Ph.D. Thesis

MODERN MINIMALLY INVASIVE DIAGNOSTIC AND THERAPEUTIC OPTIONS FOR THE CHRONIC INFLAMMATION MEDIATED DISEASES OF THE AIRWAYS

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LIST OF PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

I. Zs. Bella, A. Torkos, L. Tiszlavicz, L. Iván, J. Jóri: Cholesterol granuloma of the maxillary sinus resembling an invasive, destructive tumor. *Europian Archives of Oto-Rhino-Laryngology*, 262(7), 531-533, 2005. **IF: 0,895**

II. L. Rovó, M. Széll, **Zs. Bella**, A. Korsós, L. Kemény, J. Jóri: The -509 C/T genotype of TGFβ1 might contribute to the pathogenesis of benign airway stenosis. *Otolaryngology-Head and Neck Surgery* 142(3), 441-3, 2010. **IF:1,463(2009)**

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IV. Kadocsa E., Koreck A., **Bella Zs**., Csoma Zs., Ignácz F., Alexa M., Dobozy A., Jóri J., Kemény L.: Intranazális fototerápia: új terápiás eljárás allergiás rhinitisben *Fül-Orr-Gégegyógyászat* 52(2), 108-114, 2006.

V. Bella Zs., Kiricsi Á., Viharosné D-R. É, Dallos A., Kiss M., Kemény L., Jóri J., Kadocsa E. Rhinofototerápia perzisztáló allergiás rhinitisben-Humán, randomizált, kettős vak, placebo kontrollált, prospektív, előzetes vizsgálat. *Fül-Orr-Gégegyógyászat* 56(2), 107-115, 2010.

VI. Zs. Bella, E. Kadocsa, L. Kemeny, A. Koreck: Narrow-band UVB phototherapy of Nasal Polyps: Results of a Pilot Study. *Journal of Photochemistry and Photobiology B: Biology* 100(3), 123-127, 2010. Available online 2 June 2010. **F:1,871**(2009)

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Publications published related to the topic, but not used in the PhD procedure:

D. Mitchell, L. Paniker, G. Sanchez, **Zs. Bella,** E. Garaczi, M. Szell, Q. Hamid, L. Kemeny, A. Koreck.: Molecular response of nasal mucosa to therapeutic exposure to broad-band ultraviolet radiation. *J Cellular and Molecular Med*, **14**, 313-322, 2008 IF: 5,228

Abstracts in reviewed papers:

Zs. Bella, L. Rovó, L. Tiszlavicz, M. Marek, J. Czigner: A combination of endoscopic laser surgery and segment resection for the treatment of cricotracheal stenosis. *European Surgical Research*, 34, Suppl. , 2002

A Koreck, A Szechenyi, A Cimpean, **Z Bella**, E Garaczi, M Raica, I Rasko, L Kemény**:** DNA Damage and Repair in Nasal Mucosa After Rhinophototherapy. *Allergy* 62 (Suppl.83): 167-551, Nr. 636, 2007.

Z. Bella, E. Kadocsa, J. Jóri: Rhinophototerapy in allergic rhinitis-clinical experiences. *Allergy* 62 (Suppl.83): 167-551, Nr. 635, 2007.

Zs. Bella, E. Kadocsa, J. Jóri: Rhinophototerapy in allergic rhinitis. Eur Arch Otorhinolaryngology 264 (Suppl 1), S265-S354, 2007.

E. Garaczi, **Zs. Bella**, M. Boros-Gyevi, E. Tóth, Zs. Csoma, L. Kemény and A. Koreck: Intranasal phototherapy is more effective than fexofenadine hydrochloride in the treatment of seasonal allergic rhinitis. *World Allergy Organization Journal*, p S76, 2007.

Zs. Bella, E Kadocsa, L Székely, J Jóri: Rhinophototherapy in grass pollen induced allergic rhinitis *World Allergy Organization Journal*: p S77, 2007.

Z. Bella, E. Kadocsa, A. Koreck, L. Kemeny**:** UV Phototherapy of Nasal Polyps: Results of a Pilot Study. *Otolaryngology - Head and Neck Surgery*, 139 (2) P170, 2008.

M. Desrosiers, **Z. Bella**, A. Javer, S. al-Mot, L. Kemeny, A. Koreck, Q. Hamid: Ultraviolet Light: A Potential New Therapy for Nasal Polyps *Journal of Allergy and Clinical Immunology* 121(2), Supplement 1, S264, 2008.

E.Kadocsa, **Zs. Bella**, G.Jakab: Evaluation of specific nasal allergen provocation test in patients with ragweed pollenosis. *Allergy* 64 (Supplement90), 179-538, No. 511, 2009.

Zs. Bella, A.Kiricsi, E.Viharosne Dosa Racz, E. Kadocsa**.:** Rhinophototerapy in persistant allergic rhinitis: Randomised double-blind placebo-controled study. *Allergy* 64(Supplement90:), 179-538, No. 1186, 2009.

Rethy L., Kadocsa E., **Bella Z.,** Kiss M., Garaczi E., Kemeny L., Benson M.,: Old molecules as new markers for poor response to local steroid treatment in ragweed-induced allrgic rhinitis. Allergy 65 (Supplement 92), 683, No1863, 2010.

Zsiros O., Kadocsa E., **Bella Z.,** Tiszlavicz L., Nemeth I., Jóri J., Kemeny L.,: The antiproliferativ effect of the UV phototherapy on nasal polyps- in vitrohystopathological examination. Allergy 65 (Supplement 92), 631, No1716, 2010.

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ABBREVATIONS:

AGS:	anterior glottic stenosis
AR:	allergic rhinitis
CD4+:	a major classification of T lymphocytes, referring to those that carry the CD4 antigen; most are helper cells, also called CD4 T lymphocytes.
CI:	confidence interval;
COPD:	chronic obstructive pulmonary disease,
CRS:	chronic rhinosinusitis
DNA:	deoxyribonucleicacid
E:	evening
ECP:	eosinophil cationic ptotein
FGF:	fibroblast growth factors
GM-CSF:	granulocyte-macrophage colony-stimulating factor
ICAM-1:	Intercellular Adhesion Molecule 1
IL:	interleukin
M:	morning
mUV/VIS	mixed ultraviolet and visible light
NB-UVB:	narrow-band- UVB wave lenght: 305-315nm
NER:	nucleotide excision repair
NI:	nasal itching
NIPF:	nasal inspiratory peak flow
NKFP:	Nemzeti Kutatási és Technológiai Hivatal
NO:	nasal obstruction
OR:	odds ratio;
PAR:	persistent allergic rhinitis
PAS-AK:	periodic acid-Schiff –alkalic blue
PCR-RFLP:	polymerase chain reaction restriction fragment length polymorphism
PUVA:	psoralen (P) and ultraviolet A (UVA)
R:	rhinorrhea
RANTES:	Regulated on Activation, Normal T Expressed and Secreted
RL:	Rhinolight® intranasal phototherapy
S:	sneezing
SNP:	sinle nucleotid polimorphism
SZTE:	University of Szeged
TGFβ1:	transforming growth factor β1;
TNS:	total nasal score
UVA:	ultraviolet-A
UVB:	ultraviolet -B
UVDE:	ultraviolet DNA endonuclease
VCAM-1:	vascular cell adhesion molecule-1
VIS:	visible light
VLA-4:	Very Late Antigen-4
WT:	wild type
XeCl:	xenon chlorid

INTRODUCTION AND AIMS:

Inflammatory diseases of the airways are illnesses of high incidence affecting large populations, bearing great social and economical importance by having an impact on workplace and at-home activities as well as the quality of life. Anatomical structures such as the nasal cavity, the larynx, the trachea and the lower airways taking part in breathing contribute to ensure physiological breathing as a whole unit, therefore their diseases develop more and more obviously in clinical practice, in correlation with each other. The *concept of 'one airway, one disease'* has also been declared by the WHO ARIA document (12).

Being the gateway in the air flow for breathing, the nose plays an indispensable part in the homeostasis of the s. It warms, humidifies and filtrates air, thus protecting the lower airways. The nose and the bronchial systems are anatomically connected to each other. Their surface and histological structures are similar as both areas are covered with multilayered ciliary epithelium almost everywhere, and they are in contact by means of numerous indirect neural and systemic mechanisms, too. The nature of the nasobronchial link and the nasobronchial reflex has yet not been fully cleared, however, it is a fact that nasal diseases (allergic rhinitis, nasal polyposis, common cold) resulting the release of inflammatory mediators may have a consequence on the lower airway, too. The link studied the most is the one between allergic rhinitis and asthma.

We have launched several projects for the study of diseases of the airways, in cooperation with joint work groups of the Department of Oto-Rhino-Laryngology and Head-Neck Surgery at the University of Szeged, the Department of Dermatology and Allergology, and the Institute of Pathology. We have researched pathophysiologically similar molecular biological processes which nevertheless presented differrent symptoms based on their anatomical localization. As a practicing clinician, during my work my primary aim and task was to study and introduce the application opportunities in practice of already acquired knowledge in the field of clinical diagnostics and therapy.

1. In the past 20 years, our clinic has become of the the leading centers in Hungary in the treatment of scarry airway stenosis in adults and children. In the case of large airway stenosis, some external agent causes the mechanical damage of the endotracheal mucosa. The often iatrogenic cause (mispositioned tracheotomy, long term intubation), initiates an inflammatory cascade mechanism, which then results scarring in the pursuit of restitution. In reference to cricotracheal resection introduced in patients with laryngeal and/or tracheal stenosis, we had

the opportunity to conduct a histological study of the removed surgical specimens. By means of the increase of „minimally invasive" endotracheal interventions preceding resection aimed at the elimination of acute asphyxia, the inflammatory cell infiltration and scar tissue formation affects deeper and deeper tissue layers. Particularly apparent is the large fibrosis extending to all tissue layers of the trachea observable during the CO_2 laser vaporisation(s) with increased number and extension, which manifests in an increased clinically stenosis predisposition. Histological examinations even in patients classified in the same group showed large individual differences. Individually patient-dependant, genetically determined molecular biological mechanisms affecting the inflammatory base process stand behind the scar formation, which has thus far been explained with exogeneous causes. Recognition of these processes result the creation of new diagnostic and therapeutic modalities. Diseases with airway scarring can be related to the expression and polymorphism of certain determined genes, just as it was suspected earlier in connection with fibrotic diseases of airways or other organs (1)

2. Ultraviolet (UV) light is one of the major environmental hazards, and its role is well-known in the triggering of skin tumors and skin aging. However, phototherapy (UV and visible light (VIS) has a significant local and systemic immunosuppressive effect, therefore it has been broadly used as a remedy for the treatment of various inflammatory, immune-mediated skin diseases (2,3). Phototherapy exerts its immunosuppressive effect in the skin by means of inducing T-cells apoptosis, inhibiting the number and function of Langerhans cells and increasing the quantity of immunomodulatory cytokines (IL-10) (4,5). Our research results suggest that irradiating the nasal mucosa with light of different wavelengths (UV and visible, mUV/VIS) is efficient in reducing the symptoms of allergic rhinitis (6-8). Based on the above results, the Hungarian Medical Research Council (ETT TUKEB) declared the mUV/VIS-based therapy of allergic rhinitis to be a medical procedure (file no.: 351/KO/02, certificate no.: 60008/20/ETT/2002). The Rhinolight® phototherapeutic device used for the treatment is a CE-marked medical device. The efficacy of the Rhinolight® intranasal phototherapy in seasonal allergic rhinitis was justified by our research work group using a randomized, double-blind, placebo-controlled clinical study (8-11). Positive therapeutic experience gained in the treatment of chronic immune-mediated diseases of the nasal mucosa inspired the creation of new phototherapeutic protocols and the designation of new indication areas and target groups.

Allergic rhinitis (AR) has become by now the most common chronic disease worldwide (12), with an estimated number of patients to be over 500 million. According to WHO figures, half of Europe's population can become hay fever sufferer by 2015 due to the global and explosive growth of incidence. Studies for prevalence in Hungary also reported on a significant increase (13-14). In addition, its social and economic importance is further intensified as severe and/or persistent cases increase asthma prevalence, remarkably constrain nighttime resting and daytime activities, which cause additional decline in the quality of life. The success of all initiatives for prevention, primarily aimed at the change of lifestyle and environment is doubtful, due to the lack of widespread social inclusion. Currently, the most successful way of alleviating the harmful consequences can be the application of effective treatment modalities.

The group of CRS disorders annually accounts as many as 22 million office visits and more than 500,000 emergency department visits in the U.S., according to some estimates. Annual CRS-related healthcare expenditures may reach as much as $3.5 billion (15). Inflammation of viral or bacterial origin are the most frequent of the above. On some occasions special and rare forms may develop, which primarily present differentialdiagnostic difficulties (16). Nevertheless, despite having established a proper diagnosis and following the therapeutic principles, we often experience treatment failure.

Nasal polyposis appear in some 20% of chronic rhinosinusitis patients. In developed countries, also in Hungary, nasal polyposis is a disease affecting large population, characterized by a high recurrence rate, bad recovery inclination, and significantly reduced quality of life. Depending on different sociocultural and environmental impacts, these days its prevalence varies between 1,3-5,6%. 20-25% occurence was documented after block dissection in cadavers, therefore the pathological deformation is much more frequent than that of the diagnozed and treated cases. Treatment of nasal polyposis has still not been solved until now. Recurrence of polyps is still high even under the generally accepted combined, surgical-steroid treatment strategy, so neither surgical treatment, nor steroid therapy administered in the long run, but not even their combination can result total recovery of the patient (17). Even the application of further conservative treatment modalities (nasal lavage, long-term low-dose systemic macrolid antibiotics and local antimycotics, aspirin desensitization, anti-IgE, anti-IL-5, etc.) cannot offer a final solution. Also, it poses a problem that possible complications and side-effects of both surgery and the steroid treatment are known.

