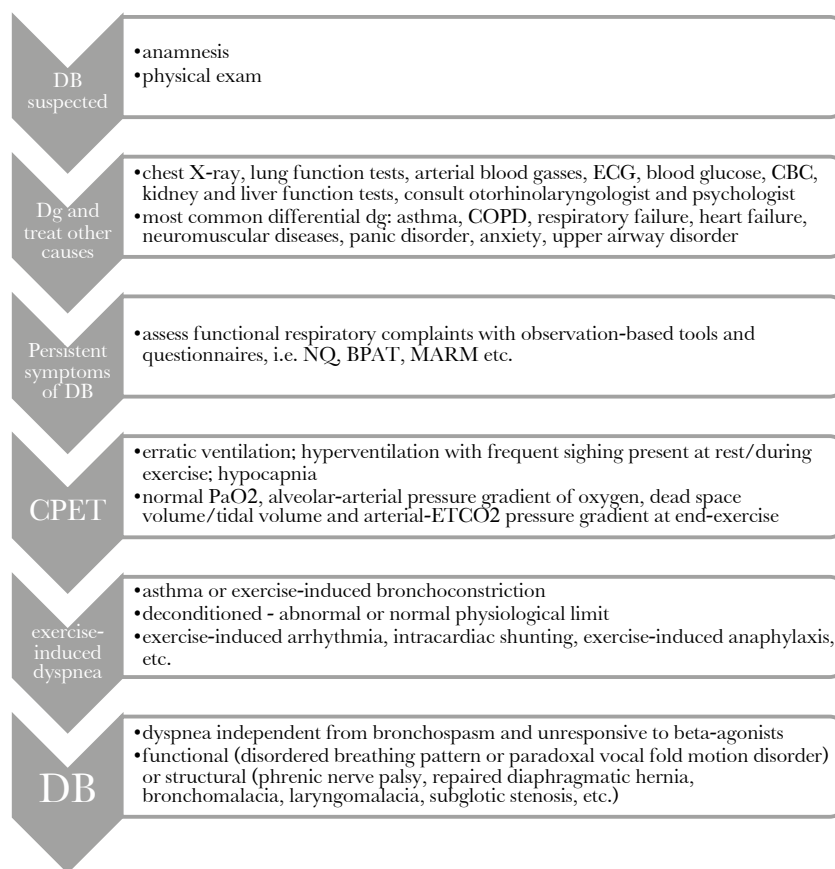
Figure 3. An example of diagnostic algorithm for DB

tives to the assessment of DB-related symptoms because it is not oriented around the direct effects of hyperventilation, the trade-off may be reduced specificity, particularly for people with respiratory medical conditions such as asthma, for whom similar symptoms might arise from pathological changes in airways rather than from disturbed biomechanical breathing patterns".

The Manual Assessment of Respiratory Motion (MARM) is a palpation technique based on the examiner's interpretation and estimate of motion identified by hands at the posterior and lateral lower rib cage. The MARM enables examiner to measure differ-ent aspects of breathing (i.e. rate, regularity). The most important part is to assess breathing pattern and the relative distribution of breathing motion between upper rib cage and lower rib cage and abdomen. The MARM is a practical and reliable tool for the breathing pattern assessment with good agreement between examiners. Moreover, one study comparing MARM with respiratory induction pletizmography found that it can better distinguish thoracic from abdominal breathing.[36]

The Brompton Breathing Pattern Assessment Tool (BPAT) is, like MARM, a tool used by the observer (i.e. physiotherapist).

The BPAT includes assessment of abdominal or apical breathing, inspiratory and expiratory flow, inspiration and expiration through mouth or nose, air hunger, breathing rate and rhythm.[17] Recently, it appeared to be a useful screening tool for identifying DB in patients with difficult-to-treat and severe asthma (with score ≥4 as a cut-off for diagnosing DB was confirmed with sensitivity 95% and specificity 78%).[37] Similarly, it is useful in evaluating DB in long COVID (12 weeks after confirmed or presumed pneumonia caused by SARS-CoV2 virus). Using the established cut-off, it showed a sensitivity of 89.5% and specificity of 78.3%.[3]

The Hyperventilation Provocation Test (HVPT) requires voluntary hyperventilation for several minutes and is considered positive if symptoms of HVS are recognized by the examinee. Earlier, the test was a gold standard for diagnosing HVS and the symptoms of HVS were largely attributable to hypocapnia (low end-tidal carbon dioxide). However, a high percentage of false-positive results during the HVPT has been found in studies with a control condition of stressful mental load.[38,39] What is more, when limiting symptoms to hypocapnia, a study in the Lancet found a high rate of false positives (66%) in patients where end-tidal pCO_2 was maintained at baseline value by manual titration of carbon dioxide from the cylinder into the inspired air.[40]

The end-tidal carbon dioxide measurement (ET-CO2) is measured using capnography with an expected low ET-CO2 in hyperventilation.[41]

The Breath Hold Test or *Breath Holding Time (BHT)* is an indicator of a person's ventilatory response to biochemical (sensitivity to hypoxia and hypercapnia), biomechanical (lung volumes), non-chemical factors, and psychologic factors, as well as training, exercise and altitude, etc. For example, divers may accommodate to the absence of respiratory movements (non-chemical factors for ventilatory response) and prolong BHT to 40 or 50s.[42] A short BHT (<30s) after normal expiration at functional residual capacity is considered to be related to DB. A physician Konstantin Buteyko, M.D., Ph.D., that developed breathing technics to reduce HVS claimed that BHT can detect chronic hyperventilation and that BHT predicts alveolar CO2 (PaCO2) according to his patented mathematical formula.[41,43]

Finally, to objectively evaluate breathing patterns in various clinical and outpatient settings, an ideal system should tend to fulfill the following characteristics: (1) Accurate calculation of volume changes without using a mouthpiece that may alter the normal breathing pattern; (2) Need of a simple, stable and repeatable calibration; (3) Possibility of use in non-collaborating subjects (during sleep, or in unconscious patients); (4) Permitting the analysis in different postures; (5) Permitting the analysis under dynamic conditions such as walking or cycling; (6) Allowing high frequency response in order to accurately describe rapid phenomena (i.e. electric or magnetic stimulation of phrenic nerves); (6) Allowing the analysis of movements and volume changing of the upper thorax, lower thorax, and abdomen; (7) Allowing the analysis of movements and volume changing of the hemi thoraces; (8) Being non-invasive and safe for the patient.[44] Different techniques with various limitations are available for measuring natural breathing at rest - tidal breathing patterns, as well as exercise-induced changes in breathing. A less frequently used techniques include pneumotachography, respiratory induction plethysmography opto-electronic plethysmography and structured light plethysmography. These vary in the source of the signal and the type of parameters that are generated, such as thoraco-abdominal asynchrony.[45–47] Only structured light plethysmography does not necessitate direct contact with patient's body and is less dependent on patient's cooperation.[47] How-
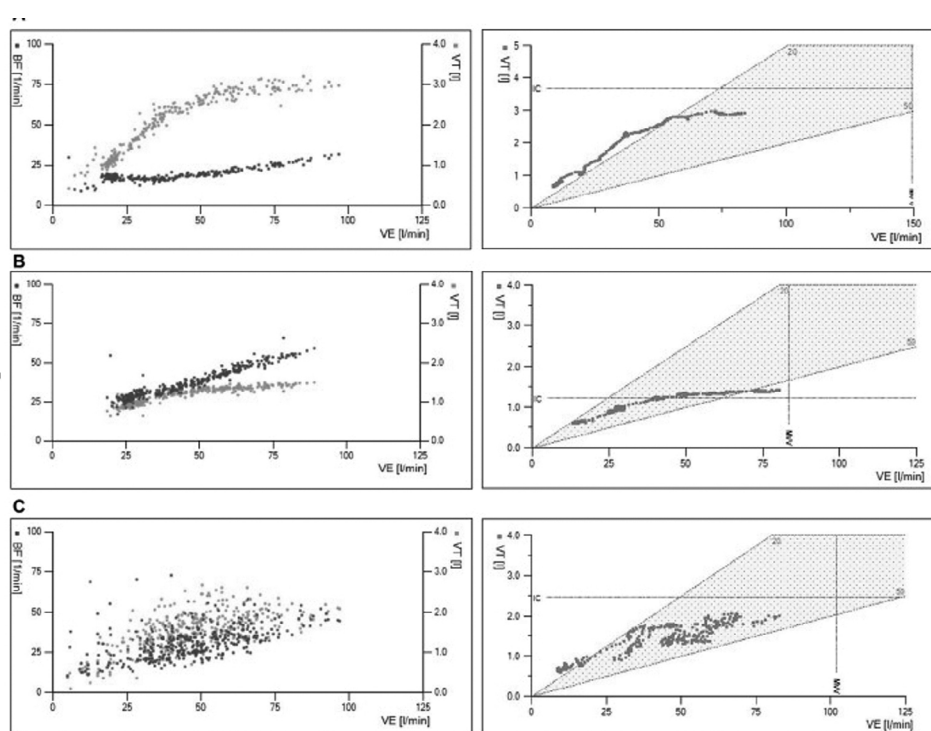
Figure 4. Ventilation slopes and Wasserman panel (VT/V'E). (A) Normal subject. (B) Respiratory limitation showing a regular, but limited increase of tidal volume with high breathing frequency. (C) Dysfunctional breathing with an erratic pattern. Plots of tidal volume (VT on the right y-axis) and breathing frequency (BF on the left y-axis) against minute ventilation (V'E on the x-axis) during incremental exercise testing. Data are not filtered in the ventilation slopes. Geratherm Respiratory combined filter is used in the Wasserman panel (VT/V'E). BF, breathing frequency; VT, tidal volume; V'E, minute ventilation.[51]

ever, these techniques are resource- and evidence-limited and need further clinical and experimental research.[48]

Cardiopulmonary Exercise Testing (CPET) is the most detailed diagnostic tool to objectify breathing patterns during exercise and it represents "an ideal candidate" for gold standard among proposed diagnostic methods for DB.[12] A major benefit of CPET is that, in contrast to the questionnaires and observation-based approaches, it offers objective measurements and plots data which can be directly analysed. Erratic ventilation, hyperventilation with frequent sighing present at rest or during exercise and recorded in the respiratory panels of CPET can bring up to a diagnosis of DB. Furthermore, CPET may unravel the mechanisms of breathlessness by simultaneously evaluating cardiovascular adaptation, ventilation, and gas exchange through exercise. CPET permits recognition of any pathophysiological cause of exertional dyspnoea which would not manifest during tests performed at rest.[49–51] Precisely, patients with DB usually present with high frequency of breathing at rest which rises swiftly at the begging of exercise, while tidal volume may remain stable. This can increase dead space ventilation and change the kinetics of multiple CPET variables. Also, decreasing PaCO2 set

point in rapid shallow breathing, as seen in hyperventilation type of DB, typically induces ventilatory inefficiency characterized by high minute ventilation/CO2 output (VE/VCO2 slope), with generally a normal dead volume/tidal volume ratio. Important to notice is that increase in end-inspiratory and end-expiratory lung volumes, as seen in hyperinflation, therefore reduces inspiratory capacity and possibly contributes to the troublesome dyspnoea sensation regardless of the existence of true hypocapnia.[2] However, other types of DB with normal PaCO2 and VE/VCO2 have been described, in particular, erratic ventilation with wide and irregular variations of tidal volumes and breathing frequency over the progression of effort during CPET.[52] Boulding and colleagues suggested a classification of DB patterns according to incremental CPET data, as well as change in breathing frequency, tidal volumes, and respiratory muscle mechanics before and after exercise.[2] Analysis of ventilation patterns on CPET may contribute in differentiating types of breathing dysregulation in people with dyspnoea present in the absence of deconditioning as a post–acute-phase sequelae of mild infection with SARS-CoV2 virus. Nonetheless, one should consider its highly demanding resources and setups in the context of the high prevalence of post-COVID-19, as well as the fact that "exercising at physiological limits may exacerbate symptoms in these patients, also referred to as postexercise malaise".[12,53] An example of CPET in a normal subject compared with a person with limited tidal volume and high breathing frequency and a person with dysfunctional breathing is showed in *Figure 4*.[51]