Histological characteristics of nasal polyposis very much resembles that of certain other immunological skin diseases of proliferative nature (eg. psoriasis), which have already been successfully treated with PUVA for some 30 years now (per os combined use of UVA-light and 8-methoxypsoralen photosensitizer). The intranasal PUVA treatment significantly reduced nasal symptoms of allergic rhinitis patients (18). During our preliminary, open- label, prospective clinical study, we however did not manage to achieve macroscopic change after a 6-week PUVA treatment, yet we experienced a significant reduction of eosinophil cationic protein (ECP) and IL-5 levels in the nasal lavage, also a significant decrease of eosinophils in the polyp tissue. (19). Similar etiological factors of allergic diseases and the favorable experience in the application of intranasal PUVA treatment hold out promising results and inspire further development of UV phototherapy and the examination of its use in nasal polyposis.

MY DUTIES AND AIMS IN THIS COMPLEX STUDY WERE:

1.1. ***Histopathological examinations of stenotic and scarry trachea specimens removed using cricotracheal resection.***

1.2. ***Examination of TGFβ superfamily playing a decisive role in the regulation of inflammatory processes, and the predisposition role of its various poyimorphisms in the formation of laryngotracheal scarring.***

2.1. The basic condition for the application of the new, promising phototherapeutic treatment was the exclusion of the nasal mucosa damaging effect of UV beams used in the therapeutic range and dose (Justification of safe usability). ***Evaluation the in vivo effect of intranasal phototherapy, by assessing DNA damage and repair mechanism in nasal mucosa***.

2.2. So far, little experience has been available regarding its applicability in persistent allergic rhinitis (PAR). The aim of our research executed with the support of the Jedlik Ányos Programme (NKFP1-00004/2005) during *a human, randomized, double-blind, placebo-controlled, prospective clinical study was* ***the development of a new RL-therapeutic protocol, and the establishment of its efficacy and safe usability in the treatment of PAR.*** (Ethical licence: SZTE, Regional Human Medical Biological Research Ethics Committee, file no.: 110/2007, permit no.: 2288.).

2.3**.** Our experimental histological studies have justified that UVB penetrates well in vivo into the polyp tissue, it reaches in the stroma the lymphocytes responsible for the inflammation, and induces apopotosis in them. On the basis of these results, the objective of our clinical studies: ***To examine the clinical efficacy and tolerability of NB-UVB phototherapy in bilateral nasal polyps***

1. HISTOPATHOLOGICAL AND MOLECULAR BIOLOGICAL STUDIES OF DISEASES WITH SCAR FORMATION IN THE AIRWAY

1.1. Histopathological and molecular biological studies of processes with laryngotracheal scarring

1.1.1. Introduction

Air enters the lung through the larynx and the trachea, thus, any obstruction of this area leads to either asphyxia, or in severe cases, to the drop in the quality of life or to a state incompatible with life. The most frequent type of the stenosis is the intraluminar scar formation due to the injuries of airways tissue or anatomical structures. Until the mid-XX. century, the most frequent cause of the scarry stenosis of the upper airways were external neck traumas and infectious diseases which recover with scars (e.g. diphtheria, syphilis etc.). However, during the last decades this symptom has been the complication of the prolonged intubation and mechanical ventillation due to traffic accidents, complicated surgical and intensive care interventions in several hundred thousand cases. This can affect a large part of the population living in a society with good health care. The cuff of the ventilatory tube may cause an ulceration on the airway.

Wound healing involves three temporally overlapping stages: an inflammatory stage, a proliferative stage, and a phase of contraction and remodeling (20). The insult provoking inflammation and the inflammation itself results the destruction of the intact tissues. In an ideal case, after the course of inflammation reparative-regenerative processes restore the integrity of sorrounding tissues, so as a result a sanation of full value is achieved. Unfortunately, in case of excessive inflammations the regeneration is unable to restore the original state, therefore in such cases the devastated tissue is replaced by connective tissue (fibrosis). During the process, the number of inflammatory cells gradually drops in the inflammatory area, while the number of fibroblasts increases, which are responsible for the production of connective tissue fibers, and these take over the location of the original parenchyma. Growth factors (TGF-β) PDGF, FGF) have a decisive role in the formation of fibrosis, which facilitate proliferation of fibroblasts, the production of matrix and inhibit the functioning of enzymes decomposing matrices. As the process pushes on, the number of cells decreases, proportion of the matrix goes up, and finally the fiber-rich scar tissue poor in cells is being formed (21). Karagiannidis et al. demonstrated the overexpression of TGF-β1 in the subepithelium. This factor bears a central role in the formation of fibrosis by means of

rearrangement of extracellular matrix components, morevoer, it is one of the strongest inductor of myofibroblasts, which have a higher collagen synthesis activity than normal fibroblasts (27).

1.1.2. Materials and methods

Thus, the process of scarring is determined by a complex inflammatory process. In the mid-90s a significant trend became widespread for the endoscopic solution of scarry stenoses (Fig.5.6.) (22). It seemed obvious that the scarring process can be solved in a minimally invasive manner. But international experience and our own results point out that the restenosis predisposition is significant, therefore the ultimate definitive solution can often be only external surgery and the removal of the stenotic part. The endotracheal endoscopic technique, and, if required, open resection have been used as a routine application for the resolution of tracheal stenoses at our clinic since 1996 (Fig.1-3.). These interventions enabled us to carry out the retrospective histological examitation of the removed scarry tracheal segments (n=27) (Fig.4.). Patients were selected into three groups based on the number and extension of CO_2 laser interventions (recanalization) conducted prior to the resection for the elimination of acute asphyxia. Group 1. is the control group (6 patients), who did not receive laser treatment. Specimens of patients with max. one described laser treatments were selected into group 2 (12 patients), while specimens of patients with 2-4 extended laser treatments affecting cartilage were selected into group 3 (9 patients). Histological sections embedded in paraffin were prepared from the surgical removed specimens, which, apart from the ordinary hematoxilline-eosine staining, were also stained with PAS-AK and examined under light microscope. Our evaluation covered all three tissue layers of the trachea: epithelium, subepithelium, cartilage/perichondrium. For the evaluation of the degree of change we set up a semiquantitative score (0-3).

1.1.3. Results:

Erosion of the epithelium was observed in all three groups, partial regeneration of the respiratory epithelium (multilayered ciliary epithelium) took place only in three cases of the control group, in 14 patients squamous metaplasia (Fig.12.) developed, squamous epithelium was formed to replace the destructed respiratory epithelium. In the third group erosion and metaplasia appeared in all cases (Fig.7/A,10.12.). A significant difference was observed between the control group and group 3.

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Irrespectively of the number and extension of laser treatments, inflammation and fibrosis were expressed in all three groups. The subepithel was characterized by chronic polymorphonuclear infiltration, and particularly the presence of plasma cells (Fig.7.8.12.) . The volume of inflammation among patients having received extended laser treatment was significantly higher than in the control group. The subepithelial layer is characterized by the tissue image of granulation tissue and intense fibrosis (Fig.8-11.14.15.) in all study groups, during which fibroblasts appear and produce connective tissue matrices.

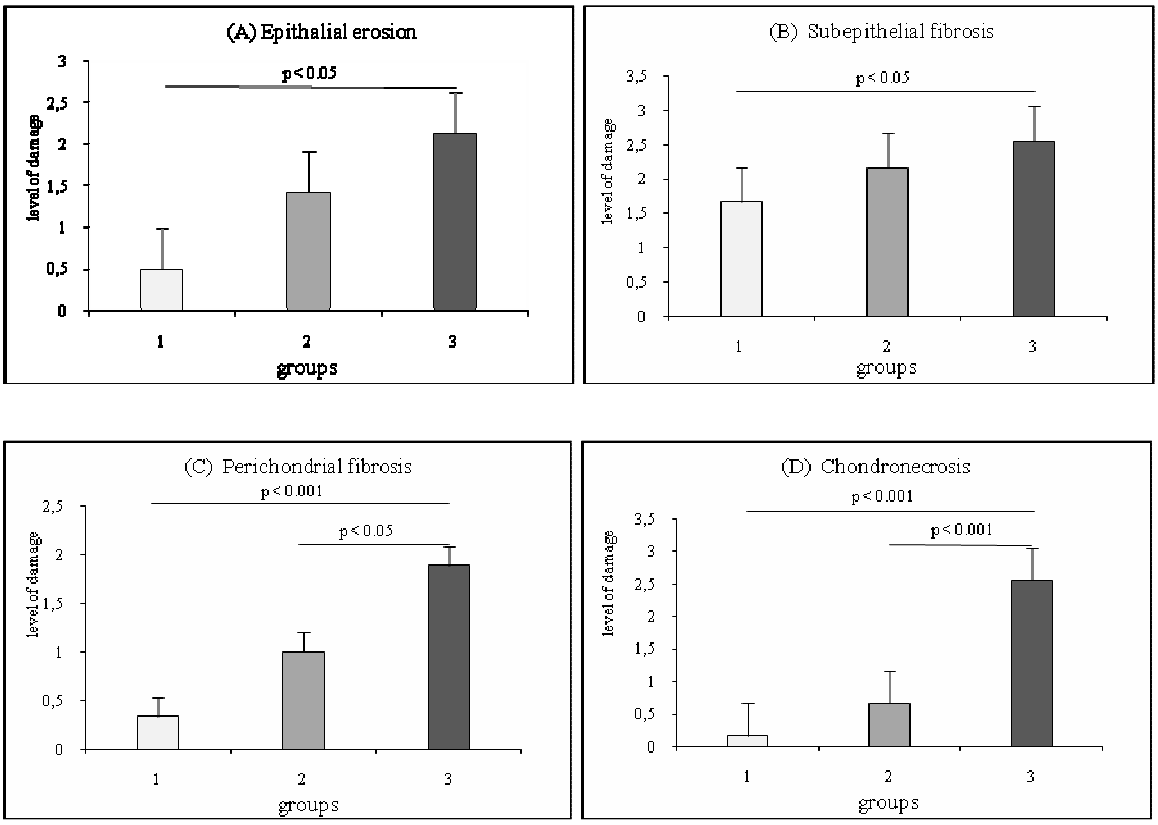

Figure 16/A-D: Damage levels of the tracheal tissue layers after different degree (groups 1-3) of the endotracheal CO_2 laser intervention

The perichondrial fibrosis in the third group was of larger degree as compared to the control group. As regards the degree of fibrosis, significant difference could be demonstrated between group 2 and 3. Cartilage necrosis, inflammation destructing cartilage was caused by the multiple, extended laser treatment. Single laser treatment caused either no or only small-degree cartilage necrosis. However, we observed minimal damage affecting cartilage in only one case in this group (Fig.16.). Special staining procedures enabled us to characterize certain tissue-specific changes. Using PAS-AK staining, the older (purple) and young (blue) connective tissue can be separated (Fig.15.)

After staining the cartilage matrix red, necrosis was better observable, and in some cases the formation of new cartilage can be observed, which, being stained blue as a sign of regeneration, further increases the grade of stenosis (Fig.14.15.) With the application of Crossmann staining, connective tissue scarring can be characterized as light blue (Fig.9. 10.).

1.1.4. Discussion:

Minimally invasive interventions gaining ground lately involve less burden on patients. However, in many cases they only result temporary solution to the problem, and, as histopathological studies proved, in the course of time they further aggravate stenosis. Contrary to the short-term advantages of laser treatments, their histological analysis showed a number of negative long-run consequences, many times the mechaism of increased development of restenosis. In most cases after the epithelial erosion the multilayered ciliary epithelium did not regenerate on the affected area, therefore the trachea lost its mucociliary clearance function. By the increasing of the number and extension if laser interventions, the occurence and degree of inflammatory, necrotic and fibrotic processes affecting deeper tissue layers (such as cartilages) rises. Our results histologically confirm clinical observations stating that there is a connection between the severity of the scarring and the size of the trauma triggered. Regarding the modern, minimally invasive treatment of stenoses, the above confirms the necessity to apply microspot and ultrapulse laser techniques offering less tissue trauma. (23).

The deviations of high volume observed in each therapy groups also indicate other possible factors affecting the grade of inflammation and fibrosis. Of these, the role of regulatory factors showing indivudal deviations and determining the scar formation process on a molecular biological level seems obvious.

1.2. Molecular biological studies to assess the genetic predisposition of diseases with airway scarring

1.2.1. Introduction:

According the literature data, proximately 1-3 % of the prolonged intubations can lead to significant airway stenosis because of the developed granulation and scar formation. Most of the obstructions usually close the larynx or the trachea directly, but the difficulty of the vocal cord movement, the injuries of the laryngeal muscules and joints (scars of the posterior commisure) can cause suffocation indirectly. The development of the postintubatory stenosis depend on many circumstances. According to our knowledge external factors, mode and duration of the intubation play a main role. The general condition of the patient and the microcirculation of the involved tissues are also considered as determinent factors. The most recent publications supported the fact that more gentle ventilatory tubes, proton-pump inhibitors which reduce the gastrooesophageal reflux, local application of fibroblast proliferation inhibitors can influence the development of the stenosis during intensive care in favourably. To preserve laryngeal function, the necessity and the timing of the tracheostomy instead of the translaryngeal ventillation is also an important question. The danger of the development of the callused airway stenoses can be reduced with these methods, but the expense of the intensive care increases significantly.

According to our observations after processing a high number of cases refered to our clinic difference can be found among the patients with similar intubatory circumstances in the frequency of stenosis.

This brings up a possibility that the different level of scar formation after the damage of the respiratory mucuos might be explained by different individual regenerative mechanisms, similarly to the keloid formation already being examined in dermatology, and can also be explained by genetic predisposition.

Members of the transforming growth factor (TGF) β superfamily are pluripotent cytokines that modify the growth and differentiation of various cell types. The β1 isoform of the TGF family (TGFβ1), one of the strongest inducers of myofibroblasts and a mitogen to immature fibroblasts, appears to be one of the major factors in fibrotic disorders of the human airway tract. Some of the polymorphisms of the TGFβ1 gene have previously been studied in diseases of the airway tract associated with fibrotic abnormalities. The fact that some polymorphisms might both be susceptibility factors for some fibrotic airway disorders and protective alleles in other disorders implies considerable complexity for TGFβ1 in the

pathogenesis of these diseases. As recently reported by Lawson and Loyd (1), the polymorphism at codon 25 (G915C causing an arginine to proline change) has been associated with post-lung-transplantation allograft fibrosis, but the association of the same polymorphism with idiopathic pulmonary fibrosis could not be demonstrated. The same applies to codon 10 (T869C causing a leucine to proline change): it was not associated with idiopathic pulmonary fibrosis, whereas its association with gas-exchange abnormalities during the progression of the disease was clear. A very similar modifying effect of codon 10 was reported by Drumm et al. (24), who demonstrated that cystic fibrosis patients harboring the codon 10 CC genotype are more prone to severe lung disease. Moreover, their data indicated that the -509 C/T polymorphism might also contribute to lung complications in cystic fibrosis. Drum et al. refer to the contrasting data of Wu et al. and Celadon et al. (25-26) observed in chronic obstructive pulmonary disease (COPD) induced by smoking, which is protected against by the same codon 10 C and -509 C alleles. The above results on the genetic background of fibrotic processes in various diseases of the airway tract stimulated us to conduct an initial study in which we compared the frequency of four TGFβ1 gene polymorphisms in patients with benign airway stenosis due to endotracheal intubation and in intensive care patients who had also undergone endotracheal intubation but had never presented tracheal stenosis. The argument for our ongoing work was supported by the recently published paper of Karagiannidis et al. (27), in which it was demonstrated that TGFβ1 mRNA expression was increased in stent-related stenoses as compared with nonstenotic control sections, as well as a strong matrix-associated subepithelial expression of TGF1 protein. Moreover, it has been also shown that anterior glottic stenosis (AGS) is also characterized increased TGFβ1 expression in a canine model system and that chitosan, an effective drug against AGS significantly down-regulated the expression of TGFβ1 (28) These data suggest that, similarly to the diseases of the airway tract discussed above, TGFβ1 might be implicated in the fibrotic abnormalities of the main bronchi, trachea and larynx following mucosal injury (e.g., stenosis following endotracheal intubation).

1.2.2. Materials and methods

Sixty-six Caucasian intensive care patients were enrolled in the prospective study (January 2004 – December 2007) Thirty-six of them were diagnosed with severe circumferential cricotracheal and tracheal postintubation stenosis (grade III and IV according to the Myer Cotton classification (29) following endotracheal intubation.

The consecutive 30 patients, who had never presented scarry airway stenosis in spite of their long-lasting endotracheal intubation, were regarded as the control population in our study. They were all treated at the Anesthesiology and Intensive Care Departments of our University Clinic, therefore this series of patients represents also a homogenious group considering how endotracheal intubation was carried out. (Table I.)

	Age (years)	Length of intubation (days)
Patients with acquired tracheal stenosis (n=36)	45,8963±17,31	7,87±4,92
Control patients (n=30)	62,07±16,44	6,93±5,32

Table I. Demographic and clinical characteristics of the enrolled patients

Genotyping: For our genomic study, venous blood was taken from the patients no other tissue specimens were collected from them. Genomic DNA was isolated from the blood samples and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method was applied to genotype four polymorhisms of TGFβ1 (Table II.). The PCR products were size fractionated on agarose gels (Cambrex, Berkshire, UK) and photographed.

The statistical significance of the association between the polymorphisms and postintubation laryngotracheal stenosis was assessed by means of the Fischer exact probability test and odds ratios (OR) with 95% confidence intervals (CI) were also calculated (30).

Polymorphism	Primers	Restriction Enzyme	Genotype Distinction
-800 G/A	Fwd: CTGGCAGTTGGCGAGAACA Rev: TAGAAAGGACAGAAGCGGTG	*MaeIII*	GG: 326 bp AA: 206+120 bp
-509 C/T	Fwd: GGAGAGCAATTCTTACAGGTG Rev: TAGGAGAAGGAGGGTCTGTC	*DdeI*	TT: 120 bp CC: 46+74 bp
codon10	Fwd: TCCGGGCTGCGGCTGCAGC Rev: CAGGATCTGGCCGCGGATGG	*PvuII*	TT: 18+135 bp CC: 153 bp
codon25	Fwd: TTCAAGACCACCCACCTTCT Rev: TCGCGGGTGCTGTTGTACA	*FseI*	GG: 318+152 bp CC: 500 bp

Table II. PCR-RFLP Detection of the TGFβ1 Polymorphisms

1.2.3.. Results

The genotype distribution of three TGFβ1 polymorphism (-800 G/A, codon 10 and codon 25) did not show difference when compared between controls and patients with scarry airway stenosis. In contracts with these the -509 C/T polymorphism presented a differential genotype distribution between the affected and control population. As shown in Table III, all three genotypes of this polymorphism could be detected. The overall allele frequency data proved to be in good agreement with those recorded for Caucasians in the NCBI internet databases (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1800469): C: 0.68, T: 0.32, where the genotype distribution is C/C: 0.500, C/T: 0.400 and T/T: 0.100, indicating that approximately half of the Caucasian population harbors the mutant allele of this locus. Since the number of homozygous mutants in our study group was extremely low, we excluded them from the statistical analysis and the proportions of the wild types (C/C) and heterozygous mutants (C/T) were compared between the benign airway stenosis patient group and the control group. This comparison revealed that the ratio of heterozygous mutants was significantly (p=0.0053) higher among the control patients who had undergone long-term endotracheal intubation but had never presented tracheal stenosis. These data suggest a protective function for the heterozygous C/T genotype against acquired tracheal stenosis, or on the other hand, the C/C genotype might be a susceptibility factor for tracheal stenosis in patients undergoing endotracheal intubation during intensive care (OR=4.5; 95% CI= 1.5123–13.3902).

A				
Variable	No and ratio (%) of patients with the genotype			
	ST	C		ST+C
C/C	21 (0.583)	7 (0.233)	p=0.0116	28 (0.424)
C/T	14 (0.388)	21 (0.700)	OR: 4.5	35 (0.530)
T/T	1 (0.027)	2 (0.066)		3 (0.045)
B				
Variable	Patients with the allele (%)			
	ST	C		ST+C
C allele	0.777	0.583	p=0.0014	0.689
T allele	0.222	0.416		0.310

Table III. Distribution of TGFb1 -509 promoter polymorphism genotypes (A) and allele frequencies (B) between patients with acquired tracheal stenosis (ST; n=36) and control patients (C; n=30) who underwent long-term endotracheal intubation without presenting stenosis

1.2.4. Discussion

Benign airway stenosis is currently considered a multifactorial disorder, in which primarily the injury of tracheal mucosa and additionally the circumstances of intubation, the general condition of the patient, the microcirculation of the affected tracheal tissue layers, etc. are considered to play pivotal roles in the pathogenesis of the disease (31,32). The results of our present pilot study, however might suggest that, besides these factors, a genetic predisposition may contribute to the pathogenesis: the presence of the wild type allele of the TGFβ1 -509 C/T polymorphism. However, the molecular mechanism of the way in which this polymorphism influences the pathomechanism is still unclear. According to Karaganniadis et al (27), high-level expression of TGFβ1 mRNA and protein were detectable in biopsy specimens from stent-related stenosis compared to non-stenotic control sections. Shah and co-workers (33,34) studied the connection between the genotypes of -509 promoter polymorphism and the level of TGFβ1 mRNA expression and found that the T allele of -509 polymorphism results in high-level mRNA expression. In contrast to this, our present study demonstrates that heterozygotes for this mutation are somehow less likely to develop postintubation benign airway stenosis and those having the low expressing C/C genotype are more likely to develop this diseases. A similar contradiction exists for COPD, where the TGF1-50β9T allele is protective (25,26), although it has been also demonstrated that TGFβ1 expression is increased in the airways of COPD patients (35,36). How the -509 T allele of TGFβ1 evolves its protective effect in COPD and in benign airways stenosis requires further investigations. The explanation may lie in the bipolar effects of TGFβ1 on immune processes (37), the complex molecular interactions of TGFβ1 with pro- and antifibrotic proteins (38) or the extremely broad spectrum of TGFβ1 influences affecting different cell types during normal wound healing and fibrotic diseases (39).

In relatively developed societies, many individuals in the acute phase of serious diseases will be subjected to intensive care which may include invasive ventilation. Accordingly, large numbers of patients are faced with the complications of intubation. One of the most severe such complication is acquired benign laryngotracheal stenosis, the

management of which imposes a further serious burden on the patients. Although minimally invasive treatment options have been developed for some of these stenoses, in most cases external surgical procedures must be applied as a final measure (28,40). However, all of these treatment modes involve an increased risk for the patients in the post-intensive period of their principal disease, so that long-lasting, and sometimes definitive tracheotomy or stenting is often necessary.

The odds ratio calculated in this pilot study indicated that patients with C/C, who represents almost the half of the Caucasian population, are 4.5 times more susceptible to acquired benign airway stenosis. However, there are some age discrepancies in our pilot study groups, these consecutive series of patients with this high odds ratio for this possible genetic predisposition may point out practical implications beside the theoretical interest. First of all, the simple molecular technique used for the detection of this allele makes it feasible as an everyday molecular diagnostic tool in intensive care. Hence, in the event of the detection of this genotype, alternative intubation management (e.g., a shorter translaryngeal intubation period, careful measurement and, if necessary, correction of the cuff pressure, etc.) could be applied. Furthermore, our data support the suggestion of Karagiannidis et al. (27), that the targeted inhibition of the profibrotic TGFβ1 could benefit intensive care patients with benign laryngotracheal stenosis. Last but not least, considering the great differences in allele frequency between different ethnic groups (e.g.: C: 0.40 and T: 0.60 in Han Chinese vs C: 0.77 and T: 0.23 in African Americans), it is plausible to hypothesize that intensive care patients from various races might exhibit different susceptibilities toward acquired tracheal stenosis.

2. SAFETY AND THERAPEUTIC ASPECTS OF INFLAMMATION-MEDIATING UV LIGHT

2.1. Effects of intranasal phototherapy on nasal mucosa-safety study

2.1.1. Introduction

One of the main mechanisms of action of UV light is induction of DNA damage in the irradiated cells. This mechanism is responsible in part for the biological effects of UV light and consequently its therapeutic use. However, DNA damage is also implicated in the mutagenic and carcinogenic potential of UV light. Knowledge of the mutagenic risk of DNA photodamage has stimulated interest to determine the wavelengths dependent distribution of different DNA photodamage types (41,42) UV light is able to cause DNA damage by direct mechanisms (absorption of photons by the DNA) or by indirect mechanisms such as generation of reactive oxygen species (42). Cells possess repair mechanism in response to UV induced DNA damage. The primary process that removes DNA damage is the nucleotide excision repair (NER) pathway. The removal of DNA base modifications via NER requires DNA damage recognition, lesion demarcation, dual asymmetrical incisions at the **5′** and **3′** sites flanking the lesion, excision of nucleotides from the single-stranded loop, containing the lesion, and gap-filling by DNA synthesis and ligation (43). Alternatively, highly damaged cells undergo cell cycle arrest, activation of the caspase cascade and finally apoptotic cell death (44).

2.1.2. Materials and methods

Intranasal phototherapy of ragweed allergic patients: The examinations were performed during the 2005 ragweed season in Szeged, Hungary, in eight ragweed allergic patients undergoing intranasal phototherapy. Each intranasal cavity was irradiated 3 times a week for 2 weeks, using gradually increasing doses of mixed ultraviolet and visible light (mUV/VIS). The irradiations were performed with a broad band light source (Rhinolight®, Hungary, 180 mW, spectral composition 5% UVB, 25% UVA and 70% visible light). Nasal cytology samples were collected with a disposable plastic curette (Rhinoprobe®, ASI, Arlington, Texas) before starting therapy, immediately after last treatment, 10 days and 2 months after last treatment.

DNA damage detection with COMET assay: DNA strand breaks and alkali-labile sites were quantified by Comet Assay (single cell gel electrophoresis). As the enzyme, ultraviolet

DNA endonuclease (UVDE) from Schizosacharomyces pombe participates in an alternative excision repair pathway in which DNA is cut immediately **50** of CPDs or (6-4) photoproducts, this endonuclease can be used in Comet Assay for identification and measurement of UV induced damage. The protocol was a modification of the previously described alkaline Comet assay (45).

Nasal epithelial cell culture: Positive control experiments were conducted on second passage *nasal epithelial cell cultures,* isolated from patients undergoing mucosal resection of the concha nasi inferior. Cell suspensions and were put in 500 **ll** PBS and irradiated with mUV/VIS light with the following doses: 225 mJ/cm^2 and 450 mJ/cm^2. After irradiation cell suspensions were collected into Eppendorf tubes and centrifuged at 200g for 4 min and processed using both the conventional and the UVDE modified Comet assay protocols.

CPD detection in nasal mucosa: Nasal cytology samples were stained with monoclonal antibodies against CPDs (anti-thymine dimer, clone KTM53, Kamiya, Seattle, WA, USA). Control samples were included in each experiment using negative control reagents that are routinely applied to histologic sections. Immunostaining was performed using an immunoperoxidase kit (mouse IgG Vectastain Elite ABC kit, Vector Laboratories, Burlingame, CA, USA). 3,30-diaminobenzidine (DAB) substrate, which results in a brown-colored precipitate, was used as chromogen. The samples were counterstained with haematoxylin.

Statistical analysis: The data between different time-points were analyzed for statistical significance using the Dunnett test. To compare the conventional Comet assay with the UVDE modified technique at the same time points paired t-test was used. Value of $p < 0.05$ was considered to be statistically significant for comparison between data sets.