Differential diagnosis should always and firstly include all diseases that can be the cause of dyspnoea in the first place, and also, may be associated with DB. The finding of erratic breathing on CPET cannot exclude accompanying disease, nor can it precisely confirm DB diagnosis. Most frequently patients with DB present with resting hypocapnia and normal PaO2, alveolar-arterial pressure gradient of oxygen, dead space volume/tidal volume and arterial-ETCO2 pressure gradient at end-exercise. These findings can help differentiate from other conditions in which chronic hyperventilation occurs, as in patients with increased dead space ventilation, such as those with heart failure or pulmonary hypertension. Similarly, an identified marker of disease severity in patients with heart failure due to left ventricular systolic dysfunction is periodic breathing. As happens in DB, it may develop at rest or during exercise and last throughout the entire period of incremental workload or disappear facing the end of exercise. However, the characteristic periodicity of waxing and waning of tidal volumes (minute ventilation, as well) present in periodic breathing is in sharp contrast to the unpredictable and irregular breathing pattern of DB. Further, thoracic-dominant patterns may be present in morbidly obese patients in response to their low abdominal compliance. At last, one must consider asthma and COPD, where patients may develop thoracic-dominant and forced expiratory breathing patterns as a physiological adaptive response to pulmonary hyperinflation, in which case they should not be regarded as dysfunctional.[12] Ionescu and colleagues proposed a diagnostic and therapeutic algorithm for patients with unexplained dyspnoea. Starting with high clinical suspicion of DB, electrocardiography, chest radiography and spirometry test should be one of the first tools to exclude or prove possible cardiopulmonary etiologies of dyspnoea. If symptoms persist after adequate management, the next step is CPET. If there is good fitness on CPET with no evidence of DB, reassure the patient and discharge. If there are abnormalities present in terms of cardiac, ventilatory, gas exchange or metabolic parameters on CPET, proceed to targeted management. If there are, in addition or alone, one or more features of DB identified on CPET, refer the patient to a chest physiotherapist, with targeted therapeutic intervention.[12]

Combining different tools possibly could be the best choice as it may increase diagnostic accuracy. For example, in the recent studies the NQ was used to evaluate symptoms related to DB, and supplemented with the BPAT to objectively assess breathing pattern in patients with difficult-to-treat asthma.[17,25] Identifying DB as a co-morbidity in difficult-to-treat asthma is of a special interest, to avoid potentially harmful or costly overtreatments such as oral steroids, or biological treatments. One group of researchers found that almost quarter of patients referred to severe asthma clinic had only DB.[17] In comparison, Sedeh and colleagues[25] firstly comprehensively and systematically verified asthma diagnosis in all participants, then assessed disease severity according to international recommendations[26] and, at last, applied the NQ, BPAT, as well as Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire (AQLQ). The researchers found that patients with uncontrolled asthma and DB were mostly female (74%), had higher body mass index (BMI), had significantly poorer asthma control and lower quality of life compared to patients without DB. After adjusting for BMI the relationship between DB and poor asthma control, did not change, meaning that symptoms of DB were not induced by obesity. Also, DB alone, the NQ score as well as the BPAT were an independent determinants of ACQ-score meaning that the adverse impact of DB on asthma control could not be explained by other factors such as more bronchial hyper-responsiveness or lower lung function in patients with DB. Moreover, patients with a low NQ, but high BPAT (objective signs of DB), had a significantly poorer asthma control, compared to patients with both low NQ and low BPAT. Similar results were found in one study on asthma patients using the NQ and ACQ-score.[54] Difficult-to-treat asthma is asthma that is uncontrolled despite medium/high dose inhaled corticosteroids with a second controller, or on maintenance oral corti-

costeroids or that requires such treatment to maintain good symptom control and reduce the risk of exacerbations.[26] The aforementioned findings reinforce the idea to routinely search for DB in the patients referred for specialist management of asthma.[25,54] Further investigations are necessary to determine a possible benefit of physiotherapeutic treatment in reduction of inhaled corticosteroids use in patients with concomitant asthma and DB.

Treatment

Patient education about the condition, reassurance
Abdominal breathing retraining
Breathing rate and depth control
Breathing retraining in progressively taxing postures such as walking
Recognition of triggers
Control of symptoms during an episode of DB and manual therapy

Various modes of breathing retraining programs guided by a qualified professional (e.g. physiotherapist) are recommended, such as breathing control, diaphragmatic breathing, yoga breathing, Buteyko breathing, biofeedback-guided breathing modification, and yawn/sigh suppression.[55,56]

Educating patients about DB is the key and the first step in the program. Helping patients differentiate symptoms of DB from the associated conditions, such as asthma, is an important goal. For instance, DB would not respond to targeted treatments for asthma.

One randomized controlled trial by Lindeboom and colleagues compared relaxation therapy versus relaxation therapy and breathing exercises.[57] According to a Cochrane review[58], the results of this study "describe a significant reduction in frequency and severity of hyperventilation attacks in the breathing exercise group compared with the control group, which demonstrated an increase in the frequency and severity of attacks. In addition, a significant difference in frequency and severity of hyperventilation at-

tacks between the breathing and relaxation group was reported."

Diaphragmatic (abdominal) breathing represents breathing in slowly and deeply through the nose using the diaphragm with the least possible movement of the chest in a supine position with one hand laid on the chest and the other on the umbilical region. Lately, a systematic review has reported that mind–body exercises, such as yoga or tai chi, which incorporate diaphragmatic breathing can lower effect of intense stress or unfavourable emotions by balancing the sympathetic and vagal tone.[59] Even through many trials have found that breathing exercises are helpful in treating COPD, asthma, and postoperative pulmonary function, the efficacy of diaphragmatic breathing in managing other diseases/disorders, i.e. cancer, heart failure, and anxiety, still needs to be studied further. To stress out, diaphragmatic breathing may worsen dyspnoea in severe COPD patients.[55]

A novel mind-body breathing therapy intervention adjunct is a capnography-assisted respiratory therapy (CART) has found application in COPD-related DB management. CART consists of patient-centered biofeedback, tailored breathing exercises, a home exercise program and motivational interviewing counselling.[60]

Primary therapeutic outcome should be improvement in quality of life. Secondary outcomes mainly include the Nijmegen questionnaire score, minute volume, tidal volume, respiratory frequency, ET-CO2 or transcutaneous CO2 measurement, and functional exercise capacity. However, over the course of the therapy, evolution from the first assessment to consecutive follow-ups is frequently recorded using NQ, SEBQ, or BPAT scores. As aforementioned, these tools subjectively differentiate symptoms and breathing pattern. What is more, there is risk of bias in process monitoring. After initiating therapy, a patient filling out a questionnaire may, either intuitively or intentionally, answer the questions as they perceive the practitioner wishes them to, lowering the scores given to describe the presence of their symptoms in order to demonstrate the treatment is working. Similarly, the practitioner knows that the patient has received therapy and so will be searching for proof that therapy has been successful. In reality, it has been noticed that patient symptoms and quality of life improve, as assessed in one study with Asthma Quality of Life Questionnaire, even though the NQ/ SEBQ/BPAT scores do not substantially increase from the baseline.[61] CPET may be effective in the objective guidance of the breathing retraining process and in the monitoring of therapeutic effect.[12]

Conclusion

Taking into account the heterogeneity, psychological and physiological aspects of DB, a multidimensional, holistic assessment would appear the most correct approach to improve understanding and diagnostic efficiency. The current narrative review was composed in a manner of brief summary of the available data considering DB, in order to promote understanding of the disorder by health care professionals. Upgrading knowledge of the etiologic and pathophysiologic factors, diagnostic tools and treatment options in DB management enables practitioners to improve health-related quality of life in people experiencing breathing pattern abnormalities.

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2

Asthma Phenotypes and Comorbidities

Aspergillus Sensitisation and Severe Asthma Clinical Outcomes

Sabina Škrgat,[1,2] Katarina Pelicon Slabanja[3]

Abstract

Fungal lung diseases represent a heterogenous group of conditions and the differing definitions are used to describe these relationships. Historically there has been the nomenclature evolution on the spectrum of lung diseases linked to sensitisation to *A. Fumigatus* including SAFS (severe asthma with fungal sensitisation) and ABPA/M (allergic bronchopulmonary aspergillosis/mycosis). It seems that AFAD (airway fungal airway disease) therefore represents an open definition of IgE sensitisation to thermotolerant fungi. It covers not only the most severe forms of the disease as SAFS and ABPA, but also milder forms of airway disease. It might represent a treatable trait which has to be seen, longitudinally observed, and treated and consequently preventing lung damage.

Keywords: allergic fungal airway disease, severe asthma, IgE sensibilisation

1 Department for pulmonary and allergic diseases, University Medical Centre Ljubljana, Slovenija

2 Medical Faculty, University of Ljubljana

3 Pulmonary Department, General Hospital dr. Franc Derganc, Šempeter, Slovenija

Introduction

Fungal lung disease represents a heterogeneous group of conditions[1]. They can be divided into infective, toxic, or allergic in nature, although there is a degree of overlap. Some of them are connected and interact in patients with asthma and particularly in severe asthma. Its behaviour in interaction with fungi results in different clinical asthma outcomes, which are under recognised and represents a clinical and diagnostical challenge.

The differing nomenclatures are used to describe these relationships. A recent proposal of various clinical outcomes of airway colonisation with thermotolerant filamentous fungi e.g. *A. Fumigatus* (table 1) includes allergic group. This allergic group can again be broadly divided into two, both of which can be associated with severe asthma. The first type is an allergenic response to environmental fungi such as non-thermotolerant *Alternaria* and *Cladosporium* which act as seasonal aeroallergens, the symptoms of which are directly related to airborne concentrations of fungal material, and which can include acute severe exacerbations. The second type involves an allergic response to thermotolerant filamentous fungi such as species from the *Aspergillus* and *Penicillium* which can act as aeroallergens, and they have the additional property of being able to germinate in the airways. Consequently, they are colonising the lungs and causing a persistent allergenic stimulus that can lead to lung damage[2-4].

Historically the clinical and immunological variability in presentation of fungal allergy to thermotolerant fungi has developed into a separate differentiation/definition of two conditions: allergic bronchopulmonary aspergillosis/mycosis (ABPA/M) and severe asth-

Table 1. Clinical outcomes of airway *colonisation* with thermotolerant filamentous fungi e.g., *A. Fumigatus (adapted from 1)*

	Basic Clinical manifestaton	Further subclasification
Upper airway	Allergic fungal sinusitis	
Lower airway	Cavitating lung disease	Aspergilloma
	Chronic Lung disease	Fungal allergy Chronic pulmonary aspergillosis Ekstrinsic allergy alveolitis
		Fungal bronchitis
	Immunocompromised host	Invasive aspergillosis

ma with fungal sensitisation (SAFS). Recent publications[1] support the idea that these presentations should not be strictly seen as a completely different entities since there is limited evidence that there are distinct mechanisms involved in the spectrum of thermotolerant fungal lung allergy. Consequently, recently an inclusive set of criteria which includes all presentations of the disease under the umbrella term allergic fungal airway disease (AFAD) is preferred[1,5].

Evolution of terminology toward AFAD

The fungi that play a role in asthma can be divided into two groups: those that can grow at body temperature, referred to as thermotolerant, which are capable of both infection and allergy, and those that cannot but can still act as allergens in IgE sensitised individuals. It is the thermotolerant group of filamentous fungi that cause AFAD[1,5]. The pathophysiology behind different clinical outcomes is the host response to airway colonising, allergenic, thermotolerant, filamentous fungi, with *A. fumigatus* as the major culprit[5].

Sensitisation ot *A. Fumigatus* has been associated with a spectrum of states including SAFS and ABPA/M. The descriptions of ABPA criteria have developed over time and the Petterson s criteria[6] were later further up-

graded with the criteria proposed by the International

Society for Human & Animal Mycology (ISHAM)[7] which are more relaxed making them more relevant to clinical practice[5].

Proposed ABPA criteria includes:

1. the presence of asthma or cystic fibrosis,
2. evidence of specific IgE to *A. fumigatus* and total IgE above 1000 IU/ml
3. at least two of raised IgG antibodies to A. fumigatus, abnormal radiology consistent with ABPA and an eosinophil count (steroid-naive patients of greater than 0.5X109/l)

In an accompanying diagnostic algorithm, total IgE was central in distinguishing between ABPA and IgE sensitization without ABPA

This structure has been very recently further upgraded[8] with the work of the Japan ABPM research program, supported by the Japan Medical Research and Development Organization. They developed new ten-component diagnostic criteria for ABPA/ABPM in non-cystic fibrosis patients (table 2) where they compared the sensitivity and specificity of the new and conventional criteria to discriminate pathological and physician-diagnosed ABPA/ABPM from related diseases, including fungus-negative mucoid impaction in bronchi, chronic eosinophilic pneumonia, fungus-sensitized severe asthma, and chronic pulmonary aspergillosis. The new diagnostic criteria, compared with existing criteria, showed better sensitivity and specificity for diagnosing ABPA/ABPM; The sensitivity for pathological ABPM with Rosenberg-Patterson criteria, ISHAM criteria, and these new criteria were 25.3%, 77.2%, and 96.2%, respectively. The sensitivity for physician diagnosed ABPA/ABPM were 49.2%, 82.7%, and 94.4%, respectively. The areas under the curve for the receiver operating characteristic curves were 0.85, 0.90, and 0.98, respectively[7].

Table 2. Clinical diagnostic criteria for allergic bronchopulmonary mycosis in patients without cystic fibrosis (adapted from 7)

1. Current or previous history of asthma or asthmatic symptoms
2. Peripheral blood eosinophilia (≥ 500 cells/mm3)
3. Elevated total serum immunoglobulin E levels (IgE ≥ 417 IU/mL)
4. Immediate cutaneous hypersensitivity or specific IgE for filamentous fungi
5. Presence of precipitins or specific IgG for filamentous fungi
6. Filamentous fungal growth in sputum cultures or bronchial lavage fluid
7. Presence of fungal hyphae in bronchial mucus plugs
8. Central bronchiectasis on computed tomography (CT)
9. Presence of mucus plugs in central bronchi, based on CT/bronchoscopy or mucus plug expectoration history
10. High attenuation mucus in the bronchi on CT

Filamentous fungi in 4-6 should be identical. Patients that meet 6 or more of these criteria are diagnosed with ABPM.