2.1.3. Results

2.1.3.1.1. Kinetics of DNA damage detected by Comet assay in patients undergoing intranasal phototherapy

Comet assay was performed on nasal cytology samples of eight allergic rhinitis patients before starting the treatment protocol, immediately after last irradiation and 10 days after last treatment. Four of the eight patients returned for the 2 month follow-up visit when nasal cytology samples were collected. DNA damage was significantly higher in nasal cytology samples collected immediately after completing the 2 weeks treatment regimen than before starting therapy ($p = 0.02$ in conventional Comet assay and $p = 0.002$ in UVDE

modified assay) (Fig. 17.). DNA damage assessed by the UVDE modified method (49.42 ± 7.97%) was compared with that obtained by the conventional Comet assay (48.48 ± 8.08%) in samples collected immediately after last treatment.

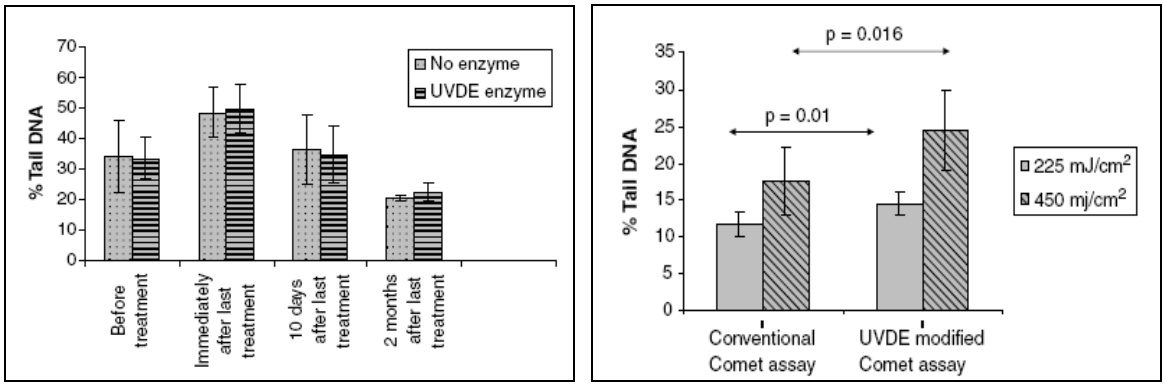

Figure 17/A: DNA damage detected by conventional and UVDE modified Comet assay in nasal cytology samples

Figure 17/B: DNA damage detected by conventional and UVDE detected Comet assay in cultured nasal epithelial cells exposed to UV light

A slight, but not significant increase was detected in all patients with the UVDE modified technique compared to the conventional Comet assay ($p = 0.7$). In positive control experiments in which nasal epithelial cell cultures were used, a significant increase ($p < 0.05$) of UVDE detected DNA damage was observed compared to data obtained by conventional Comet assay (Fig.17/B). DNA damage of control, non-irradiated nasal epithelial cells was low and no difference was detected between conventional and UVDE modified Comet assay (5.26 ± 1.82% and 6.07 ± 1.43%, respectively). Ten days after the last intranasal treatment a significant decrease in DNA damage was observed compared to data obtained immediately after finishing the treatment protocol with the UVDE modified Comet assay method ($p = 0.003$) (Fig.17/A) and a slight, statistically not significant ($p = 0.057$) decrease was observed using the conventional Comet assay technique (Fig.17/A). No significant difference in DNA damage detected by UVDE modified and conventional Comet assay was observed ($p = 0.52$) at this time-point. The difference between baseline (before treatment) and 10 days after last treatment was not statistically significant ($p > 0.05$ for both Comet techniques). Four of these patients were also examined off-season, at the 2 month follow-up. All patients were symptom-free when the samples were collected. The DNA damage detected by both Comet techniques

was significantly decreased compared to that detected 10 days after last treatment ($p = 0.04$ in conventional Comet assay and $p = 0.04$ in UVDE modified assay) and was similar to previously reported data from healthy individuals (Fig.17/A)

2.1.3.2. Kinetics of CPD detection in nasal mucosa of patients undergoing intranasal phototherapy

Nasal cytology samples of the 8 patients undergoing rhinophototherapy were stained for CPDs before starting therapy, immediately after last irradiation and 10 days after the last treatment. None of the samples collected before starting intranasal phototherapy stained positive for CPDs. In all samples collected immediately after last treatment strong positive staining for CPDs was detected. The number of positive cells decreased significantly 10 days after last treatment, but scattered residual positive cells were present in all examined samples. Cytology samples of 4 patients treated during the season with rhinophototherapy which were examined off-season (two months follow-up) showed no positive CPD staining cells. (Fig.18).

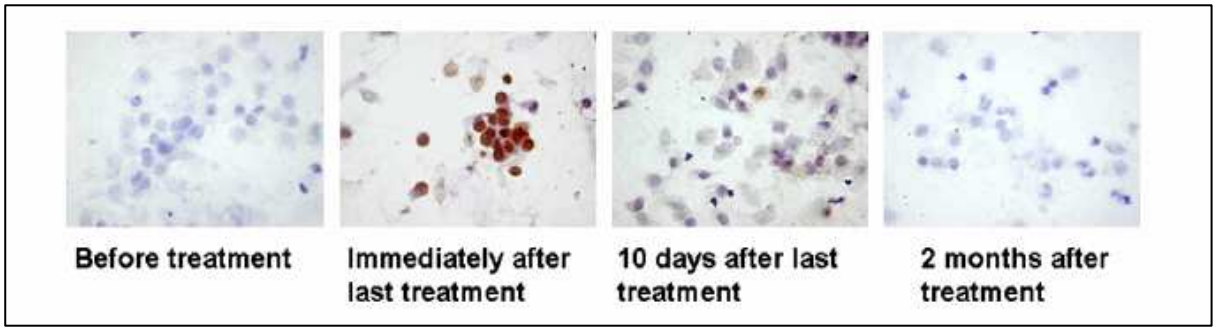

Figure 18.: Detection of CPDs in nasal citology samples, before starting intranasal phototherapy, immediately after last treatment, 10 days and 2 months after treatment

2.1.4.. Discussion:

Although, UV light has been previously successfully applied for the treatment of diseases of the oral and nasal mucosa, no data exist regarding DNA damage and repair of oral and/or nasal mucosal epithelial cells. The study conducted in allergic rhinitis patients undergoing intranasal phototherapy is the first assessing UV induced DNA damage in nasal epithelium. The DNA damage detected by Comet assay in the present study can be attributed in part to strand brakes as the direct effect of UV on DNA, but may represent also detection of apoptosis and necrosis not related to UV light. A portion of the cells collected are undergoing apoptosis or necrosis, which reflects in part physiologic apoptosis occurring in human tissues related maintainance of normal homeostasis and also cell death due to the procedure of

collecting cells and most important due to inflammation. DNA repair mechanisms also induce new strand brakes which are detected by Comet assay (46,47). Considering that at baseline and immediately after last treatment most of the patients were still symptomatic, Comet assay results reflect also DNA damage caused by the disease itself and therefore is not detecting UV specific damage. The addition of the UVDE enzyme increased the specificity of the modified Comet assay in detecting the UV specific damage attributed to photoproducts (CPDs and 6-4 PPs). This was mirrored by the significantly increased damage detected by the UVDE modified Comet assay technique in cultured epithelial cells exposed to mUV/VIS light. Cultured cells exhibited a significantly lower baseline DNA damage level of non-irradiated cells compared to DNA damage of cells collected from allergic subjects with active disease state. Although immediately after last treatment a slight increase in DNA damage was observed in all subjects with the UVDE technique compared to the classical Comet assay, the difference was not significant. These results suggest a limited contribution of UV specific DNA damage to the overall cell damage of nasal mucosa in symptomatic allergic rhinitis patients detected at this time-point with the Comet assay technique. Ten days after last treatment the DNA damage detected by the UVDE modified technique showed a significant decrease while the DNA damage detected by the conventional method proved to be not significant. However, when comparing DNA damage results detected by the UVDE modified technique with those obtained with the classic Comet assay, no significant difference was detected. Although, these results suggest that 10 days after last treatment repair of UV specific photoproducts occurred they also highlight the limitations of Comet assay (both techniques) in analyzing the superimposed DNA damage (specific to UV light) in an inflamed nasal mucosa which is characterized by high baseline DNA damage values characteristic to the disease itself. Therefore, direct detection of UV specific photoproducts by techniques such as immunostaining of tissue specimens is of outstanding importance for evaluating DNA damage and repair of nasal mucosa. For an accurate evaluation of the UV specific DNA damage, CPD staining was performed. Based on the long half life of CPDs and their persistence in the tissue they are considered the most important photoproducts for mutagenesis. Our results showed that CPDs can be detected in nasal mucosa samples immediately after irradiation and residual staining was present 10 days after last irradiation. These results are in concordance with previously published data in the skin. Samples taken 2 months after the last irradiation showed no residual damage in nasal mucosal samples, suggesting that after intranasal phototherapy no long-term residual CPDs remain in nasal cells. Collection of nasal epithelial cells using Rhinoprobes is a very convenient and easy

technique and have been previously used to asses the effect of air pollution on DNA damage of nasal mucosa (48-49). However, for a complete evaluation of the safety of phototherapy in airway diseases the results reported here should be replicated in future studies by staining for CPDs in biopsy specimens of the nasal mucosa exposed to UV light with special emphasis on DNA damage and repair of the basal cell layer. UV light-induced carcinogen effect is linked to the cumulative doses of UV light (usually requiring many years). Development of skin cancers has always been a concern when applying long-time phototherapy in dermatological patients. Therefore, several prospective and retrospective studies regarding association of phototherapy with increased risk for skin cancer have been conducted in psoriasis, atopic dermatitis and vitiligo patients. Recently, Lee at al performed a complex study based on extensive literature search (MEDLINE between 1966 and 2002) to asses the risk of skin cancer associated with UVB phototherapy (50). No significant increase in the risk of developing skin cancer was found. They stated that evidence suggest that UVB phototherapy remains a very safe treatment modality. Since skin and mucosa are characterized by differences in structure and cell turn-over rate we have compared in a separate study the ability of these tissues to repair UV specific photoproducts (CPDs and 6-4 PPs). We found that skin and airway mucosa exhibit similar kinetics in repairing UV induced DNA damage (62). Cumulative doses in rhinophototherapy are lower compared to that used in skin phototherapy which suggests that the safety profile of mucosal phototherapy is similar to that of skin. In this pilot study we have shown for the first time that nasal mucosa exposed to UV light possess the capacity to repair DNA damage which suggests that the multistep process of carcinogenesis has not been triggered. However more studies are needed in the future to characterize UV specific DNA damage and repair of the nasal mucosa.

2.2. mUV/VIS rhinophototherapy in persistent allergic rhinitis

2.2.1. Introduction

The efficacy of the combined UVB-UVA-high intensity VIS intranasal phototherapy (Rhinolight®) in seasonal allergic rhinitis was reported by our research work group in a randomized, double-blind, placebo-controlled clinical study (8). (Medical Research Council (ETT-TUKEB) certification on the application of Rhinolight® as a medical therapeutic procedure, file no.: 351/KO/02, certificate no: 60008/20/ETT/2002).

The role of phototherapy in the treatment of perennial allergic rhinitis (PAR) has not yet been investigated. The aim of our present research was to evaluate the efficacy and safity of mUV/VIS for the tretament of PAR in a, randomized, double-blind, placebo-controlled prospective clinical study.

2.2..2. Materials and methods

The target population was represented by PAR patients having moderate or severe symptoms for at least 4 weeks and occuring at least 4 days a week, with allergies (diagnosed with a prick test of specific IgE) to house dust mite, mould or (in case or permanent contanimation) animal epithelial antigen (51). Patients sensitized exclusively to epithelial antigen were not enrolled. Between November 2007 and March 2008, 34 patients were included and randomized into our study; 25 patients finished the study (female/male= 7/18; average age: 34,56 yrs). 9 patients had to be excluded due to upper-airways infection occuring in the treatment period;data of these patients wasnot included in the assessment. Based on the inclusion and exclusion criteria, selection of patients took place by rigorously taking into account the list of prohibited drugs (photosensitizers, non-steroidal anti-inflammatory drugs, antibiotics etc.), and washout periods (nasal and systemic steroids: 1 month, antihistamine: 2 weeks, oral or intranasal decongestants: 2 weeks, leukotriene antagonists: 1 month).

The one-month follow-up visit of the last patient was performed inJune, 2008. Enrollement of patients, randomization and the clinical visits were carried out at the Department of Otorhinolaryngology and Head-Neck Surgery at the University of Szeged, while phototherapy, laboratory evaluation of nasal and oral mucosa samples were performed at the Department of Dermatology and Allergology at the University of Szeged. During the „run in" period, patients kept a diary of symptoms for a week in the mornings and evenings, and scored their nasal symptoms on the basis of the preceding 12 hours (on a scale of 0-10).

If symptom scores were 4 or over for at least two symptoms (of which one had to be either nasal obstruction or rhinorrhea) during four consecutive days (moderate, severe PAR), the patient was enrolled (1. visit). Based on *randomization* three groups were formed: group A: 11 patients (3 droputs) – evaluated number of patients: 8, group B: 10 patients (4 dropouts) - evaluated number of patients: 6, group C: 13 patients (2 dropouts) - evaluated number of patients: 11.

During a 6 week period subjects received altogether 13 intranasal phototherapy treatments (first week 3x/week, then 2x/week for 5 weeks). Based on randomization, , patients in group A and B received RL treatment(1.6-2.7 J/cm^2/nostril/treatment). In group C, similar to our previous studies, low-intensity visible white light (placebo group) was administered as a placebo. Treatments were perfomred at the Department of Dermatology and Allergology between 9 a.m. and 4 p.m., by two trained technicians in turns in a random and standard way, according to the directives of the Rhinolight® user manual The low intensity visible light emitting device was obtained by insertion of a special light filterin a Rhinolight device. . In order to achieve the RL:placebo=2:1 ratio, based on a drawing of lots, one group received placebo, the other two got RL treatment. Optical settings of the devices were checkedon a weekly base. Treatment assignment of the groups was only revealed to the patients and the investigators at the end of the follow-up visits (double blind method). During the treatment period and the follow-ups, patients kept a *diary of symptoms* twice a day, right after waking up and before going to bed, based on the symptoms of the previous 12 hours (rhinorrhea, nasal itching, sneezing, nasal obstruction, on a scale of 0-10). For the examination of *nasal obstruction* the nasal inspiratory peak flow (NIPF) was measured. This is a stable, well-reprodusible, fast and cost-effective method which can be acquired easily by the patient (52). Patients measured the flow two times a day in their homes (the highest value of three, previously measurements was recorded each time) using a Youlten (Clement Clark, England) instrument.