Many fungal sensitised individuals with severe asthma do not fulfil the criteria for ABPA, so in 2006 the term SAFS was introduced. Denning and colleagues thus proposed the term severe asthma with fungal sensitisation (SAFS) to describe this aspect of troublesome asthma and used criteria in opposition to the ABPA criteria by including an IgE of 1000 IU/L[8]. However, SAFS includes asthmatics with sensitisation to any fungus.

It seems that AFAD therefore represent an open definition of IgE sensitisation to thermotolerant fungi, therefore a treatable trait which has to be seen, longitudinally observed and treated as appropriate. It covers not only the most severe forms of the disease as SAFS and ABPA, but also milder forms of airway disease. It is important to stress that many patients with clinically significant fungal allergy do not have severe asthma. Nevertheless, all patients with IgE sensitisation to thermotolerant fungi in the context of asthma and other airway disease are at risk of progressive lung damage, and as such should be monitored closely irrespective of a diagnosis of ABPM[7].

The terminus AFAD reminds a clinician, that the disease might progress in other forms including lung damage and that »watch and see« strategy might be not enough.

Pathophysiological abnormalities and clinical outcomes related to airway fungal allergy

Basic immunology

Fungal sensitisation occurs in about 3–10% of the general population[9] and 7–20% of asthmatics. The prevalence is higher in patients with severe asthma (rates between 35–75%)[10]. The hallmark of AFAD is exaggerated T2 immunity causing IgE sensitisation to filamentous fungi and eosinophilic inflammation[7]. Airway epithelium is exposed to proteolytic enzymes from fungi following deposition of the spores/hyphae or smaller particles on the surface. Those enzymes augment the permeability of the epithelial layer by digesting the proteins of tight junctions, destroying the integrity of epithelial cells and by digesting the structural proteins of the basement membrane. Selective production of TSLP, IL-25, and IL-23 by epithelial cells and inhibition of IL-12 production by dendritic cells (DCs) may be responsible for the shift toward Th2 responses[11]. In the study of Balenga and co-workers they have shown that a major *A. fumigatus* allergen, Asp f13, which is a serine protease, alkaline protease 1 (Alp1), promotes airway hyper-responsiveness by infiltrating the bronchial submucosa and disrupting airway smooth muscle (ASM) cell-extracellular matrix (ECM) interactions[12]. The group later demonstrated that Alp1 quantities were significantly higher in sputum from patients with Af sensitivity than those without, regardless of clinical severity of the disease. But the amount of Alp1 in the lower airways of asthmatics correlated with severity of disease and interestingly with sputum neutrophil, but not eosinophil counts. They suggested that it is proteolytic destruction of lung tissue, which could promote influx of neutrophils into the airway lumen[13].

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Mucus impaction

Mucus impaction in AFAD is most strikingly evident in those patients who present with lobar collapse due to inspissated mucus but is also seen in the smaller airways on CT scans[7]. The precise pathway by which IgE sensitisation to thermotolerant filamentous fungi may cause production of viscid mucus is not clear but could be related to excess production of MUC5AC by goblet cells because of vigorous T2 hyperimmune stimulation[7,14]. Evolution of mucin synthesis is complex and include activated eosinophils as well since there is evidence that they induce mucin synthesis in human airway epithelial cells via EGFR (epidermal growth factor receptor)[15].

Immaging, functional impairment and comorbidities

Aspergillus fumigatus sensitization defined by a specific IgE of 0.35 kU/L or greater was associated with functional and radiological abnormalities: 83.4% had an abnormal HRCT with bronchial wall thickening (41.3%), bronchiectasis (35.3%), air trapping (20.3%) and bronchial dilatation (16.5%). Radiological evidence of airway disease was also associated with more obstructive spirometry. *A. fumigatus* sensitization was associated with a 2.01 increased hazard ratio of bronchiectasis and more obstructive spirometry. They suggested that patients with *A. fumigatus* sensitization had variable clinical and radiological characteristics that frequently did not conform to the conventional diagnostic criteria for ABPA[16].

All patients with IgE sensitisation to *A. fumigatus* are at risk of lung damage irrespective of whether they meet the criteria for ABPA[17]. A large cohort (n = 431) of asthmatics enriched for IgE sensitisation to fungi were recruited in a cross-sectional study to determine the relationship between immunological biomarkers of fungal allergy and evidence of lung damage in asthma[17]. The patients with AFAD had higher rates of early-onset dis-

ease and as a result almost twice the duration of asthma. Those with AFAD had overall about a 10% deficit in FEV1 which was not related to atopy and not seen in patients sensitised to non- thermotolerant or non-filamentous fungi. Significant differences in radiological appearances between those sensitised and non-sensitised to fungi included bronchiectasis (50% versus 29%), tree-in-bud (17% vs 4%) and collapse/ consolidation (35% vs 21%). Authors suggested that IgE sensitisation to thermotolerant filamentous fungi, in particular *A. fumigatus* but not total IgE, is associated with fixed airflow obstruction and several radiological abnormalities in moderate to severe asthma.

The group of Kurukulaaratchy[18] reported that *A. fumigatus* sensitisation in patients with difficult asthma identifies a more severe form of disease associated with older age, male sex, longer duration of disease, lung function impairment, bronchiectasis, higher inflammatory parameters, greater treatment needs but less psychophysiologic comorbidities.

Fungal bronchitis

Fungal bronchitis describes chronic purulent sputum production due to non-invasive infection with thermotolerant fungi in the context of a relatively immunocompetent host. It is not widely used in the medical literature. A positive sputum culture for thermotolerant fungi is critical for the diagnosis of fungal bronchitis. In a recent report[19] the group of Wardlaw and co-workers have recognised a clinical presentations of often chronic exacerbations of airway disease which were unresponsive to standard treatment with broad spectrum antibiotics or high dose oral corticosteroids, in which sputum culture was positive for either *A. fumigatus* or *Candida spp.* Usually the sputum was white/creamy or brown rather than the green associated with bacterial infection, and was very mucoid or rubbery in consistency[19].

Management of AFAD

To a large extent management of AFAD is similar to the management of the underlying airway disease with personalised approach. An approach toward treatment of T2 treatable trait (eosinophilic pattern of disease) includes inhaled corticosteroids. They are a cornerstone of therapy. There exist a theoretical risk of augmentation od fungal colonisation, but with the approach toward using the lowest dose of inhaled corticosteroids to achieve a control of disease this might not be serious problem in clinical practice. In severe cases low dose continuous or intermittent oral corticosteroids (OCS) are necessary to achieve control.

Since OCS are seen as a last resort in asthma therapeutic algorithms, anti-T2 biological therapy is a possible option in AFAD treatment. Evidence on omalizumab, but also mepolizumab, benralizumab and dupilumab are based mostly on case series and reports. Favourable reported responses include significant reduction in OCS burden, reduction in acute exacerbations, improvement in lung function and improvement in patients outcomes[120–23].

The place of antifungal therapy in AFAD remains uncertain. Whilst open studies have often reported a benefit, placebo controlled, blinded studies have shown either no benefit or a modest improvement at best compared to standard of care, which these days probably includes biological therapy. Clinical practice would suggest that in the majority of patients with AFAD the benefits of azole therapy are not outweighed by side effects. However, where fungal bronchitis is present, particularly in the context of difficult to treat exacerbations, they are an important adjunct to therapy and can lead to a dramatic improvement in symptoms in relatively short time. Positive sputum fungal culture seems to be a useful biomarker of a response to antifungal therapy even in the case of *Candida* species if it is persistent[5]. There are no definitive guidelines on how long a course should be, but clinical recommendation from the group of Wardlav[1] recommends that three months is necessary and usually sufficient. Repeated courses are sometimes necessary.

Conclusions

The term AFAD has a liberal definition, based on the presence of IgE sensitisation to thermotolerant fungi and evidence of fungal-related lung damage[23]. As such it is more inclusive than ABPA or SAFS, not being focused on total IgE and not restricted to severe asthmatics only. The recommendation supports close patients follow up due to detecting and preventing long term lung damage[17].

Furthermore, unlike SAFS, AFAD distinguishes between sensitisation to thermotolerant and non-thermotolerant fungi[5].

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OSA in Patients with Severe Asthma-Alternative Overlap Syndrome

Ivan Čekerevac[1,2], Bojan Djokić[2]

Abstract

Bronchial asthma and obstructive sleep apnea (OSA) are common chronic diseases of the respiratory system. During the last decade, there has been a growing interest in the connection between these two disorders. Studies show that asthma patients are at increased risk for OSA, and the prevalence is on average around 70% in severe asthma patients. Rhinitis, gastroesophageal reflux disease and obesity are common comorbidities for both entities. OSA is an independent factor in the exacerbation of asthma and each condition in itself can contribute to the exacerbation of the other. Asthma, by its mechanical effect, has a direct impact on OSA, leading to greater collapse of the upper airway and worsening snoring and apnea symptoms in patients with OSA. On the other hand, OSA directly affects asthma through nerve reflexes, intermittent hypoxia, increases inflammation, increases the production of leptin and vascular endothelial growth factor as well as sleep fragmentation. Indirect effects in a bidirectional interaction are reflected in the prolonged effects of systemic corticosteroids, chronic diseases of the upper respiratory tract, tobacco use and increased body weight in asthmatics, which leads to worsening of OSA symptoms. It remains unclear whether OSA in asthmatics is merely a comorbidity or a specific new phenotype of asthma. In patients with asthma and OSA, CPAP treatment reduces asthma symptoms, improves morning expiratory flow, and improves quality of life parameters.

Keywords: severe asthma, OSA, alternative overlap syndrome

Bronchial asthma and obstructive sleep apnea (OSA) are frequent chronic diseases of the respiratory system. During the last decade, there has been a growing interest in the connection between these two disorders. For this reason, the term Alternative Overlap Syndrome (Asthma and OSA) was introduced in 2013, to distinguish it from the classic Overlap Syndrome (COPD and OSA). There is more and more evidence that OSA is associated with increased bronchial hypersensitivity[1] and inflammation[2] and thus may be an independent risk factor for exacerbation of bronchial asthma[3]. Studies show that asthma sufferers have an increased risk of OSA, and the prevalence is on average around 70% in severe asthma sufferers[4]. Many patients with asthma report poor sleep quality, daytime sleepiness and higher frequency of snoring during sleep than in the general population[5]. These symptoms are common in patients with OSA, indicating a connection between the two disorders[6]. Similar pathophysiological mechanisms are observed in both disorders, which are manifested by an increase in local and systemic inflammation, and common comorbidi-

ties such are gastroesophageal reflux, obesity, and rhinitis[7–9].

Asthma and OSA-alternative overlap syndrome

OSA is the most common breathing disorder during sleep, typically occurring in obese people[5]. Like asthma, OSA has its own phenotypes depending on the craniofacial morphology. Common risk factors for OSA include male gender, age, obesity, increased neck circumference (greater than 17 inches in men and 16 in women), craniofacial abnormalities (micrognathia, retrognathia), and the presence of cardiovascular comorbidities[4]. Certain studies point out that the presence of OSA in patients with asthma can be a separate phenotype of asthma[10,11]. The frequency of OSA in severe asthma and difficult-to-treat asthma ranges from 50 to 95%[4]. Such a large difference in frequency can be explained by the different methodology of the studies. In earlier studies, the methodology was based on self-reporting of snoring during sleep and periods of apnea[12,13]. Recent studies have included polysomnography in their methodology. After a four-year follow-up period, patients with asthma had a 40% higher risk of sleep apnea compared to patients without asthma[14]. In one retrospective study, asthma patients with frequent exacerbations, high doses of inhaled corticosteroids and frequent use of systemic corticosteroids had a more frequent diagnosis of OSA (15). Studies using polysomnography reported a higher incidence of sleep apnea (88 to 95%) compared to studies using a respiratory polygraphy (49% in severe asthmatics)[16]. The significant difference in frequency can be explained by the following: the respiratory polygraphy can underestimate the severity of OSA in patients with asthma; asthma can have an impact on the phenotypic expression of OSA by reducing the Aurosal index[14]. All this shows that more prospective research is necessary to evaluate the development of these two disorders.

Frequent comorbidities in asthma and OSA

Rhinitis: the prevalence of both allergic and non-allergic rhinitis in asthma sufferers is estimated at 80 to 90%, and rhinitis is a risk factor for the development of asthma[17,18]. Rhinitis causes chronic inflammation and nasal obstruction, which results in an increase in negative oropharyngeal pressure during inspiration and predisposes to airway collapse, increased apnea-hypopnea index (AHI) and OSA symptoms[19]. Chronic inflammation in the upper and lower respiratory tract can potentiate the development of OSA[20].