The amount of the rescue medication (levocetirizin 1x5mg/day.) consumption was recorded, as well as side-effects and adverse events. Investigators assessed the patients in the 3.week of the treatments (2. visit), after 24 hours following the end of treatments (3. visit), and after a month (4. visit) (Table IV.).

EVALUATION	Screening	Inclusion (1. visit)	Treatment phase 1-6. week	Treatment phase at the end of 3. week (2. visit)	24 hrs after last treatment (3. visit)	One-month follow-up control visit (4. visit)
Approval after briefing	**X**					
Inclusion/exclusion criteria	**X**	**X**				
Pregnancy test		**X**			**X**	
Nasal symptom score /0-10/	**X**	**X**	**X**	**X**	**X**	**X**
Quantitative smell test		**X**			**X**	**X**
Mucociliar transport /saccharin test/		**X**			**X**	**X**
ENT examination	**X**			**X**	**X**	**X**
NIPF	**X**	**X**	**X**	**X**	**X**	**X**
Biopsy from nasal mucosa		**X**			**X**	**X**
Adverse events			**X**	**X**	**X**	**X**
Rescue medication/Concomitant medication	**X**	**X**	**X**	**X**	**X**	**X**

Table IV. Schedule of assessments

For the quantitative determination of the *smell threshold*, a standardized Smell Threshold Test developed by the University of Pennsylvania was used. A thresholds is set out using a standardized solution series of phenyl-ethyl-alcohol (PEA) stimulating the olfactory nerve, separately in each nostril, in several steps. The mathematical average of the concentration values determined during the test are considered to be the threshold concentration of the given nostril (48). This average is then compared with the previously determined average values of the age group of a healthy population. The *mucociliar transport function* was measured by means of a saccharin test. The saccharin-time shows a good correlation with the mucociliar function. Mucociliar cells of the nasal mucosa secure the flow

and transport of mucus. Saccharimide-Na (3x3 mm) placed on the anterior surface of the inferior nasal concha it first solutes in the mucus and advances back to the pharynx due to the mucociliar transport, where it generates a sharp and sweet flavour. The time passing by is called saccharin-time (11).

Based on a separate patient consent, 18 patients agreed to nasal mucosa sampling for the determination of *ICAM-1 adhesion molecule expression.* The ICAM-1 expression of nasal mucosa epithelial cells is a very sensitive marker of nasal mucosa inflammation in AR (49,50), therefore it may represent an objective measure for the efficacy of intranasal phototherapy. Nasal mucosa sam,ples were collected by scraping from the front-lower part of the inferior nasal concha using the Rhinoprobe® device (56). Samplings were conducted before the treatment (1. visit), after the completion of the treatement (3. visit) and at the end of the follow-up phase (4. visit). 7 of the 18 patients examined received placebo, 11 of them received RL treatment. Percentage figures gained during the 1. sampling were considered as 1, and values gained from the 2. and 3. samplings were correlated to this. Nasal mucosa epithel cells were used to stain the ICAM-1 (The anti-ICAM-1 antibodies (monoclonal anti-human ICAM-1 Alexa Fluor 647, Santa Cruz Biotechnology) were used.) (54).

Statistical analysis: Using a Repeated Measures ANOVA test, we analysed the change of RL treatment results compared with placebo results, and the change of values in time at the end of the treatments and the follow-up period in each groups as compared with the baseline value. The Fischer LSD (Post hoc) tests (Table V.) was also performed at the end of the treatment (3. visit) and after the completion of the follow-up phase (4. visit).

2.2.2. Results

Symptom scores for morning and evening *nasal symptoms* significantly improved compared to baseline in both groups by the end of the treatment ($p<0,05$), which lasted until the end of the one-month follow-up therefore the significant role of time for each symptoms can be demonstrated using the Repeat Measures ANOVA statistical method. After further analyzing our data in RL group was compared with Placebo group too (Fischer LSD, post hoc test).

By the end of treatment symptom scores for morning sneezing ($p=0,034$), rhninorrhea ($p=0,0019$), nasal obstruction ($p=0,021$), total nasal score (TNS, $p=0,019$), NIPF ($p=0,0019$) and evening sneezing ($p=0,017$) and NIPF ($p=0,0077$) showed significant improvement in the

RL group compared with placebo group. By the end of the four-week follow-up period morning and evening nasal itching (p=0,004, p=0.0003), evening rhinorrhea (p=0,0034) and TNS (p=0,0017) scores of the RL group and that of the placebo group reached also statistcial significance. By the end of the follow-up no significant difference was registered between the scores of morning and evening sneezing, both of which improved siginficantly by the end of treatment (p=0,12, p=0,077). Evening nasal obstruction proved to be the most stubborn symptom. It improved significantly, however, it did not improve significantly compared with placebo at any time examined.

	6. week At the end of treatment		**RL vs Placebo**	**10. week At the end of follow up**		**RL vs Placebo**
	RL	**PLACEBO**	**p**	**RL**	**PLACEBO**	**p**
Sneezing_M	-2,16	-1,44	**0,03446**	-1,97	-1,43	0,12313
Itching_M	-2,04	-1,95	0,78344	-2,40	-1,47	**0,00432**
Rhinorrhoea_M	-2,46	-1,38	**0,00188**	-3,16	-1,75	**0,00006**
N.obstraction_ M	-2,24	-1,52	**0,02108**	-3,16	-1,99	**0,00021**
TNS_M	-8,84	-6,30	**0,01859**	-10,62	-6,66	**0,00027**
NIPF_M	19,31	6,79	**0,00185**	27,28	11,82	**0,00007**
Sneezing_E	-2,36	-1,57	**0,01738**	-2,35	-1,72	0,05631
Itching_E	-2,28	-1,95	0,31600	-2,73	-1,53	**0,00030**
Rhinorrhoea_E	-2,07	-1,54	0,09565	-2,93	-1,99	**0,00342**
N.obstarction_E	-1,69	-1,47	0,49021	-2,47	-1,91	0,07767
TNS_E	-8,38	-6,54	0,07768	-10,47	-7,19	**0,00174**
NIPF_E	20,85	11,56	**0,00770**	28,14	13,30	**0,00003**

Table V. Average change of nasal symptoms and NIPF as compared with baseline scores at the end of treatment (6. week), and at the end of the follow-up (10. week)

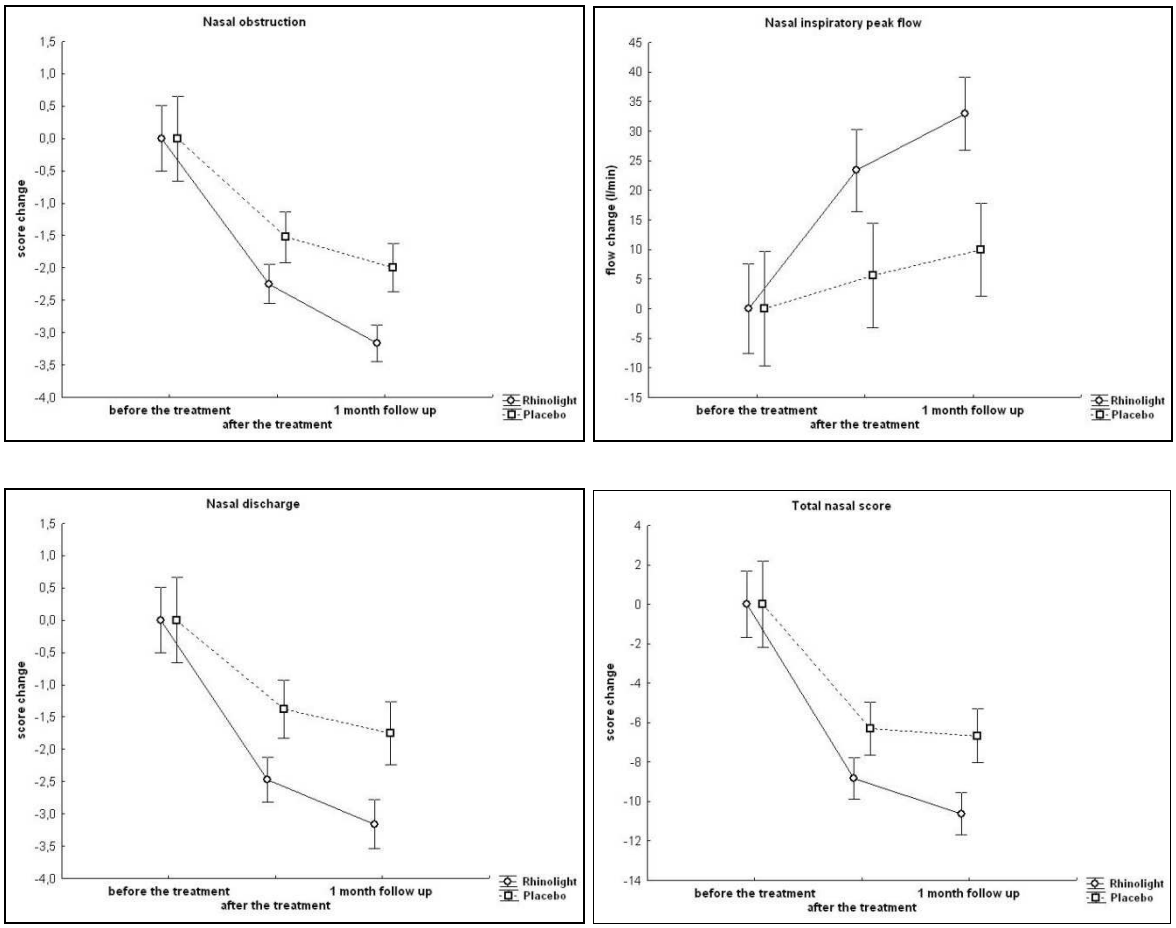

Figure 19/A-D: Nasal obstruction in the morning, NIPF, rhinorrhea and TNS scores change based on values registered at baseline, at the end of treatment (6. week) and follow-up periods (10. week).

Measured values of the *mucociliar function* and the *quantitative smell threshold* showed a large variations, and no significant change was observed either in the course of time, or regarding the comparison between the groups (p>0,05). Usage (consumption) of the rescue medication (in the RL group: 4.21 tbl/person/treatment cycle, in the placebo group: 3,45 tbl/person/treatment cycle) did not was not statistically different in the two group (p>0,05)*ICAM-1 expression*, wasdecreased in patients treated with RL compared toplacebo. This decrease was detected at the end of the tretament period and it became even more remarkable at the end of the follow-up phase (4. visit), yet it did not reach the significant level at any time (**Figure 20.**).

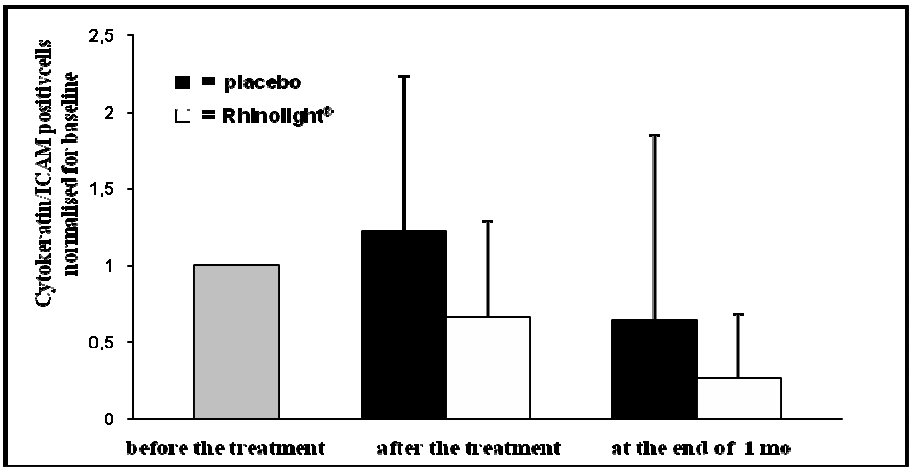

Figure 20. Proportion of ICAM-1 positive cells of the nasal mucosa in groups treated with Rhinolight® and placebo light .

No severe *side-effects* were observed. Three patients in the RL group reported mild dryness of the nasal mucosa, which disappeared in a few days after using vitamin A oil. In both groups 2-2 patients were observed to have mild nose bleed when they blew their nose, which was not considered directly related to the treatment, and have not requiredtreatment. Temporary, spontaneously disappearing nasal pain occured in three patients in the RL group, and two patients in the placebo group, while headache and diarrhea occured in 1-1 patients in both groups. No difference existed between the frequency of side-effects in the two groups.

2.2.4. Discussion

More than 70% of the Rhinolight® light falls into the range of visible light. The amount of UV-A light released is below 25%, proportion of UV-B range is less than 5% of the total light (8,10,57,58). The RL phototherapy inhibits the antigen-induced histamine release frommast cells, which has been reproted by clinical studies. It induces apoptosis in T-lymphocytes and eosinophil cells, thus reducing the number of eosinophil cells, ECP and Interleukin-5 levels (57). Based on our previous randomized, placebo-controlled, double-blind study we found the RL treatment is effective in the treatment of ragweed-induced seasonal AR (8,53,54). During our current study we aimed to find out whether the RL procedure can be used in a safe and effective way for moderate and severe PAR. The diary of symptoms kept by patients served as a basis for the treatment assessment (0-10). Taking into account the symptoms of the preceding 12 hours, the morning and evening registrations each characterize separately the severeness of symptoms during the daytime (activity) and the night (resting) periods. Data collection twice a day promotes the sensitive follow-up of daily, weekly and

monthly changes in terms of time, as well as the interpretation of individual and occasionally outstanding values.