Gastroesophageal reflux disease (GERD): a common disorder found in about 58 to 65% of patients with OSA and as many as 80% of patients with asthma[7,21]. Persistent symptoms of GERD lead to inflammation of the upper respiratory tract, which can cause sleep fragmentation, snoring during sleep. Frequent microaspirations and direct injuries to the airways cause worsening of asthma by increasing the tendency to bronchial obstruction[4].

Obesity: Obesity is a risk factor for the development of OSA, but it is also an independent risk factor for asthma[4]. As a complex entity, it affects breathing through various mechanisms and physiological processes. Accumulation of fatty tissue in the upper parts of the respiratory tract leads to an increase in resistance and collapsibility, while in the region of the chest and abdomen it leads to restrictive disorders where functional residual capacity is reduced and ventilation is weakened[22]. OSA is more common in obese men, while asthma is more common in obese women, which suggests a potential influence of hormones[23].

Pathophysiological correlation between OSA and severe asthma – bidirectional interaction

OSA is an independent factor for the exacerbation of asthma and each condition in itself can have an effect on the worsening of

the other in alternative overlap syndrome. Asthma, with its mechanical effect, has a direct impact on OSA, leading to a reduction in lung volume by reducing the diameter of the airway, as well as by affecting the structure and function of the smooth muscles of the airway. All of this leads to greater collapse of the upper airway and worsens snoring and apnea symptoms in patients with OSA[4]. OSA also directly affects asthma through nerve reflexes, intermittent hypoxia, increases inflammation, increases the production of leptin and vascular endothelial growth factor as well as sleep fragmentation[4]. Intermittent hypoxia leads to systemic oxidative stress and the development of systemic inflammation, where an increase in tumor necrosis factor alpha (TNF-ὰ), interleukin-6(IL-6) and C reactive protein was observed in patients with

Indirect effects in a bidirectional interaction are reflected in prolonged effects of systemic corticosteroids, chronic diseases of the upper respiratory tract, use of tobacco and increased body weight in asthmatics, which leads to worsening of OSA symptoms[4]. GERD and cardiovascular comorbidities in patients with OSA affect the poor course of bronchial asthma. (Figure 1)

Clinical significance of alternative overlap syndrome

It remains unclear whether OSA in asthmatics is only a comorbidity or a specific new phenotype of asthma. On the one hand, allergic asthma is accompanied by a T2 inflammatory response and excessive production of interleukin 5 and interleukin 13, which lead to eosinophilia and hyperreactivity of airway
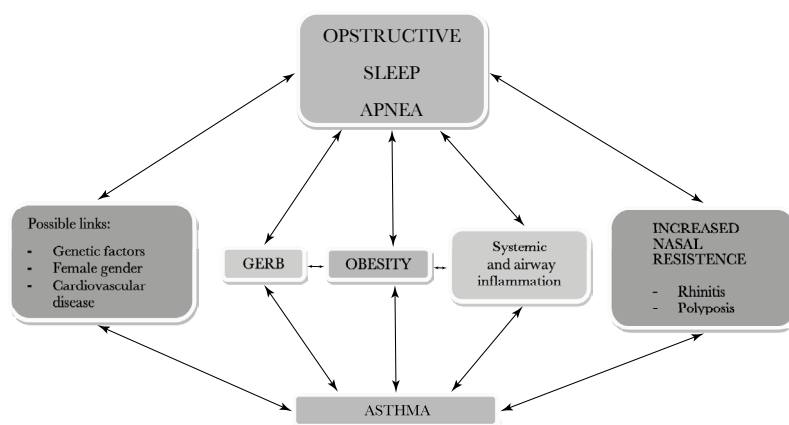
Figure 1. Obstructive sleep apnea and asthma: pathophysiologic links

AHI greater than 15[24]. Also, intermittent hypoxia can lead to stimulation of receptors of the carotid body and initiate reflex bronchoconstriction and participate in the occurrence of nocturnal symptoms associated with asthma[25]. Leptin, a hormonal protein produced by adipose tissue, has a proinflammatory effect and stimulates the release of IL-6 and TNF-ὰ from adipocytes[25].

smooth muscles and mucus hypersecretion, which are complicated by obesity and OSA. In contrast, obese patients who developed non-allergic asthma with late onset develop mechanical changes that lead to lung function disorders and favor the onset of obstructive apnea[5]. In these patients, the adipose tissue secretes several cytokines and adipokines that have direct effects on the airway epitheli-

um and can be a trigger for bronchial hyper-activity[26,27]. From the above, polysomnography is recommended for asthma patients with inadequate control of night symptoms despite proper treatment[28].

Mortality in patients with OSA and asthma is poorly researched. In one study, it was shown that patients with asthma and sleep disorders have a higher risk of mortality compared to asthma patients without sleep disorders[29]. In patients with asthma and OSA, CPAP treatment reduces asthma symptoms, improves morning expiratory flow, and improves quality of life parameters[30]. In one prospective study, it was shown that the proportion of adult patients with uncontrolled asthma dropped from 41.4 to 17.2% with CPAP treatment. It was also shown that the proportion of patients who had worsening asthma decreased from 35.4 to 17.2% after six months of CPAP machine use[7].

Conclusion

The association between OSA and severe asthma is based on coincident pathophysiological mechanisms, bidirectional interactions and the presence of similar comorbidities. Similar to asthma, OSA also promotes an inflammatory response through hypoxia and hypercapnia and sleep fragmentation leading to an irreversible increase in C reactive protein, tumor necrosis factor and other proinflammatory cytokines involved in airway collapse and hyperreactivity. Proinflammatory factors tend to decrease when these patients are treated with a CPAP device, which leads to improvement in asthma symptoms and a better quality of life.

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3

Asthma Monitoring and Evaluation

Induced Sputum Role in Severe Asthma Phenotyping

Sanja Popović-Grle[1,2], Gordana Pavliša[1,2], Marina Lampalo[1,3], Anamarija Štajduhar[1], Branko Pevec[1],
Mira Pevec[1], Dina Rnjak[1], Valentina Fiket[1], Ivana Rubil[1], Silvana Smojver-Ježek[1,4]

Abstract

Induced sputum is a method which, by inhaling hypertonic saline, provoke a person to expectorate certain amount of bronchial secretion. Sputum sample obtained consists of liquid and cellular phase. It is used over the years to investigate airway inflammation in patients with asthma and chronic obstructive pulmonary disease (COPD). Results of cell counts in induced sputum is essential in differentiation of airway eosinophilia or neutrophilia or both, or a determination of paucigranulocytic phenotype. Obtained severe asthma phenotype due to airway inflammation help us to select biological therapy and predict responders.

The correlation between "noninvasive" measures such as blood eosinophilia, or fraction of exhaled nitric oxide (FeNO) with sputum eosinophils is suboptimal. Induced sputum is a good and reproducible discriminator for eosinophilic asthma. There are some safety concerns as induced sputum procedure can provoke adverse effects such as bronchoconstriction and dyspnea, which is reversible to standardized therapy. Sputum induction is safe and well tolerated by patients with severe asthma, which supports its use in clinical and research practice.

Keywords: severe asthma, sputum induction, asthma phenotypes, safety

1 University Hospital Centre Zagreb, Clinical Department for Lung Diseases Jordanovac, Zagreb, Croatia

2 School of Medicine, University of Zagreb, Zagreb, Croatia

3 Faculty of Health Studies, University of Rijeka, Rijeka, Croatia

4 University Hospital Centre Zagreb, Clinical Department of Pathology and Citology, Zagreb, Croatia

Introduction

Asthma is a chronic inflammatory disease of the airways that causes variable airflow obstruction and bronchial hyperresponsiveness. Many cells and cellular elements play a role in the pathogenesis of asthma. Cellular quantification in sputum samples is one of the noninvasive methods of assessing asthmatic airway inflammation. Sputum induction with hypertonic saline is used frequently for investigation of airway inflammation in patients with asthma or chronic obstructive pulmonary disease (COPD). The method was introduced in asthma by Pin et al. in 1992 and has been evolving ever since[1]. As it is relatively non-invasive technique which provides repeatable and valid results[2] sputum induction quickly gained an important place in clinical practice. The correlation between "noninvasive" measures such as blood eosinophilia, or fraction of exhaled nitric oxide (FeNO) with sputum eosinophils is suboptimal, making them poor surrogate measures of airway eosinophilia[3]. Based on sputum eosinophil and neutrophil proportions in induced sputum we could categorized four inflammatory subtypes of an airway inflammation in asthma: neutrophilic asthma, eosinophilic asthma, mixed granulocytic asthma and paucigranulocytic asthma[4]. Asthma phenotypes based on different types of airway inflammation allow us to individually tailor treatment. It has been well known for decades that sputum eosinophilia can predict response

to corticosteroid treatment, but the phenotyping of the inflammatory response in asthma has gained even greater clinical significance with the introduction of biological therapy. A great advantage of the technique is that it enables sampling of the airways in a less-invasive manner, in contrast with other methods such as bronchial biopsy, bronchial brushing and bronchoalveolar lavage, all of which require bronchoscopy. This is particularly important in the examination of patients with severe airways disease where endoscopy poses significant risk to oxygen desaturation[5]. These techniques allow sputum to be obtained from 80 to 90% of patients, which is significantly more than patients can expectorate spontaneously. It has been shown that cells in induced sputum reflect well the findings in bronchial wash and lavage samples and are more viable than in spontaneous sputum[6]. Fahy et al. studied markers of inflammation and cells in samples obtained by sputum induction, bronchial washing and lavage samples from healthy and asthmatic subjects. Concentrations of cells and inflammatory markers were higher in induced sputum samples than in bronchial washings or lavage materials. Induced sputum samples contained higher percentages of neutrophils and eosinophils, and higher concentrations of eosinophil cationic protein, albumin and mucin-like glycoprotein, probably because they were less diluted[7]. Induced sputum was first developed as a research tool, and in the meanwhile, it became a valuable clinical tool[8].

Sputum induction procedure

Induced sputum is quite a technically demanding procedure. Each sputum induction should proceed by spirometry, and patients with FEV_1 <40% of predicted, or less than 1 L, have to be excluded from induction procedure.[35] The highest FEV_1 value as well as PEF value obtained were considered as baseline and were used to calculate a relative fall in FEV_1 and PEF during the procedure. Each

subject with severe asthma who is going to do the induced sputum should be premedicated with 200 μg salbutamol. Sputum induction is performed by inhalation of increasing concentrations of aerosolized saline (0.9%, 3%, 4% and 5%) through a mouthpiece without a nose clip. In our clinic we use an Omrone NE U07 ultrasonic nebulizer (Omron Healthcare Europe) with an output of 1.0 mL/min and particle size of 3.5 μm mass median diameter to generate an aerosol. Each concentration should be inhaled over 7 minutes. After each inhalation, the patient should be asked to expectorate into a container for an analysis and FEV_1 or PEF is needed to be measured again. The procedure should be interrupted if dyspnea or wheezing occurred and immediately appropriate treatment should be provided. If there is a fall in FEV_1 or PEF on measurement between different concentrations inhalation of 10-20% versus baseline, the same concentration of saline should be used in the next inhalation interval. If the fall is greater than 20%, the procedure should be terminated.[30] Patients should be instructed to interrupt the inhalation if they need to expectorate (in this case the clock was stopped and inhalation continued after expectoration) or experience dyspnea or wheezing. The volume of the induced sputum should be recorded. Sputum sample should be immediately trasferred into cytological laboratory.

Processing of induced sputum for cytological analysis and differential cell counting

It is recommended to process induced sputum a soon as possible or within 2 hours to ensure optimal cell preservation. Two different approaches can be followed for processing, entire sputum analysis or more often selected sputum plugs method. Selected sputum plugs or „fishing" method means selection of dense viscid portions of samples for analysis with minimising of saliva contamination[9,10].

It is necessary to weight sputum plugs before the homogenisation. Homogenisation is performed by the use of fresh solution of 0,1% dithiothreitol (DTT) that breaks disulphide bonds in mucin molecules and preserves cells morphology. The added DTT volume is 2-4 times of recorded weight of the plugs, tubes with mixture are placed in the shaking water bath on 37°C for 15 minutes to ensure complete homogenisation. It is necessary to stop the effect of DTT and preserve cells morphology by adding buffer solution (PBS) in a volume equal to the sputum volume plus DTT volume.[36]

Filtration of the fluid mixture through 48-52 μm nylon gauze can remove remaining debris and mucus. Next step is recording of the filtrate volume, accessing cell viability and measuring total cell count/millilitre using haemocytometer.