Nasal) symptom scores in our PAR patients significantly improved in paralel with the treatment both in the RL and the placebo group. Significant improvement in the placebo group may be explained with the fact that continous medical attendance (weekly 3, then 2 treatments, altogether four doctor's visit and if necessary, and repeated keeping in contact by phone calls, psychological patient conseilling) can have a significantly positive impact on subjective symptoms. By the end of treatment and the follow-up periods, morning nasal obstruction, rhinorrhea, TNS values, and both the morning and evening NIPF, in the *RL treatment group proved to be significantly more effective than placebo light* (Table V., Figure 19.). The difference between the two treatment groups (significance levels) further increased for all the above symptoms and the NPIF values This latter phenomenon can be explained either with gradual disappearance of the placebo effect of treatments in the follow-up period or with the efficacy of RL treatments extending beyond the treatment period. These presumptions are confirmed by the fact that certain symptom scores (nasal itching in the morning and evening, rhinorrhea in the evening) and the evening TNS values showed significant difference between those treated with RL and the placebo group by the end of the follow-up period. Morning and evening sneezing scores improved by the end of the treatment in the RL group, but changes proved to be not significant by the end of the follow-up period. Evening nasal obstruction proved to be the most stubborn symptom as its change did not reach a significant level by the end of either the treatment or the follow-up period. However, based on the NIPF values which objectively measures nasal breathing, the RL treatment proved to be effective in the evening. The frequent contradiction between the subjective sensation of nasal obstruction and objective parameters describing nasal breathing thus NIPF values has long been known (60). Similar results were reported by us in seasonal allergic patients treated for 4 weeks with RL in whichRL treatment proved to be significantly more efficient than placebo in all nasal symptoms and TNS values, except for nasal obstruction (8,10,57,58). It must be noted that during this period nasal obstruction scores significantly decreased in the placebo group. In our current study the sensitivity of symptom changes were significantly reduced by the well-known fact that in persistent moderate-severe and severe perennial allergy patients patients symptoms are of less intesity on average, comparedto seasonal (eg. ragweed) allergic rhinitis,. Patients find it more difficult to evaluate changes of smaller absolute value during the treatment. The registered improvement of symptoms scores

exceeding 50% in the placebo group suggests the bilogical efficacy of low-intensity visible white light (VIS), and it might question its usability as a placebo light. However, previous in vitro studies on cell cultures unequivocally proved the therapeutic separability of RL and VIS with regard to the main apoptosis-inducing therapeutic effect (57,59). Based on the results of these basic studies we applied VIS treatment as placebo in our previous studies and in our current work.

The objective of our previous safety studies was the determination of the rate of UVB-induced DNA damage and the risk of carcinogenity (61,62). During our study we had an opportunity for the first time to examine in a standardized way smelling as the physiological function of the neuroectodermal endonasal epithelium structure which is exposed during the treatment to UV light (9,53,63). We observed an improving trend in the smell in the RL tretaed group, which did not reach a significant level. The low number of subjects and the high varaitions in measurement results made assessment difficult. The improvement observed can primarily be attributed to the appeasement of allergy symptoms (64,65). No decrease of smell function was observed in any of the patients, which indicates that intranasal RL treatment in the applied does not damage the olfactory epithelium. It is worth continuing observations in seasonal-type allergens, where comparing measurement results made during the symptomfree period, symptomatic period before treatment, symptomfree periods after treatment and the season can give us more accurate results. We can examine the impact of allergic symptoms on smell functions as well as the impact of Rhinolight phototherapy on the olfactory epithelium with allergic symptoms excluded.

The effect of RL on the mucociliar function has also been examined for the first time. The saccharin-time indicates shows a good correlation with the mucociliar function . A number of publications deal with the inhibition of this function by AR, and studies the regeneration of the reversibly damaged function after the termination of seasonal symptoms (11). We observed a non-significant improvement of the „mucociliar clearance” function in the RL group. Phototherapy applied did not affect either the mucociliar or the smell functions detrimentally, which is another proof for the safety of the RL treatment. Improved the nasal symptoms because of, reduced the inflammatory processes so decrease the oedema and secretion of the nasal mucosa. In parallel with this, we expected an improvement in smell and mucociliar functions, which actually was well observable in a part of the patients, however, regarding the total treatment groupwe were not able to Show any significant changes.

We observed a decrease in the number of ICAM-1 positive cells in patients treated with RL compared with those treated with placebo. The decrease in ICAM-1 expression of the nasal epithelium in the RL group implies a decrease in the inflammatory process, which actually coincides with the improvement of the allergy symptoms, however, this difference was statistically not significant. The high variations observed and the low number of subjects may be responsible for not reaching statistical significance.

According to clinical observations, if drug treatment (local steroid and/or antihistamine) is stopped in patients suffering from PAR, they will become more susceptible to upper-airways infections (51,66). In case of some patients the treatment period unluckily overlapped with the flu pandemic period, so this can explain that more than a quarter of the randomized patients had to be excluded from the study due to intercurrent upper-airways infection.

Nasal dryness observed in the RL group was the only treatment-specific side-effect (8,10,61), which was not observed in the placebo group. Nasal dryness was not severe, and it did not increase the likelihood of nasal bleeding. Both our previous studies and experience gained during clinical practice in seasonal allergic rhinitis showed a 50-70% incidence of nasal dryness during the two-week, 3x a week treatment. Our experience suggests that regular treatment of the nasal mucosa with vitamin-A oil and the 2x a week treatment regimen reduces the probability of nasal dryness.

Due to the high-rate placebo effect, analysis of differences during the 4-week follow-up is particularly important. When the placebo effect already does not prevail, a significant difference can be seen between the two groups both for subjective and objective measurements. Statistical evaluation is mainly difficult due to the low number of cases. A large multicentric study is neededin order to refine the preliminary results gained so far.

In case of severe symptoms, based on its special mechanism of actionand its good side-effect profile, the RL phototherapy might be combinedwith other therapeutic modalities. According to our study, RL phototherapy is also effective in monotherapy for the treatment of persistent allergic rhinitis.

2.3.. UV phototherapy of nasal polyps

2.3.1. Introduction

Immunosuppressive effect of UV light has its most efficient part in the UVB range, so the so-called narrow-band („narrow-band”, NB-UVB: 305-315nm wavelength) light source is the one most widely used in phototherapy. It was the research group at the Department of Dermatology and Allergology at the Universitiy of Szeged to use 308 nm xenon-chloride laser (XeCl) first to treat psoriasis, vitiligo, and atopic dermatitis (67,68,69).

In some 80% of nasal polyposis cases a persisiting cellular inflammation of eosinophils is in the background. There are degranulated mastocytes and eosinophils in the polyp tissue, and the survival period of the latter increased. Deposition of plasma proteins (eg. albumin) and fibronectin regulated by subepithelial eosinophil infiltration was observed. Regardless of whether originated from atopic or non-atopic patient, always significantly more IL-5 can be detected in the polyp tissue than in the control mucosa. IL-5 of growing quantity and eotaxin can be responsible for the eonisophil cell activation, accumulation and increased survival, all the above resulted regardless of allergy. However, probably adhesion molecules also take part in this mechanism (expression of VCAM-1 and VLA-4 are increased). Presumably TGF-β1 exerts an effect contrary to IL-5, and it is present in the polyp tissue in a reduced quantity, and can be responsible for the deposition of extracellular matrix proteins and fibroblast activation. According to a new observation (70) a significant part of patients have a high amount of total tissue and multiclonal IgE, and Staphylococcus aureus enterotoxin A and B, in addition, there are more asthma and aspirin intolerance patients in this group. Staphylococcus aureus enterotoxins are pyrogenic proteins of high molecular weeght, the so-called superantigens, which have a firm T-cell stimulating effect. and induce cytokine release from macrophages, mastocytes, eosinophil and epithel cells. (71,72). In in vivo and in vitro studies Staphylococcus aureus enterotoxins can be connected to the measure of airway hyperactivity and tissue eosinophilia, as well as the severity degree of perennial rhinitis and asthma, therefore the disease must be regarded as an affecting factor. Based on another concept (73) similarly to as described in CRS, the inflammatory process develops as a result of the „failed” elimination reaction launched against fungus antigen detectable in the nasal lavage of healthy individuals.

Intranasal phototherapy has been shown to be effective in inflammatory mucosal diseases such as oral lichen planus and seasonal allergic rhinitis (61,74). The therapeutic

effect of UVB light is primarily attributed to its local immunomodulatory action. One of the most important mechanisms that explain the effects of UVB light is induction of apoptosis in inflammatory cells (57,59,75,76) Therefore, UV phototherapy may represent a new therapeutic tool for the management of nasal polyps. A pilot feasibility study was performed to assess the clinical efficacy and tolerability of NB-UVB phototherapy in bilateral nasal polyps.

2.3.2. Materials and methods

An open-label prospective pilot study was conducted in patients with grade 1-3 nasal polyps (77). The study was approved by the Central Ethics Committee of Hungary and the Ethics Committee of University of Szeged. All subjects signed an informed consent. We excluded potential subjects from the study who had a diagnosis of cystic fibrosis, oral steroid-dependent asthma, or had upper or lower respiratory infection within 4 weeks prior to the beginning of the study or had any significant nasal structural abnormalities which can interfere with the delivery of intranasal phototherapy. We also excluded patients with known photo-sensitivities or photo-allergies to natural or artificial sunlight and those who were receiving any form of light therapy, or had used any of the following drugs: systemic and topical corticosteroids, leukotriene modifiers and/or immunosuppressive drugs within 4 weeks, membrane stabilizers and/or antihistamines within 2 weeks, prior to the beginning of the study.

Patients were exposed to gradually increasing doses of NB-UVB light (300mj/cm^2 to 1200mj/cm^2) over a 12 week period. UV light was delivered targeted to the nasal polyp tissue under endoscopic visualization (Allux Medical Inc, Menlo Park, CA, USA). Patients were treated 3x/week. Subjects rated their nasal obstruction symptom scores weekly on a visual analogue scale from 0 (none) to 6 (very severe) (77). The NOSE quality of life questionnaire was used at baseline and end of treatment period (score range from 0 to 100) (78). Adverse events were monitored by endoscopy. After finishing the treatment period patients had two follow-up visits, at one and three months after final treatment. At the 1 month follow-up visit the investigators had the option to prescribe mometasone furoate nasal spray or to keep the patients without any topical steroids until the 3 month follow-up visit (decision was based on patients symptoms and endoscopic status). At both follow-up visits symptom scores and the NOSE questionnaire data were collected.

Statistical analysis: The two-sided Dunnett test was used for statistical analysis, $p < 0.05$ was considered statistically significant. Correlation between nasal obstruction scores and NOSE scores was assessed by the Pearson correlation test.

2.3.3. Results

Thirteen subjects were enrolled in the study (6 men, 7 women; ages: 38 to 61 years, mean 47). Ten subjects completed the study, 3 patients dropped out because of non-device related causes (one subject receiving anticoagulant medication presented spontaneous bleeding not related to the treatment, one patient with active seasonal allergic rhinitis and asthma and one patient with superior respiratory tract infection required treatment with prohibited medication). From the ten subjects who finished the whole treatment regimen 3 were de-novo nasal polyps and 7 had at least one sinus surgery (performed at least one year before enrollment).

Nasal obstruction symptom scores significantly improved at end of treatment compared to baseline ($p = 0.009$) (Fig. 21/A). Significant improvement of quality of life (NOSE) at end of treatment compared to baseline was also noted ($p = 0.018$) (Fig. 21/B). Changes in nasal obstruction scores showed a good correlation with changes in NOSE scores ($r = 0.81$). Treatments were well tolerated and no device related adverse events were reported. During the one month follow-up period none of the subjects received any treatment, including intranasal steroids. Nasal obstruction and NOSE scores remained stable during this period (Fig. 21). A statistically significant difference was observed in nasal obstruction and NOSE scores recorded at the one month follow-up visit compared to baseline ($p = 0.002$ for nasal obstruction and $p = 0.001$ for NOSE).

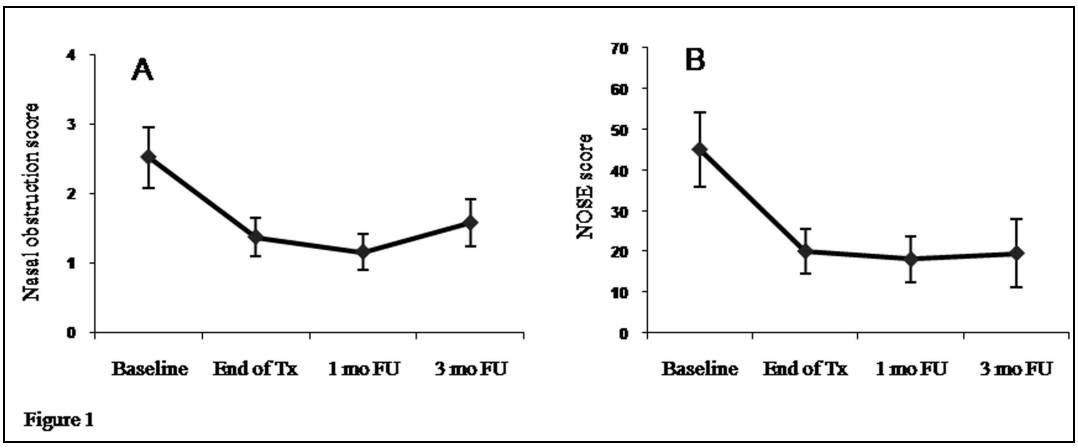

Figure 1

Figure 21.: Effect of narrow-band UVB phototherapy on nasal obstruction symptom scores (A) and NOSE scores (B) at end of treatment, 1 month (1 mo FU) and 3 months (3 mo FU) follow-up.

At the one month follow-up no topical steroid was prescribed in seven patients, the other 3 subjects received mometasone furoate nasal spray. No significant worsening was observed in nasal obstruction and NOSE scores at the 3 months follow-up visit (Fig. 21.). Differences between the 3 months follow-up nasal obstruction and NOSE scores and scores recorded at baseline were statistically significant ($p = 0.035$ for nasal obstruction and $p = 0.015$ for NOSE). A detailed analysis showed that subjects with prior sino-nasal surgeries had a better access to the polyp disease which significantly impacted the therapeutic outcome (Fig. 22.).

2.3.4. Discussion

Nasal polyps can induce severe symptoms such as nasal obstruction, rhinorrhea and loss of smell (17). Quality of life of NP patients is severely impaired and it has been shown to be comparable or even worse than that of patients with chronic obstructive pulmonary disease, coronary artery disease and asthma (79).

NP is an inflammatory disease with the majority of polyps belonging to the eosinophilic type. Data suggests that persistence of tissue eosinophilia is of central importance in the pathogenesis of NP (79,81). Eosinophils are terminally differentiated cells which differentiate, migrate and accumulate in the tissues following release of various cytokines and chemokines, such as IL-5, IL-3, GM-CSF, RANTES. These factors can further enhance the inflammatory process by contributing to stromal fibrosis, epithelial damage, increased edema and increased extracellular matrix protein production. Thus, eosinophils potentially damage cells and their prolonged survival in NP is a key factor in the pathogenesis. Apoptosis is critical in the regulation of eosinophil removal and delayed apoptosis has been reported as an important mechanism for tissue eosinophilia in several diseases, including nasal polyps (82). IL-5 is one of the major cytokines which promote eosinophil maturation, activation and survival. CD4+ T cells are considered to be the major source of IL-5, but other cell types including mast cells and eosinophils also release this cytokine. In this manner eosinophils themselves may promote cell survival. Therefore, apoptosis of eosinophils and T cells may represent a therapeutic target in NP.

Being an inflammatory disease, treatment of NP is primary medical with anti-inflammatory drugs, such as topical and oral steroids, but even with maximal, well controlled medical therapy a significant percentage of patients with NP fail and had to undergo sinus surgery (17). Unfortunately, NP have a strong tendency to recur after surgery even when aeration is improved and proper medical treatment is administered. NP recurrence rates reported by different studies vary largely. In their literature review Dalziel et al found that disease recurrence rate varied from 4% to 60% (median 20%), one major factor responsible for these differences being the length of the postoperative follow-up (83). Therefore, early treatment of recurrent disease is of outstanding importance to avoid or significantly delay further surgeries.

Several studies suggest that apoptosis of inflammatory cells is one of the major mechanisms of action of systemic and topical corticosteroids (84,85). It has been shown that long-term treatment of NP with topical corticosteroids reduced the number of eosinophils in vivo (86). Watanabe et al have shown that increased eosinophil apoptosis is present in NP cultured in the presence of corticosteroids (87). However, in a recent study Bloom et al have demonstrated that in the presence of high levels of IL-5, which are more closely related to those detected in clinical situations, glucocorticoids may actually decrease, rather than increase, the number of apoptotic eosinophils, suggesting that in in-vivo situations the local cytokine (or cytokine combination) levels can rescue eosinophils from glucocorticoid-induced apoptosis (87). Topical corticosteroids are also characterized by limited penetration in the polyp tissue and are frequently impossible to deliver to problematic areas such as the ethmoids, sphenoid, frontal and maxillary sinuses. Therefore, despite continuous topical steroid treatment a significant part of patients show a gradual worsening of the polyp disease. A modality to reverse the growth of NP and to delay surgery is the use of high dose systemic steroids (17). Systemic steroids are effective in reducing the severity of symptoms associated with nasal polyps but are also characterized by strong recurrence tendency and is not unusual for patients to receive more than one course of systemic steroids per year in the attempt to delay surgery. Systemic steroids are known for severe side effects especially if used repeatedly and in higher doses and the medical community is looking for therapies that can reduce their use in NP (88).

In addition, it has been shown that a subset of NP patients is refractory to corticosteroid treatment and this can be linked to genetic polymorphism of the glucocorticoid receptor

(overexpression of the glucocorticoid GRbeta splice variant). Therefore, this population may be predisposed genetically for medical failure and repetitive surgeries (86)

NB-UVB is considered a gold standard for the treatment of inflammatory skin diseases and is recommended by guidelines around the world (89,90). The safety profile of NB-UVB has been widely studied. Recently, Lee et al performed a complex study based on extensive literature search (MEDLINE between 1966 and 2002) to assess the risk of skin cancer associated with UVB phototherapy (91). No significant risk of developing skin cancer was found. The authors stated that evidence suggests that UVB phototherapy remains a very safe treatment modality. Recent studies performed on nasal epithelium and in patients receiving intranasal phototherapy showed that the UV-induced DNA damage response of respiratory epithelium is very similar to that of the human epidermis. It was also shown that nasal mucosa is able to efficiently repair UVB induced DNA damage (61,62). These data suggest that intranasal UVB phototherapy has a similar safety profile as skin phototherapy.

NB-UVB phototherapy may represent an alternative, steroid-sparring treatment for patients with nasal polyps. The severity of NP has generally been correlated to the degree of nasal obstruction and therefore any new treatment has to have a significant effect on this symptom. We have shown that both nasal obstruction and quality of life (NOSE) of patients improved significantly after NB-UVB phototherapy. The therapeutic outcome was stable for at least 3 months after end of treatment despite the fact that most of the patients had not received any specific therapy. In the current study the dose of UV light was increased very slowly for safety reasons, however the good tolerability suggests that the treatment can be started at higher doses and/or the dose escalation may be done in a more rapid fashion which would allow achieving clinical results more rapidly. As the treatment is delivered under endoscopic visualization, in a targeted fashion, this type of therapy may be especially appealing for patients with recurrent disease after sinus surgery. In these patients the reconstructed airways allows a better access for delivering the treatment and therapy can be applied in a highly controlled way.

Our preliminary data suggest that targeted NB-UVB phototherapy may represent a promising new therapeutic modality in nasal polyposis especially in patients with previous sinus surgeries, but a larger, double-blind study is warranted in the future to assess the therapeutic power of a shorter regimen and to further assess the safety profile of this new treatment.

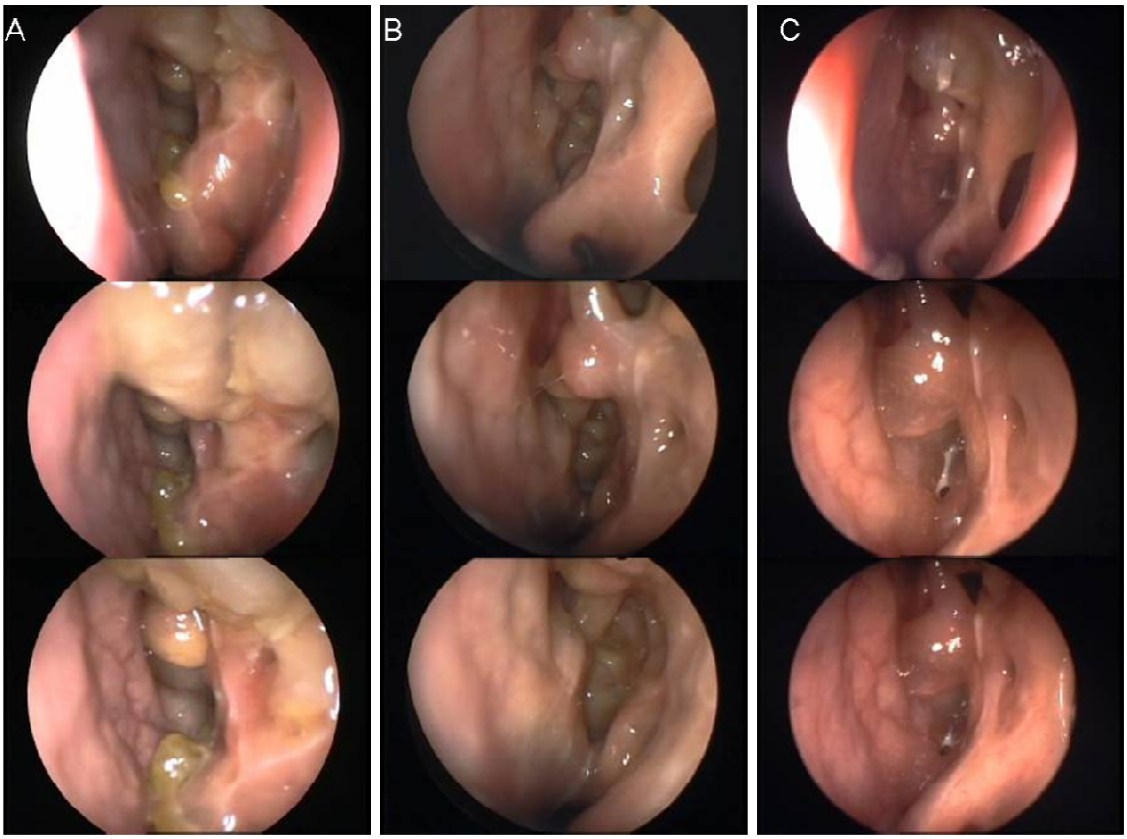

Figure 22.: Effect of NB-UVB phototherapy in a patient with recurrent polyp disease after sinus surgery. Phototherapy significantly improved the disease state at end of the treatment period (B) compared to baseline (A) and improvement was stable during the 1 month follow-up period (C)

CONCLUSION AND NEW RESULTS

The appearance and course of diseases are determined by genetic and environmental factors together. 99% of the genome is the same in every human. The genetic variability (heterogenity) accounting for less than 1% determines the individual responses and the versatility of biological and physiological processes generated to the same noxa formed in the human body, which are traceable with molecular biological methods. Identification of etiological factors and knowledge of genome-level predilection factors in modern medicine prepares the potential to introduce a custom-made therapy tailored to the individual.

The better understanding of chronic inflammatory processes taking place in the background of airway diseases may contribute to the reassessment of thus far less successful treatment methods. Our accurate knowledge allow the creation of new prevention programmes and the introduction of modern conservative and minimally invasive therapeutic treatment modalities.

Reviewing the objectives and new results of our studies, let us summarize our conclusions:

Hystopathological examination of stenotic, scarry trachea specimens removed with cricotracheal resection:

We have introduced a new semiquantitative method for the classification of the hystopathological severity of benign laryngotracheal stenoses, which is capable of evaluating the effect of different treatment types.

The occurence and degree of inflammatory, necrotic and fibrotic processes affecting deep tissue layers such as the cartilage increases with the growth of the number and extension of endotracheal laser interventions. **We have histologically justified the** clinical observation stating that **there is a connection between the severity of the scarring and the size of the trauma triggered.**

The deviations of high volume observed in each therapy groups also indicate other possible factors affecting the grade of inflammation and fibrosis. Of these, **the role of regulatory factors showing indivudal deviations and determining the scar formation process** on a molecular biological level seems obvious.

Examination of TGF-β superfamily playing a decisive role in the regulation of inflammatory processes, and the predisposition role of its various polymorphisms in the formation of laryngotracheal scarring.

Acquired benign laryngotracheal stenosis associated with abnormal fibrotic processes is one of the most severe complications of endotracheal intubation. We aimed to conduct an initial study to identify genetic susceptibility factors for this disease. The results of our preliminary study suggests that in addition to other factors, **genetic predisposition may contribute to the pathogenesis of acquired benign laryngotracheal stenosis**: the presence of the wild type allele of the TGFβ1 -509 C/T polymorphism that has a high rate of heterozygosity in the general population may play a role in the pathogenesis of this disease. Confirmation our findings by further studies on larger groups of patients may help optimizing the intensive patient care to avoid or diminish the risk of this hardly manageable complication.

The evaluation the in vivo effect of intranasal phototherapy, by assessing DNA damage and repair mechanism in nasal mucosa.

The DNA-damaging effect of high-energy ultraviolet light irradiation is well known, and this effect may indicate the first step of carciogenesis. The majority of literature data refer to the damage formed after UV irradiation of the skin, this is why the parallel study of the impact exerted by UV light on keratinocytes and nasal epithelial cells is important. We gained the epidermal keratinocytes from the skin of donors who had undergone plastic surgery in our institute. Nasal epithelial cells were separated and bred from samples of mucosa resection surgeries. We detected the measure of UV-induced DNA damage using an alkalic Comet assay. During the application of the method, linearly with *the raising of UV doses, the extent of DNA damage also increased in both cell types, therefore we justified that the* ***Comet assay chosen is capable of demonstrating the DNA damage caused by UV light.***

We detected the effect of the intranasal mUV/VIS phototherapy on DNA damage and repair mechanisms in vivo. In our studies, we processed tha nasal mucosa samples of 26 allergic rhinitis patients in a double-blind experimental system using a Comet assay. Our results have shown that in the experimental study design we chose **the *UV/VIS phototherapy did not cause significant DNA damage.***

These results suggest a limited contribution of UV specific DNA damage to the overall cell damage of nasal mucosa in symptomatic allergic rhinitis patients detected at this time-

point with the Comet assay technique. Therefore, ***direct detection of UV specific photoproducts*** *by techniques such as immunostaining of tissue specimens* ***is of outstanding importance for evaluating DNA damage and repair of nasal mucosa*** *(CPD staining).* Our results showed that CPDs can be detected in nasal mucosa samples immediately after irradiation and residual staining was present 10 days after last irradiation. We found that skin and airway mucosa exhibit similar kinetics in repairing UV induced DNA damage.

In this pilot study we have shown for the first time that ***nasal mucosa exposed to UV light possess the capacity to repair DNA damage*** *which suggests that* ***the multistep process of carcinogenesis has not been triggered.***

Preparation of a new therapeutic protocol, establishment of its efficacy and safe usability in the treatment of PAR.