Dissolved induced sputum should be processed as any other liquid cytology sample in cytocentrifuge and centrifuge. Cytospin slides are usually May Grunwald-Giemsa (MGG) stained and used for cell counting. Remaining supernatant can be stored on -80°C for additional analyses[4]. Cytological examination of the slides should be performed under light microscope high power magnifications (400x, 1000x). Adequate are samples with less than 20% of squamous cells counting on 300-500 of all cells. Differential cell count (%) should be calculated on 300-500 non-squamous cells: eosinophils, neutrophils, macrophages, lymphocytes, columnar epithelial cells and mastocytes, if any. Both counts (%) should be recorded in final report[9,10]. On the basis of cell differential counts in induced sputum different inflammatory phenotypes of acute asthma can be divided in four types[4]:

- eosinophilic asthma (EA): >3% sputum eosinophils
- neutrophilic asthma (NA): >61% sputum neutrophils and <3% eosinophils
- mixed granulocytic asthma: >61% sputum neutrophils and >3% eosinophils
- paucigranulocitic asthma: <61% sputum neutrophils and <3% eosinophils

Asthma inflammatory phenotypes

Asthma inflammatory subtypes are characterized by some important clinical differences. Eosinophilic asthma is the most common phenotype. It is very prevalent in individuals with nonsevere disease, and also accounts for approximately 50% to 60% of the total severe asthma population[11]. It has classically been associated with allergic sensitization and a T2-dominant inflammatory response. Eosinophilic group is characterised by the highest degree of airway hyperresponsiveness. Woodruff et al demonstrated that the percentage of eosinophils in induced sputum was independently associated with more severe airflow obstruction and methacholine reactivity[12]. Eosinophilic phenotype of asthma mainly responds well to corticosteroid treatment. Strategies that are based on sputum examination to guide treatment decisions have been effective in improving lung function and decreasing asthma symptoms and exacerbations. Normalization of induced sputum eosinophil counts has been shown to be an effective strategy for preventing severe asthma exacerbations and hospitalizations. Sputum examination can detect an increase in airway eosinophils up to 3 months before the development of a clinical exacerbation[13]. This approach requires frequent sputum analyses and is impractical for routine clinical use in most centres. However, the ability to analyze sputum is necessary in centers dealing with more severe forms of asthma. The effectiveness of a treatment strategy based on assessment of airway inflammation was not as clear in patients with mild asthma, suggests that sputum analysis is not necessary in those with milder asthma that responds well to initial therapy[14].

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Eosinophilic inflammation in the airway mucosa that persists despite the use of high doses of inhaled corticosteroids or systemic corticosteroids is recognized as a separate phenotype, severe asthma. It has been shown that eosinophilic inflammation despite vigorous antiasthma treatment is associated with remodelling of the airways, impaired lung function, and near-fatal asthma attacks[15]. In addition to the classic allergen-mediated Th2 paradigm, innate immune stimuli such as environmental factors, air pollution, weather changes, and viral infections may be capable of eliciting Th2 responses associated with eosinophilia[16]. Recruitment of eosinophils into the airway in allergic asthma is mediated by the coordinated action of cytokines and chemokines including IL-5, IL-13, eotaxins, and the adhesion molecules P-selectin and vascular cell adhesion molecule-1. IL-5 is a critical cytokine for eosinophil generation in the bone marrow, as well as eosinophil recruitment, activation, and survival[17]. The effects of IL-13 include induction of goblet cell metaplasia and increased mucus secretion, increased airway hyperreactivity, and, indirectly, trafficking of eosinophils to the site of tissue injury via chemotaxis[18]. Based on that, sputum eosinophil count provides an effective method to identify patients who will benefit from biological therapy. Drugs targeting specific Th2 cytokines, including monoclonal antibodies against IL-5 and IL-13, have shown a promising effect in the treatment of refractory eosinophilic asthma[19]. Steroid usage in severe asthma could mask the underlying eosinophilic inflammation. There are investigations showing that single sputum measures underestimate the likelihood of asthma classification as eosinophilic phenotype.[37] On some occasions, like decreasing corticosteroid therapy or after antibiotic treatment, it is neccessary to repeat the induced sputum after few weeks, to see if the eosinophils demasking will appear.Asthma in most patients in reality is eosi-

nophilic, due to results of severe asthma patient cohorts.[38]

Neutrophilic inflammation is also a common finding among adults who have persistent asthma symptoms despite inhaled corticosteroid treatment and particularly during asthma exacerbations[20]. Douwes et al. also found that only around 50% of asthma cases was associated with eosinophilic inflammation, and that in most other cases asthma was accompanied by an increase in airway neutrophils and interleukin 8 (IL-8)[21]. Increased neutrophils have been reported in subjects with severe asthma requiring intubation and sudden onset fatal asthma, indicating a role for neutrophils in the most severe forms of asthma[22,23]. The results of the study by Jatakanon et al. indicate that an increase in the number of neutrophils is associated with a greater degree of severity of symptoms[24]. Li et al. reported that a significant proportion of asthma and wheezing illness in both adults and children is associated with neutrophilic airway inflammation and that this pattern is not limited to individuals with severe symptoms[25]. Neutrophils in severe asthma were significantly increased compared with those with mild asthma and healthy controls but not when compared with those with moderate asthma[26]. This raises important and interesting questions regarding the mechanisms and consequences of neutrophilic inflammation, as well as presenting a novel and inviting therapeutic target. Neutrophilic inflammation is most frequently induced by infection or pollutant exposure [27]

The possibility of bacterial infections as a cause of the neutrophilia was evaluated by examining for intracellular bacteria, which is a validated technique that correlates with quantitative microbiology in the detection of respiratory tract infections. Interestingly, intracellular bacteria levels were higher in asthma compared with healthy controls, which may indicate increased exposure of the lower airways to bacteria in asthma. This may

be a consequence of airway impairment and impaired local defence caused by airway mucosal damage in asthma[28].

Induced sputum analysis in research and clinical practice

Sputum induction analysis is non-invasive, but quite a technically demanding procedure. Besides, it has safety concerns, as inhaled hypertonic saline can cause adverse effects such as coughing, vomiting, bronchoconstriction and lung hyperinflation[29]. The mechanism of the effect is unknown but may involve the activation of airway mast cells or sensory nerve endings. This makes the examination uncomfortable for some patient, and monitoring of lung function during the procedure is necessary. The safety of the method was thoroughly assessed in patients with airway obstruction characteristic for asthma[29,30] and COPD[31,32]. Pretreatment with beta-agonists is a routine part of the procedure and is intended to prevent bronchospasm. In our practice some subjects reported adverse events, mostly dyspnea followed by drop in lung function, but after bronchodilator and corticosteroid therapy all recovered. During the sputum induction procedure, the patient must be carefully monitored by the medical staff, patients should be encouraged to expectorate quality and sufficient sample for cytological analysis, for which the procedure is rather demanding and complicated. That makes this procedure time-consuming. For these reasons, it is performed only in specialized respiratory clinics and as a part of research projects.

Induced sputum analysis has brought new insights into innate and adaptive immunity processes in airways, but did not answer all questions[33]. It also allowed investigations in transcriptomics, proteomics, and genomics, which are in progress, hoping to elucidate more about the complexity of inflammation[34].

Conclusion

Induced sputum is a valuable tool for determining the asthma inflammatory phenotypes. Monitoring of airway inflammation provide additional data which enables individual adjustment of treatment to each patient. With the introduction of biological therapy, precise immunological phenotyping becomes even more significant. For this reason, we would recommend that every severe asthma center should be familiar with this method and its use in clinical practice.

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Monitoring and Evaluation of Therapeutic Response in Patients with Severe Asthma on Biologics

Sanda Škrinjarić Cincar[1,2]

Abstract

Five biologics with different mechanisms of action have been available for uncontrolled severe T2 high asthma in clinical practice worldwide for years, and the first drug in a class of new biologics targeting the top of the inflammatory cascades, thymic stromal lymphopoietin (TSLP) is available from the beginning 2022. The results of randomized and real-life studies as well as experience in daily clinical practice confirm that the safety profile of biological drugs is very good. Biologics have a good potential to achieve remission during treatment, but not all patients respond equally well. The effectiveness of all biologics in severe asthma is approximately 60% in the real life conditions. An important task of the clinician is the correct assessment of the therapeutic response to biologics and the evaluation of patients who have a satisfactory response to treatment and those who do not. Therapeutic response to biologics should be assessed individually according to pre-defined goals every 3 to 6 months. In patients with a good response to biological drugs, continuation of treatment and continuous monitoring of efficacy and safety is recommended. If there is no satisfactory response to the initially introduced biologic, switching to another biologic is a rational option. During therapy with biologics, it is necessary to closely monitor the effect on exacerbations and symptoms. Research has shown that improving the overall quality of life is the most important outcome for most patients with severe asthma. Also, one of the most important effects of biological therapy is the possibility of excluding or reducing the dose of corticosteroids in patients who need them for disease control. The effect of biologics on improving lung function is important, but not critical for evaluating the effectiveness of treatment. However, previous reports have not yet provided precise instructions for long-term treatment with biologics in daily clinical practice, and there are questions still need to be answered.

Keywords: severe asthma, biologics, therapeutic response, evaluation

Introduction: a new treatment options for uncontrolled severe asthma

Severe asthma is a disabling disease that accounts for not more than 5% of all asthmatics[1]. Although patients with severe asthma represent a minority of the total asthma population, they carry a majority of the morbidity and healthcare costs. Accurate treatment with biologics in the form of monoclonal antibodies has made it possible to attack certain pathogenic pathways or mechanisms and modifies them in order to control disease. In recent years the treatment of severe asthma with biological drugs in selected patients with T2-high inflammation has become very successful. The development of biological drugs for T2-low asthma was not so successful[3].

Patients with "problematic" asthma should be carefully examined and distinguished from those whose asthma is uncontrolled due to poor adherence and/or poor inhalation technique as well as difficult to treat for other reasons such as uncontrolled asthma triggers and comorbidities. Comorbidities in patients with asthma need to be treated appropriately. Some comorbidities, such as nasal polyps, allergic rhinitis, or atopic dermatitis, are favorably affected by biologics, while others, such as gastroesophageal reflux disease, obesity, and bronchiectasis, do not diminish the therapeutic response to biologics. Before assessing the severity of asthma itself, it is necessary to address all factors that have a potential effect on health status, and also to have them under control during treatment with biologics[1,4].

It is well known that asthma is a heterogeneous disease. Clinical diversity and inflammatory phenotype are reflected as a consequence of the pathogenetic mechanism and histopathological characteristics of asthma. Clinical features such as frequent exacerbations, emergency room visits, hospital admissions, lost days from work, school, or leisure time, poor asthma symptom control, poor lung function and poor quality of life, as well as oral corticosteroid use are characteristics of severe asthma[5]. The clinical features and inflammatory phenotype of severe asthma are currently fundamental determinants for assessing the indication for introduction as well as assessing the effect of treatment with biologic drugs. The phenotyping of severe asthma is today embedded in clinical practice and is used to assess the feasibility of available biologics[6]. The use of monoclonal antibodies against immunoglobulin E (IgE) and interleukin (IL) -5 and recently IL-13/IL-4 has been shown to be efficient and safe in clinical trials as well as in everyday clinical practice[7].

Efficacy and effectiveness are not identical concepts and it is not possible to investigate both in the same type of research. Efficacy can be tested in randomized controlled trials (RCTs) under controlled study conditions. Effectiveness can be tested in real-life trials under real living conditions. Efficacy results show what we can expect in a population that has the characteristics of the examined sample, and effectiveness results show what we have really observed[8]. Both types of research have shown that biologics are very successful in treating patients with severe asthma[1]. Biologics have good potential for achieving remission during treatment, but not all patients are good responders and usually not all criteria for complete remission or good asthma control are met[9]. An important task for clinicians is to properly assess the therapeutic response to biologics and to identify which patients have a satisfactory response to treatment and which do not.

Licensed biologics for uncontrolled severe asthma

A years ago, five biological drugs with different mechanisms of action are available in clinical settings worldwide for uncontrolled severe asthma with high T2. These are: omalizumab (Xolair, Genentech/Novartis), mepolizumab (Nucala, GlaxoSmithKline), reslizumab (Cinqair, Teva), benralizumab (Fasenra, AstraZeneca) and dupilumab (Dupixent, Sanofi/Regeneron). Tezepelumab (Tezspire, Amgen/Astra Zeneca) is available in clinical practice from the beginning of 2022 in the United States of America.

The Institute for Clinical and Economic Review (ICER) analyzes the value of first five biological drugs treating moderate to severe asthma associated with T2 inflammation. The ICER Report suggests that all five approved biologics are effective and safe. Each of the five analyzed drugs significantly reduced the exacerbation of asthma compared with placebo and improved patient quality of life. Treatment with omalizumab and mepolizumab is carried out for the greatest length of time. Thanks to long-term effi-

cacy and safety data from extended studies of key trials as well as experience from everyday clinical practice, the uncertainties associated with these treatments are very small[10].

The EAACI 2021 use the GRADE approach in making recommendations for each biologic both in terms of its use and therapeutic effect assessment[11].

According to the 2021 EAACI Guidelines, a reduction in exacerbations, an improvement in quality of life, a reduction in the use of ICS as well as the use of rescue drugs, and global efficacy can be expected with high certainty in patients treated with omalizumab. Improvement in asthma control is expected with moderate certainty[11]. Real-life studies have shown the efficacy of omalizumab regardless of blood eosinophil status[12,13]. Long-term treatment with omalizumab did not increase the risk of side effects, especially anaphylaxis[14].

The EAACI recommendations for treatment with mepolizumab to reduce asthma exacerbations and to resolve or reduce OCS are strong. The effect of mepolizumab on asthma control, quality of life and lung function in studies was good, but less clear[15,16]. The greatest positive change in symptoms and lung function observed during the first months of treatment with mepolizumab[17]. No serious side effects associated with mepolizumab were reported in real-life studies[18].

According to the EAACI 2021 recommendations, there is high certainty that benralizumab reduces asthma exacerbations and OCS in a subgroup of adult asthmatics with severe asthma with > 150 eosinophils/μL. There is also great certainty for patients treated with benralizumab that asthma control and quality of life will improve[11]. Follow-up of 1,600 asthma patients treated with benralizumab for 2 years did not indicate an increased risk of infections or malignancies, but further long-term follow-up is needed to assess possible risks of eosinophil depletion during benralizumab treatment[19]. Dosage of benralizumab every 8 weeks may also be important for some patients.

A weight-based dosing regimen of reslizumab may provide a good response in cases that have failed to respond to other anti-IL5 biologics. Only reslizumab has to be given intravenously, which may be important for some patients. Very rare cases of anaphylaxis have been reported in clinical studies within the first 20 minutes after reslizumab infusion[20].

Long-term efficacy of dupilumab has been demonstrated in both allergic and eosinophilic phenotypes. The good safety profile of dupilumab is known from previous studies for atopic dermatitis. Dupilumab is well tolerated, but ocular side effects are common[21].

In endemic areas, patients treated with anti IL-5 biologics, should be screened for parasitic infections[11].

Tezepelumab is the first drug in a class of new biologics that targets and blocks thymic stromal lymphopoietin (TSLP), which sits at the top of the inflammatory cascades. The mechanism and site of action of tezepelumab stops the release of inflammatory cytokines at the very source and therefore this drug has the potential to treat a wide population of patients with severe asthma regardless of phenotype. Randomized trials of tezepelumab showed fewer exacerbations, better lung function, better asthma control, and better health-related quality of life in patients with severe asthma who received it[22]. Considering that it has only recently been available in daily clinical practice, the experiences of clinicians are still expected.

Comparison between biologics

The assessment of the therapeutic effect of biologic drugs in severe asthma depends on a number of factors that differed significantly between studies and that can modulate the therapeutic response. In differed studies, there are different inclusion or exclusion criteria related to asthma severity, lung function,

definition of atopic status, eosinophil count, frequency and severity of exacerbations, and duration of asthma[1,11]. Due to this, the expert opinion is that the indirect treatment comparison between these five biologics may be erroneous or biased and should not be done[11]. Studies that directly compare the therapeutic response of approved biologics have not yet been performed.

Key elements for evaluating responses to biological treatment in clinical practice. How and when to evaluate? Estimation of response sizes to biological treatment of severe asthma

The effectiveness of all the biologics in severe asthma is approximately 60% in a real-world setting[23,24,25]. Most of the afore mentioned clinical features of severe asthma represent the elements for assessing the outcomes and clinical benefits from treatment with biologics (Figure 1.). Asthma control includes objective clinical outcomes (lung function parameters, number of exacerbations), but also includes subjective outcomes assessed by the patient, such as symptoms, activity level, quality of life and satisfaction.

Assessments of the therapeutic response could be hampered by disease heterogeneity, comorbidities, complexity of care, and differences in national and regional health systems. The recommendations for assessing the therapeutic response to biologics assumes that the diagnosis of severe asthma is correct and all co-morbidities and factors influencing asthma control are correctly addressed. Baseline asthma severity and duration, dose of oral corticosteroid, atopic status definition, eosinophil cut-off, exacerbation severity and rate history as well as lung function are all important in assessing treatment efficacy or effectiveness[11].

The therapeutic response to biologics should be assessed individually according to pre-defined limits for outcomes focused on the
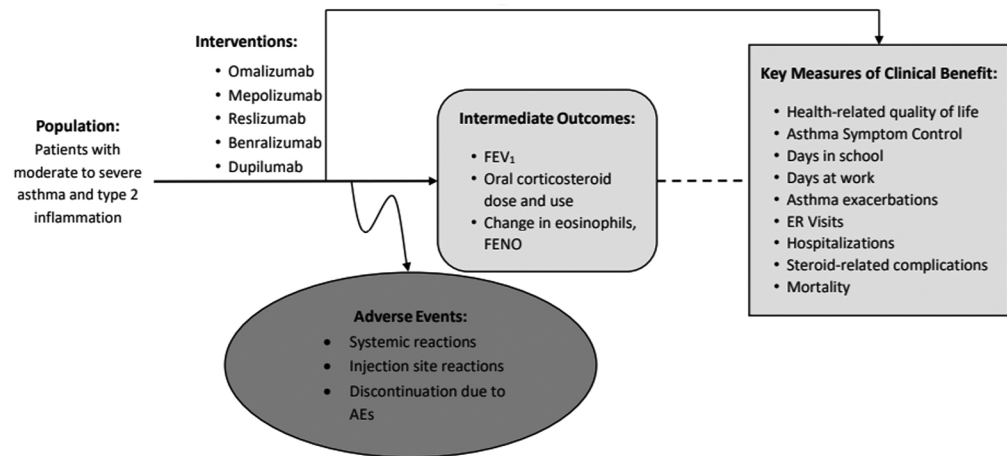

Figure 1. Monitoring of the therapeutic response after the introduction of biological drugs for severe asthma (10) (Taken with permission from *ICER Rewier 2018*).

Note: AEs: adverse effects; FENO: fractional exhaled nitric oxide; FEV_1: forced expiratory volume in one second; SAEs: severe adverse effects

goals of asthma control[11]. All changes since the previous visit should be carefully analyzed in terms of frequency and severity of exacerbations, all elements of asthma symptom control (frequency of symptoms, use of SABA, nocturnal awakening due to asthma, activity limitation), treatment intensity (including OCS dose), quality of life, adherence and inhalation technique, pulmonary function, patient satisfaction, side effects and possible concerns.

GINA and EAACI Guidelines for the treatment of severe asthma with biologics differ slightly about the time period for monitoring the therapeutic response, but agrees that it should be done between 3 and 6 months[1,11]. The GINA 2020 guidelines recommend that the first assessment of the therapeutic response to biologics in severe asthma be made after 4 months. If the response is good, it is recommended to continue treatment with a reassessment every 3-6 months. The GINA 2021 guidelines corrected the recommendation to re-evaluate the therapeutic response every 3-4 months. The EAACI 2021 guidelines recommend an evaluation every 4-6 months.

If therapeutic response to biologics is good and asthma is well controlled, it is recommended to consider reducing and eventually discontinuing oral corticosteroid treatment, then other add-on therapies, and finally inhaled corticosteroids. Oral corticosteroids and all other asthma drugs should be reduced gradually. Inhaled corticosteroids should never be completely ruled out, but at least a moderate dose should be maintained. If after step-down during treatment with biologics there is a loss of symptom control and/or exacerbations reoccur, the therapy should be intensified to the previous dose to re-establish good asthma control[1].

Well-defined criteria for assessing the therapeutic response to biological therapy in severe asthma do not currently exist, but
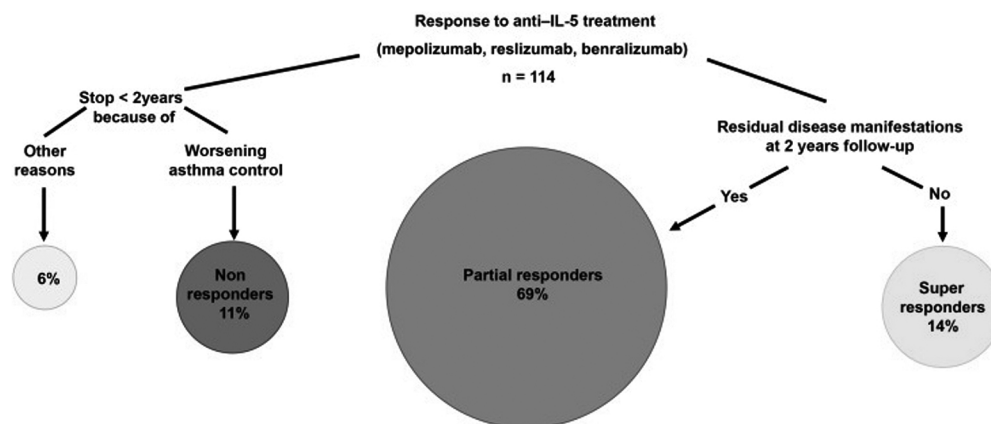

Figure 2. Response to treatment of severe asthma with biologics (all three anti-IL-5 drugs) after 2 years (26) (Taken from *Eger K et al. J Alerg Clin Imunol Pract 2021*)

it is clear that not all outcomes are of equal strength and importance. According to the latest EAACI 2021 guidelines, expected outcomes are classified into three groups. The most critical outcomes are considered to be exacerbation of severe asthma, control of asthma symptoms assessed using the ACQ (Asthma Control Questionnaire) or ACT (Asthma Control Test), quality of life measured by the quality of life of the Asthma Questionnaire

(AQLQ) and safety. Lung function, particularly FEV_1 (forced expiratory volume in the first second) and dose reduction of OCS and ICS, as well as the use of rescue drugs are considered important outcomes, while FeNO and eosinophils in sputum and blood are considered less important. The EAACI Guidelines Development Group (GDG) for the treatment of severe asthma with biologics has formulated strong recommendations regarding dose reduction of OCS and conditional recommendations with respect to other outcomes[11].

In the observational cohort study by Eger and coworkers, 11% of patients were considered as non-responders, 69% as partial responders, and 14% as super-responders after 2 years of anti–IL-5 treatment for severe eosinophilic asthma[26].

In patients with a good response on biologics to individually predetermined goals, continued treatment is recommended, in accordance with local regulatory authorities and continuous monitoring of effectiveness and safety. The rationale for this recommendation is the evidence that after discontinuation of biologics, their beneficial effect is lost[11]. A few clinical studies to date have shown that in many patients, after discontinuation of biologic therapy, symptom control deteriorates and / or exacerbations recur[27,28]. So far, there are no precise instructions on how long treatment with biologics should last.

If there is no therapeutic response to biologics, the clinicians are advised to find possible reasons. Uncontrolled asthma, after applied biological therapy, requires verification of adherence because some patients, following first few administrations of the biologics, stop taking anti-inflammatory medications without consulting their health care physicians and become non-adherent to the overall management plan.

If there is no satisfactory therapeutic response to the initially introduced biological drug after sufficient time, but the criteria for targeted biological treatment of severe asthma

are still met, switching to another biological agent is a rational option.

Studies have shown that switching to another biologic drug can have a significant effect on improving FEV1, controlling asthma symptoms, and reducing OS in patients with an initially poor response to a previous biologic drug[25,29,30]. The exact time and manner of switching from one biological drug to another have not yet been defined. More precise recommendations in this regard are expected from large ongoing studies aimed at switching biologic drugs. In a report by Numate et al, an analysis of switching from one biological drug to another in randomized studies (omalizumab to mepolizumab, mepolizumab to benralizumab, all three biologics from the IL5 / IL-5 receptor group to dupilumab) showed efficacy. Most reports suggested switching to another drug after approximately 4 months. In real-life studies, the effectiveness of biologics stabilized after 16 weeks in 80% of cases and within 24 weeks in 90% of cases. The median time to change the first biologic in subjects who did not respond to treatment was after 8.6 months and for the second after 2.7 months. In clinical practice, if justified, the large number of different biologics available for severe asthma makes it possible to reduce switching interval to each subsequent biologic treatment[25].

A sustained suboptimal therapeutic response to a biological drug requires re-phenotyping and re-examination of biomarkers and immune response pathways. Exacerbations in patients who do not respond to biologic therapy do not necessarily have to be eosinophilic and the type of exacerbation cannot be inferred without confirmation. The inflammatory phenotype of asthma exacerbations may be distinguished using FeNO[31], but induced sputum is a more desirable option for reassessing whether airway inflammation is eosinophilic or neutrophilic. If the response to treatment is unsatisfactory and a reassessment shows that there is no airway eosino-

philia, biologics should be discontinued and T2 low asthma treatment measures should be considered[11].

It is important to keep in mind that biologics may induce the production of antibodies against drugs (ADAs) that may affect the loss of a therapeutic response or hypersensitivity reaction. The measurement of ADA in everyday clinical practice has not yet been implemented. As the detection of ADAs is essential for immunogenicity assessment, future tasks is to determine how and when to routinely measure them in clinical practice[32]. If the reason for non-response is the development of neutralizing anti- drug antibodies[33,34] or other dysfunctions and autoimmune response, it is justified to switch to another biological drug following the specific characteristic[35]. Possibilities of combining two biological drugs with different mechanisms were also considered but the rationale for such use is still lacking.

Effect of biologics on exacerbations

Asthma exacerbations are the most undesirable adverse event that can occur during illness and that can be life-threatening to the patient. Such exacerbations in patients with severe asthma are frequent and significantly impair their quality of life. According to EAACI 2021 recommendations, the impact on exacerbations of severe asthma during biologic treatment are among critical, the most important outcomes for assessing treatment success[11]. Most studies, including a number of new ones, report that biologics significantly reduce the number and severity of exacerbations in patients with severe asthma[15–20,23,24,28, 36–39]. The results of a recently published MEX study call into question the routine use of oral corticosteroids to treat all asthma exacerbations without recognizing an inflammatory phenotype of asthma exacerbations which are not always eosinophilic[31].

The effect of biologic therapy on exacerbations should be closely monitored during the therapy with biologics. In some patients, exacerbations may not decrease significantly in a short period of time, so the other endpoints should be used to define a therapeutic response[11,40].

Effects of biologics on symptoms and quality of life

Symptoms of patients with severe asthma that remain uncontrolled despite the maximum intensity of treatment are the most obvious indicator of the severity of the disease if all other measures are taken to exclude the factors responsible for uncontrolled disease. Given the subjectivity, they need to be assessed with questionnaires. A clinically significant smallest difference in the ACT score is considered to be three points[41]. The sum of symptoms in severe asthma is usually very low, and it is difficult to assess the actual improvement based on differences in the ACT score. ACT has known limitations in severe asthma. It is recommended to reduce the ACT cut-off for uncontrolled asthma in severe asthma to score 16[42]. Improvement of symptoms during the treatment of severe asthma should be assessed by their qualitative and quantitative characteristics as well as by their timing, location, aggravating or alleviating factors, and associated manifestations. Of particular importance is the assessment and treatment of co-morbidities as they may contribute to poor disease control by aggravating or mimicking symptoms of asthma[4].

Symptoms greatly impair quality of life associated with health status (HRQoL) in patients with severe asthma, and the use of biologics has proven promising in this sense. More than 60% studies, dealing with the treatment of severe asthma included a HRQoL questionnaire as a primary, secondary, or research outcome[43]. Research has shown that improving overall quality of life is the most important outcome for most patients with severe asthma (44).

Safety

The results of randomized and real-life studies as well as experience in everyday clinical practice confirms that the safety profile of all five biologics is very good. Side effects during treatment with biologics in clinical studies were mild in most cases for all approved biologics. Sometimes they did not differ from the side effects seen in patients receiving placebo. The most common side effects were lower respiratory tract infection, nasopharyngitis, sinusitis, worsening of asthma, headache, pain or reaction at the injection site and arthralgia[14–21,36–39].

Common immediate side effects after administration are local pain and discomfort at the injection site. Very rarely, anaphylactic reactions occur during administration, which is why drugs should be given under the supervision of health professionals and patients should be monitored for some time after administration[14]. Reports of such adverse reactions state that they have been successfully treated.

Effects of biologics on corticosteroids treatment

One of the most important effects of biologic therapy is the possibility to exclude or reduce the dose of corticosteroids in patients who need them for disease control. The consequences of long-term of systemic corticosteroids use are widely recognized. Price and coworkers recently investigated that patients with asthma prescribed oral corticosteroid (OCS) had a significantly increased risk of osteoporosis and osteoporotic fracture, pneumonia, cardio and cerebrovascular diseases, cataract, sleep apnea, renal impairment, depression and anxiety, type 2 diabetes and weight gain[45]. The short courses of systemic corticosteroids are much safer, but are still associated with increased risk of adverse events[46]. In project ROSA the majority physicians have a favorable perception towords using biological agents whenever patients are eligible

and the most of them are more willing to accept some degree of lung function deterioration compared to other outcomes (worsening of symptoms, quality of life) when reducing OCS dose[47]. If the patients show a good response to biologics, it is recommended to consider reducing OCS carefully and gradually. Reduction of corticosteroids in cases of a good response to biologics should be gradually assessed at intervals of several months. Studies have shown that it sometimes takes a long time for a dose of corticosteroids to be significantly reduced or completely ruled out[48].

Allowing a reduction in OCS therapy by half or complete exclusion is the main criterion for response to treatment. It is also recommended to try to stop taking other additional medicines, but to maintain a medium dose of inhaled corticosteroid at all times.

According to the new GINA guidelines, the introduction of a biologic is recommended before the use of systemic corticosteroids in order to prevent their side effects. In patients with uncontrolled severe asthma who are not eligible for biologics or those who do not respond to biologic therapy, OCS treatment is still an important alternative to achieving control. Patients who do not respond to biologics may also not respond to systemic corticosteroids[1].

Effects of biologics on lung function

Severe asthma that is refractory to treatment usually significantly affects lung function and the use of biologic drugs often significantly improves it. In some patients, the changes may be permanent due to airway remodeling and result in fixed airway obstruction.

Pulmonary function in patients with severe asthma is very important, but during biologic treatment a certain degree of impairment of pulmonary function is considered more acceptable compared to other outcomes such as exacerbations, symptoms and quality of life if oral corticosteroids may be excluded or reduced[47].

According to EAACI Guidelines, the effect of biologic drugs on the improvement of lung function is an important but not critical outcome for assessing the effectiveness of treatment[11].

Effects of biologics and biomarkers

According to the Working Group of the National Institute of Health (NIH), a biomarker is defined as "… a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". Simply, a biomarker indicates an alteration in physiology from normal[49]. Ideally, a biomarker might be the pathophysiological therapeutic target itself. Identification of specific biomarkers such as peripheral blood absolute eosinophil count, total IgE, specific IgE and fractional exhaled nitric oxide (FeNO) in biological materials allowed precise treatment of patients with severe T2 high asthma. Biomarkers indicate certain asthma endotypes and predict responses to biological therapies[5]). During treatment with biologics, a reduction or complete eosinophil depletion is observed which is usually accompanied by a good clinical response to treatment, but this response to biomarkers is considered a less important outcome than other clinical outcomes. Routine monitoring of IgE levels is not recommended during omalizumab treatment[1,9]. In many patients with T2 high asthma, known biomarkers overlap as shown in Figure 2. Overlapping of biomarkers often leads to doubts about drug choice and existing biomarkers are often considered insufficient for a precise decision about the best biologic to administer. In the current situation, the overlapping of known biomarkers, in case of poor response to the initial biological drug allows switching to another biologic that targets another capture point, which often results in a good response. In studies that looked at the association between the efficacy of biological therapy and predictive bio-

markers, there was a significant difference in response rate between the groups of biologics depended on the biomarker on which the drug was selected. When the biologics were selected based on peripheral blood eosinophil counts (PBEC), IgE and FeNO the response rate was 33% (PBEC), 36% (IgE) and 50% (FeNO) for omalizumab, 65% (PBEC), 67% (IgE) and 64% (FeNO) for mepolizumab/benralizumab and 64% (PBEC), 50% (IgE) and 73% (FeNO) for dupilumab[25]. One of the goals of future research is to identify better clinically relevant biomarkers in terms of patient selection and prognosis of therapeutic response to biologics, and which will better reflect the clinical response during treatment.

Conclusion

All previous clinical studies as well as experiences from everyday practices have shown that biologics in severe asthma are highly efficient and safe in precisely selected patients with severe T2 high asthma. However, all patients treated with biologics are not good responders and there are still many doubts and unknowns regarding the treatment.

It is estimated that the effectiveness of biologics on severe asthma in real world settings is about 60%[11]. For now, there are no clearly defined criteria for assessing the effectiveness of biologics in everyday practice. Patients treated with biologics should be closely monitored and evaluated against baseline and against pre-defined treatment outcome goals. The critical outcomes of treatment with biologics are considered to be the impact on reducing exacerbation, improving symptoms and quality of life as well as the safety of biological therapy. Important treatment outcomes are considered to be the effect on reducing the intensity of treatment (oral and inhaled corticosteroids, recue medications) and the effect on improving lung function[11].

Biologic drugs allow specific inhibition of certain asthma pathways, which does not always meet all set treatment goals equally suc-

cessfully[51]. Previous reports on therapeutic responses have not provided accurate answers to a number of questions about long-term biological treatment in daily clinical practice. Precise definition of therapeutic response, criteria for optimal and suboptimal response, criteria for continuation or discontinuation of biologics, duration of biological treatment in patients who responded to therapy, rules for switching to other biological drugs in patients who did not respond to therapy, rules for combining biological drugs and the biological treatment of allergic comorbidities are just some of the questions that need to be answered.

It is also necessary to clarify issues related to the identification of factors associated with treatment failure and the possibility of increasing the therapeutic response rate. In particular, there is a great need to identify new molecular targets in order to offer effective treatments for those patients who do not respond to currently available biologics[11].

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Contributors

Assoc. Prof. Ivan Čekerevac, MD, PhD

Ivan Čekerevac was born in Čačak in 1970. He graduated from the Faculty of Medicine, University of Kragujevac and earned a phD degree at the age of 40. He has been educated abroad on several occasions such as Germany and Hungary. He is a full professor of the Faculty of Medicine Sciences, University of Kragujevac. He has published more than 80 publications, of which 25 cited in international databases, and more than 160 times at the Scopus base. He co-authored 3 books and main author of the book Basics of echosonography, chapter named Thoracic ultrasound and COPD and systemic inflammation. He has given invited lectures at international meetings. Special interest in asthma, chronic obstructive pulmonary disease, obstructive sleep apnea and non-invasive ventilation and thoracic ultrasound and ergospirometry.

Valetnina Fiket, bacc. med. techn.

Valentina Fiket was born on May 25th 1977 in Zagreb, Croatia. After finishing Medical high School she started her career at ICU department at the Special Hospital for chronic pulmonary diseases and later in the ICU unit of the Thoracic surgery, University Hospital Centre Zagreb. During that time she finished University of Applied Health Sciences and obtained the title "Bachelor of Nursing" (2001). After that she has been working at Pulmonary Disease Clinic at different departments such as Chemotherapy Unit, Pulmonary department, including Outpatient units. Her current position is a Head nurse of Outpatient Unit Clinic for Thoracic surgery. For the past few years she has actively participated in several clinical trials and acquired knowledge in other European countries since last five years. She has been a the pioneer nurse at enforcement of induced sputum in Croatia on Clinic for Pulmonary Diseases, Zagreb.

Assoc. Prof. Ivan Kopitović, MD, PhD

Professor of Internal Medicine at University of Novi Sad – Faculty of Medicine. Specialist in Internal Medicine and subspecialist in Pulmonology at Institute for Pulmonary Diseases of Vojvodina (IPDV) – Sremska Kamenica, Serbia. He has been working for more than two decades in ICU, HDU, lung function testing unit and sleep lab (sleep disordered breathing - SDB). He is a consultant in the

field of asthma, COPD, critical care medicine and SDB. He is an author and coauthor of monographic publications, book chapters and more than 250 papers published in national and international journals. He has been a principal investigator in clinical trials related to obstructive lung diseases, PTE, PAH, SDB. He is currently a Head of Department for Lung Pathophysiology with Sleep Medicine Lab at IPDV.

Prof. Mitja Košnik MD, PhD

He graduated at the Medical Faculty, University of Ljubljana in 1987. In 1992 he finished a specialisation of the internal medicine, latter he was nominated as a specialist in pulmonology and allergology. He earned a Ph.D. degree in 1998. In 2012 he was nominated as a full professor of internal medicine. He is a medical doctor and a head of the Department of Clinical Research at the University Clinic of Respiratory and Allergic Diseases Golnik and a head of the Chair of Internal medicine at the Medical Faculty, University of Ljubljana. His research interests are anaphylaxis, venom and drug allergy. He is a project manager of a national research project P3 − 0360: Slovenian network of allergy and asthma: from epidemiology to genetics. He was a mentor of 8 Ph. D. students. He is a president of Slovenian Association of Allergology and Clinical Immunology and od Slovenian Respiratory Society, a member of State expert body of internal medicine and a member of UEMS, section allergology. He published over 150 articles in journals with impact factor, his H-index is 42 and has 10.000 pure citations.

Assist. Prof. Marina Lampalo, MD, PhD

Marina Lampalo was born in Šibenik, February 1st, 1975, graduated from The University of Zagreb School of Medicine in 2001. She has been working as an internist since March of 2008, and as a subspecialist in pulmonology at Jordanovac Clinic for Pulmonary Diseases since 2010. In March of 2019, she obtained the title of Primarius. She earned the PhD title by defending her dissertation „The effect of ABO blood genotypes and tissue plasminogen activator inhibitor on lung ventilation in asthma" in 2017. She has been actively participating in numerous national and international scientific and professional meetings as an invited lecturer. Thus far, she has published multiple scientific and professional papers. She is also the author of various chapters in books on pulmonology. She received the Toraks Award for Best Scientific Work in 2016. Since 2019 she has been a research associate at the School of Medicine, University of Zagreb. In July of 2022, she was awarded the Title Assistant Professor, in recognition of scientific and teaching merits. The specialty area she has been dealing with for many years is obstructive lung diseases - primarily asthma, chronic obstructive pulmonary disease, as well as lung function and allergic diseases.

Assoc. Prof. Zorica Lazić, MD, PhD

Dr. Zorica Lazic graduated from Medical faculty at the University of Belgrade (1981). She has been the specialist in internal medicine and pneumonology since 1989 and 2005 respectively. Dr Lazic earned the MSc degree (1993) at the University of Belgrade and the PhD degree at the University of Kragujevac (1996). She

was appointed as a Professor of internal medicine at the Faculty of Medical Sciences, University of Kragujevac (2006). She has held several senior positions at the University Clinical Centre Kragujevac, including the Director of Clinic for Pulmonary Diseases from 1992 until 2022, when she retired. She has been involved in many educational programs covering topics including asthma, COPD, and interstitial lung diseases. She has participated as a guest lecturer at many national and international conferences throughout her career. She has been involved in drafting national guidelines for treatment of Asthma and COPD in Serbia. Prof. Lazic has been a member of various medical associations including the ERS, CHEST, ATS, Respiratory Society of Serbia, and Fellowship American College of Chest Physicians (FCCP). She was active as the National Lead of the Severe Heterogeneous Asthma Research collaboration, Patient-centred (SHARP) ERS Clinical Research from 2019 to 2022.

Assoc. Prof. Gordana Pavliša, MD, PhD

Gordana Pavliša, MD, graduated in 1997 from the University of Zagreb, School of Medicine. She is a specialist in internal medicine (2007), pulmonology (2009) and intensive care medicine (2020). In 2006 she got her PhD degree at the University of Zagreb with the the topic of research on erythropoietin, angiogenic factors and inflammatory cytokines in severe hypoxemia in COPD patients. She is a professor of internal medicine at the University of Zagreb, School of Medicine. From 2011 to 2022, she was the head of the Intensive Care Unit at the Clinic for Respiratory Disease, UHC Zagreb. Her primary area of interest is obstructive pul-

monary disease, respiratory infections, acute and chronic respiratory failure. From 2020-2022. she was the coordinator of the COVID-19 departments of UHC Zagreb. Since 2022, she has been working at the Department for Allergic and Obstructive Diseases. She is a member of the ERS Severe Heterogeneous Asthma Research Collaboration.

Assoc. Prof. Sanja Popović-Grle, MD, PhD

Sanja Popović-Grle is a Professor of Respiratory Medicine at University of Zagreb, Croatia, and Chief of Department for allergic and obstructive lung diseases at University Hospital Centre Zagreb. After obtaining her Doctor of Medicine degree from the University of Zagreb in 1983., prof. Grle started her professional career in the central state Pulmonary hospital Jordanovac in Zagreb, as an employee from the Medical faculty, followed obligatory praxis for 2 years as a GP. Prof. Grle main research interest is in the field of asthma, allergy, and COPD, especially in diagnostic methods and management. She has published papers in peer-reviewed journals and book chapters for students, residents, specialists or patients, and held many educative lectures on the topic. In addition to the clinical duties, prof. Grle was involved in scientific projects and trials. She has been Member of Organizing and Scientific Committees of many professional meetings and congresses.

Dina Rnjak, MD

She was born on the 11th of December 1989 in Osijek, Croatia. She graduated from the School of Medicine in Osijek in 2014. She has been a specialist in pulmonology since 2021 at University Hospital

Centre Zagreb, Clinic for Lung Diseases. Currently, she works as a pulmonologist in Intensive and postintensive care unit. She is an author of many abstracts and articles, mainly in field of asthma and interstitial lung diseases. She is a member of Croatian Thoracic Society, Croatian Pulmonology Society and European Respiratory Society.

Ivana Rubil

She was born in Zagreb, Croatia on April 27th 1979. From 1999. She started with her career in 1999 as a nurse on ICU on Clinic for lung diseases Jordanovac. She has been active participant on many international and domestic congresses. After enrolment the University for baccalaureate in 2000 (graduated with B grade) she enrolled the Masters study in Zagreb and got the title Master of nursing in 2003. Currently she is a head nurse of day hospital on Clinic for lung disease Jordanovac, University hospital center Zagreb. She has been a secretary of patient association Udah Života (Breath of Life) since 2020 , which promotes and raises awareness for a population health. Its important point is rising the quality of patients life through education of patients and their families.

Assist. Prof. Silvana Smojver-Ježek, MD, PhD

Head of Clinical Unit of Pulmonary Cytology, Department of Pathology and Cytology, University Hospital Center Zagreb, Croatia. Graduated on Medical School Zagreb, University of Zagreb. Specialist in medical/clinical cytology, Laboratory for Cytology, University Hospital for Lung Diseases Jordanovac, Za-

greb. 2004. to now: Head of Laboratory – Clinical Unit of Pulmonary Cytology. Assistant professor on Postgraduate Studies, Medical School Zagreb, University of Zagreb. Teacher on University of Applied Health Studies, Zagreb. Member of Croatian Medical Association, Croatian Society for Clinical Cytology (co-vice president), Croatian Respiratory Society. Member of the Organizing Committee of Croatian Congress of Clinical Cytology, Toraks Congress, co-organizer of the 37th European Congress of Cytopathology 2012., invited speaker. Author of 150 scientific and professional papers, book chapters, poster and oral presentations dedicated to lung, mediastinum and pleural cytology.

Assist. Prof. Maja Šereg Bahar, MD, PhD

Maja Šereg Bahar has finished study of medicine at University of Ljubljana (UL), Slovenia. She obtained her MSc (2006), and PhD (2015) degree at the same university. Since 1996 she has been employed at the University Medical Centre Ljubljana, Department of ORL & HNS in Ljubljana, Slovenia. She finished her training in otorhinolaryngology and started working also at Faculty of Medicine, UL in 2002. At the moment she works in the Center for Voice, Speech and Swallowing Disorders at the University hospital as a phoniatrician and in the Department of Otorhinolaryngology at Faculty of Medicine, Ljubljana, Slovenia. She is the author of more than 120 published papers.

Assoc. Prof. Sanda Škrinjarić-Cincar, MD, PhD

Assoc. Prof. Sanda Škrinjarić-Cincar was born in 1959 in Osijek. She has many

years of work experience as a pulmonologist at Clinical Medical Center Osijek and as a teacher at the School of Medicine, University of Osijek. She held the position of head of the Department of Pulmonology until 2020. She is currently employed at the Osijek Health Center. Her special area of interest is obstructive pulmonary diseases. She participated as the main researcher in numerous clinical studies, published a large number of scientific and professional papers and is one of the authors of the Croatian guidelines for asthma. From the very beginning of the biological treatment of severe asthma in Croatia, she participated in the assessment and follow-up of patients with severe asthma and established the Severe Asthma Team within the Department of Pulmonology of Clinical Medical Center Osijek.

Assist. Prof. Sabina Škrgat MD, PhD

Sabina Škrgat, MD, graduated from the Faculty of Medicine at the University of Ljubljana (1996). She is a specialist of internal medicine (2004) and pneumonology (2011). She got her PhD degree at the University of Ljubljana (2009) with the topic of investigation, related to angiogenesis and complement activation in asthma and chronic obstructive pulmonary disease. Her main clinical work consists of management of patients with severe asthma and other obstructive lung diseases. She is an Assistant professor at Medical Faculty of Ljubljana and she curently has a leading position in Slovenian National recommendations for asthma management. She is the clinical lead of Severe asthma Clinic at University Medical Centre Ljubljana, Slovenia. Beside clinical work, she is active in research of severe asthma by PhD mentoring. She is also the ERS SHARP (Severe Heterogenous Asthma Research Collaboration) National lead. Dr. Škrgat is a member of Slovenian Respiratory Society steering committee and a member of SHARP steering committee since 2021. She launched the first Severe asthma forum-joint meeting of South East Europe which was held in Bled, Slovenia in 2018.

Anamarija Štajduhar, MD

She was born in 1989 in Zagreb, and graduated from the School of Medicine, University of Zagreb in 2014.After graduation, she worked in the family medicine offices until 2016. She became a pulmonologist in 2021, and she has been working at the Clinic for Pulmonary Diseases Jordanovac. Currently she is PhD student at the Faculty of Medicine, University of Zagreb, under the mentorship of prof. S. Popović - Grle. She has been participating in the implementation of medical studies in English (School of Medicine, University of Zagreb) since 2023. Her particular field of interest are obstructive lung diseases, she is a member of the Croatian Thoracic Society, the Croatian Pulmonology Society, ERS and EAACI as well.

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Conflict of Interest
Authors have no relevant conflicts of interest to declare in relation to the content of this monograph.

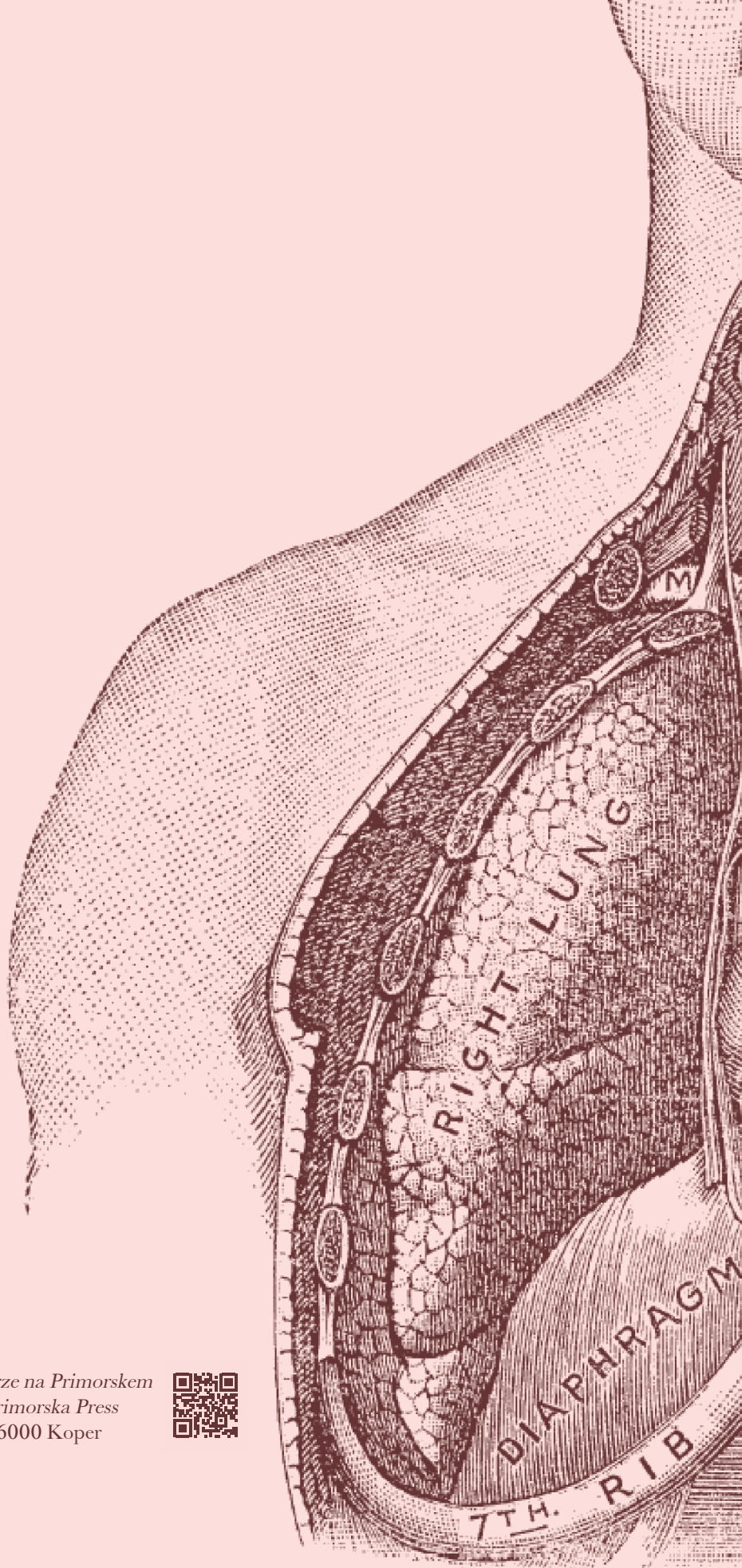**Assist. Prof. Sabina Škrgat, MD, PhD**

UNIVERSITY MEDICAL CENTRE LJUBLJANA
FACULTY OF MEDICINE, UNIVERSITY OF LJUBLJANA

Sabina Škrgat, MD, graduated from the Faculty of Medicine at the University of Ljubljana (1996). She is a specialist of internal medicine (2004) and pneumonology (2011). She got her PhD degree at the University of Ljubljana (2009) with the topic of investigation, related to angiogenesis and complement activation in asthma and chronic obstructive pulmonary disease. Her main clinical work consists of management of patients with severe asthma and other obstructive lung diseases. She is an Assistant professor at Medical Faculty of Ljubljana and she curently has a leading position in Slovenian National recommendations for asthma management. She is the clinical lead of Severe asthma Clinic at University Medical Centre Ljubljana, Slovenia. Beside clinical work, she is active in research of severe asthma by PhD mentoring. She is also the ERS SHARP (Severe Heterogenous Asthma Research Collaboration) National lead. Dr. Škrgat is a member of Slovenian Respiratory Society steering committee and a member of SHARP steering committee since 2021. She launched the first Severe asthma forum-joint meeting of South East Europe which was held in Bled, Slovenia in 2018.