During our current studies we find out whether the ***RL procedure can be used in a safe and effective way for moderate and severe PAR, either****.* Apart from the improvement of *subjective nasal symptoms*, the NIPF presents the efficacy of the RL phototherapy. Abatement of inflammatory processes in the nasal mucosa are also indicated by the decreasing tendency of *ICAM-1 adhesion molecule expression*. *Despite using the* **new, 6-week** (13 treatments) **format of the RL therapy** *and doubling the total dose (2 weeks/6 treatments), we still did not observe any side-effects which were different from that of the placebo.* Neither did we observe a decrease in either the *smelling or the mucociliar functions,* which is another proof for the safety of the RL therapy. However, it must be noted that due to the heterogenity of the allergic patient group, the resumption of studies with large patients numbers involved are necessary for the elevation of our results' value.

The examination of the clinical efficacy and tolerability of NB-UVB phototherapy in bilateral nasal polyps

Our experimental histological studies have justified that UVB penetrates well in vivo into the polyp tissue, it reaches in the stroma the lymphocytes responsible for the inflammation, and induces apopotosis in them. Intranasal phototherapy showed that the UV-induced DNA damage response of respiratory epithelium is very similar to that of the human epidermis and that nasal mucosa is able to efficiently repair UVB induced DNA damage (57,87). NB-UVB phototherapy may represent an alternative, steroid-sparring treatment for

patients with nasal polyps. The severity of NP has generally been correlated to the degree of nasal obstruction and therefore any new treatment has to have a significant effect on this symptom. We have shown that both ***nasal obstruction and quality of life (NOSE) of patients improved significantly after NB-UVB phototherapy***. The therapeutic outcome was stable for at least 3 months after end of treatment despite the fact that most of the patients had not received any specific therapy. Our preliminary data suggest that targeted NB-UVB phototherapy may represent a promising new therapeutic modality in nasal polyposis especially in patients with previous sinus surgeries, but a larger, double-blind study is warranted in the future to assess the therapeutic power of a shorter regimen and to further assess the safety profile of this new treatment.

REFERENCE LIST

1. Lawson WE, Loyd JE. The genetic approach in pulmonary fibrosis: can it provide clues to this complex disease? *Proc Am Thorac Soc* 2006; 3: 345-9.

2. Bonis B, Kemeny L, Dobozy A *et al*. 308 nm UVB excimer laser for psoriasis. *Lancet* 1997; 350: 1522.

3. Scheinfeld NS, Tutrone WD, Weinberg JM *et al*. Phototherapy of atopic dermatitis. *Clin Dermatol* 2003; 21: 241-8.

4. Kang K, Gilliam AC, Chen G *et al*. In human skin, UVB initiates early induction of IL-10 over IL-12 preferentially in the expanding dermal monocytic/macrophagic population. *J Invest Dermatol* 1998; 111: 31-8.

5. Nghiem DX, Kazimi N, Mitchell DL *et al*. Mechanisms underlying the suppression of established immune responses by ultraviolet radiation. *J Invest Dermatol* 2002; 119: 600-8.

6. Csoma Z, Ignacz F, Bor Z *et al*. Intranasal irradiation with the xenon chloride ultraviolet B laser improves allergic rhinitis. *J Photochem Photobiol B* 2004; 75: 137-44.

7. Koreck A, Csoma Z, Boros-Gyevi M *et al*. Inhibition of immediate type hypersensitivity reaction by combined irradiation with ultraviolet and visible light. *J Photochem Photobiol B* 2004; 77: 93-6.

8. Koreck AI, Csoma Z, Bodai L *et al*. Rhinophototherapy: a new therapeutic tool for the management of allergic rhinitis. *J Allergy Clin Immunol* 2005; 115: 541-7.

9. Jiang RS, Su MC, Liang KL *et al*. Preoperative prognostic factors for olfactory change after functional endoscopic sinus surgery. *Am J Rhinol Allergy* 2009; 23: 64-70.

10. Kadocsa E, Koreck IA, Bella Zs *et al*. Intranasalis fototerápia: új terápiás eljárás allergiás rhinitisben. *Fül-, orr-, gégegyógyászat* 2006; 52: 108-14.

11. Kirtsreesakul V, Somjareonwattana P, Ruttanaphol S. The correlation between nasal symptom and mucociliary clearance in allergic rhinitis. *Laryngoscope* 2009; 119: 1458-62.

12. Bousquet J, Khaltaev N, Cruz AA *et al*. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 2008; 63 Suppl 86: 8-160.

13. Balogh K, Argay K, Radich K. A rhinitis allergica prevalenciájának alakulása Budapest III. kerületében 1997 és 2005 között. *Allergol Klin Immunol* 2007; 10: 7-11.

14. Bittera, I., Kadocsa, E., Roman, R., and Túri, S. The changing prevalence of allergic rhinitis among school-children in Szeged in the past 20 years (1987-2007). Allergy 2008; 64 Suppl. 90: 212..

15. Benninger: What is Chronic Rhinosinusitis? Defining Chronic Rhinosinusitis (CRS). *Medical news today:* Main Category: Allergy Article Date: 27 Apr 2004
http://www.medicalnewstoday.com/articles/7698.php

16. Bella Z, Torkos A, Tiszlavicz L *et al*. Cholesterol granuloma of the maxillary sinus resembling an invasive, destructive tumor. *Eur Arch Otorhinolaryngol* 2005; 262: 531-3.

17. Fokkens W, Lund V, Mullol J. European position paper on rhinosinusitis and nasal polyps 2007. *Rhinol Suppl* 2007; 1-136.

18. Csoma Z, Koreck A, Ignacz F *et al*. PUVA treatment of the nasal cavity improves the clinical symptoms of allergic rhinitis and inhibits the immediate-type hypersensitivity reaction in the skin. *J Photochem Photobiol B* 2006; 83: 21-6.

19. **.** A. Koreck, Zs. Bella, E. Kadocsa, A. Perenyi, T.R. Olariu, L. Tiszlavicz, I. Nemeth, M. Kiss, J. Jóri, L. Kemény: Intranasal PUVA phototherapy in nasal polyposis – a pilot study. *Romanian Archives of Microbiology and Immunology*, 1/2010, accepted

20. Dr. Quinn's Online Textbook of Otolaryngology, Series Editor: Francis B. Quinn, Jr, MD Subglottic Stenosis [11-13-2002]
http://www.utmb.edu/oto/Grnds.dir/GrndsIndex.html

21. Koppler l., Schaff Zs. *Pathológia.* Gyulladás, krónikus gyulladás, fibrosis és hegesedés Budapest: Medicina, 2004.

22. Shapshay SM. Laser applications in the trachea and bronchi: a comparative study of the soft tissue effects using contact and noncontact delivery systems. *Laryngoscope* 1987; 97: 1-26.

23. Monnier P, George M, Monod ML *et al*. The role of the CO2 laser in the management of laryngotracheal stenosis: a survey of 100 cases. *Eur Arch Otorhinolaryngol* 2005; 262: 602-8.

24. Drumm ML, Konstan MW, Schluchter MD *et al*. Genetic modifiers of lung disease in cystic fibrosis. *N Engl J Med* 2005; 353: 1443-53.

25. Wu L, Chau J, Young RP *et al*. Transforming growth factor-beta1 genotype and susceptibility to chronic obstructive pulmonary disease. *Thorax* 2004; 59: 126-9.

26. Celedon JC, Lange C, Raby BA *et al*. The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD). *Hum Mol Genet* 2004; 13: 1649-56.

27. Karagiannidis C, Velehorschi V, Obertrifter B *et al*. High-level expression of matrix-associated transforming growth factor-beta1 in benign airway stenosis. *Chest* 2006; 129: 1298-304.

28. Fang R, Sun J, Wan G *et al*. Comparison between mitomycin C and chitosan for prevention of anterior glottic steno after CO2 laser cordectomy in dogs. *Laryngoscope* 2007; 117: 2057-62.

29. Monnier P, Lang F, Savary M. Partial cricotracheal resection for pediatric subglottic stenosis: a single institution's experience in 60 cases. *Eur Arch Otorhinolaryngol* 2003; 260: 295-7.

30. VassarStats http://faculty.vassar.edu/lowry/VassarStats.html

31. Cooper JD, Grillo HC. Analysis of problems related to cuffs on intratracheal tubes. *Chest* 1972; 62: Suppl-27S.

32. Koshkareva Y, Gaughan JP, Soliman AM. Risk factors for adult laryngotracheal stenosis: a review of 74 cases. *Ann Otol Rhinol Laryngol* 2007; 116: 206-10.

33. Shah R, Rahaman B, Hurley CK *et al*. Allelic diversity in the TGFB1 regulatory region: characterization of novel functional single nucleotide polymorphisms. *Hum Genet* 2006; 119: 61-74.

34. Shah R, Hurley CK, Posch PE. A molecular mechanism for the differential regulation of TGF-beta1 expression due to the common SNP -509C-T (c. -1347C > T). *Hum Genet* 2006; 120: 461-9.

35. Frith MC, Bailey TL, Kasukawa T *et al*. Discrimination of Non-Protein-Coding Transcripts from Protein-Coding mRNA. *RNA Biol* 2006; 3: 40-8.

36. Kokturk N, Tatlicioglu T, Memis L *et al*. Expression of transforming growth factor beta1 in bronchial biopsies in asthma and COPD. *J Asthma* 2003; 40: 887-93.

37. Wahl SM. Transforming growth factor-beta: innately bipolar. *Curr Opin Immunol* 2007; 19: 55-62.

38. Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. *FASEB J* 2004; 18: 816-27.

39. Wang XJ, Han G, Owens P *et al*. Role of TGF beta-mediated inflammation in cutaneous wound healing. *J Investig Dermatol Symp Proc* 2006; 11: 112-7.

40. Rovo L, Venczel K, Torkos A *et al*. Endoscopic arytenoid lateropexy for isolated posterior glottic stenosis. *Laryngoscope* 2008; 118: 1550-5.

41. Cadet J, Sage E, Douki T. Ultraviolet radiation-mediated damage to cellular DNA. *Mutat Res* 2005; 571: 3-17.

42. Kuluncsics Z, Perdiz D, Brulay E *et al*. Wavelength dependence of ultraviolet-induced DNA damage distribution: involvement of direct or indirect mechanisms and possible artefacts. *J Photochem Photobiol B* 1999; 49: 71-80.

43. de Laat WL, Jaspers NG, Hoeijmakers JH Molecular mechanism of nucleotide excision repair. *Genes Dev* 1999; 13: 768-85.

44. Tuchinda C, Lim HW, Strickland FM *et al*. Comparison of broadband UVB, narrowband UVB, broadband UVA and UVA1 on activation of apoptotic pathways in human peripheral blood mononuclear cells. *Photodermatol Photoimmunol Photomed* 2007; 23: 2-9.

45. Singh NP, McCoy MT, Tice RR *et al*. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 1988; 175: 184-91.

46. Olive PL, Banath JP Sizing highly fragmented DNA in individual apoptotic cells using the comet assay and a DNA crosslinking agent. *Exp. Cell Res*. 1995. 221 19-26.

47. Patton WP, Chakravarthy U, Davies RJ *at al.* Comet assay of UV-induced DNA damage in retinal pigment epithelial cells. *Invest Ophthalmol. Vis. Sci.* 1999 40 3268-3275.

48. Gluck U, Gebbers JO The Comet Assay of Nasal Epithelial: measurement of DNA damage for assessment of genotoxic air pollution. *The Laryngoscope* 2000 110 123-125

49. Calderon-Garciduenas L, Osnaya-Brizuela N, Ramirez-Martinez L *at al.* DNA strand breaks in human nasal respiratory epithelium are induced upon exposure to urban pollution. *Environ Health Perspect.* 1041996:160-8.
50. Lee E, Koo J, Berger T UVB phototherapy and skin cancer risk: a review of the literature. *Int. J Dermatol* 2005 44 355-360.

51. Bousquet J, Annesi-Maesano I, Carat F *et al*. Characteristics of intermittent and persistent allergic rhinitis: DREAMS study group. *Clin Exp Allergy* 2005; 35: 728-32.

52. Starling-Schwanz R, Peake HL, Salome CM *et al*. Repeatability of peak nasal inspiratory flow measurements and utility for assessing the severity of rhinitis. *Allergy* 2005; 60: 795-800.

53. Doty RL. Office procedures for quantitative assessment of olfactory function. *Am J Rhinol* 2007; 21: 460-73.

54. Gorska-Ciebiada M, Ciebiada M, Gorska MM *et al*. Intercellular adhesion molecule 1 and tumor necrosis factor alpha in asthma and persistent allergic rhinitis: relationship with disease severity. *Ann Allergy Asthma Immunol* 2006; 97: 66-72.

55. Liu CM, Shun CT, Cheng YK. Soluble adhesion molecules and cytokines in perennial allergic rhinitis. *Ann Allergy Asthma Immunol* 1998; 81: 176-80.

56. Lin RY, Nahal A, Lee M *et al*. Cytologic distinctions between clinical groups using curette-probe compared to cytology brush. *Ann Allergy Asthma Immunol* 2001; 86: 226-31.

57. Kemeny L, Koreck A Ultraviolet light phototherapy for allergic rhinitis. *J Photochem Photobiol B* 2007; 87: 58-65.

58. Koreck A, Csoma Z, Ignacz F *et al*. [Intranasal phototherapy for the treatment of allergic rhinitis]. *Orv Hetil* 2005; 146: 965-9.

59. Novak Z, Berces A, Ronto G *et al*. Efficacy of different UV-emitting light sources in the induction of T-cell apoptosis. *Photochem Photobiol* 2004; 79: 434-9.

60. Smal F, Deitmer T Evaluation of nasal patency. *Larygorhinootologie* 1993; 72: 611-3.

61. Koreck A, Szechenyi A, Morocz M *et al*. Effects of intranasal phototherapy on nasal mucosa in patients with allergic rhinitis. *J Photochem Photobiol B* 2007; 89: 163-9.

62. Mitchell D, Paniker L, Sanchez G *et al*. Molecular response of nasal mucosa to therapeutic exposure to broad-band ultraviolet radiation. *J Cell Mol Med* 2010; 14: 313-22.

63. Simola M, Malmberg H. Sense of smell in allergic and nonallergic rhinitis. *Allergy* 1998; 53: 190-4.

64. Guilemany JM, Garcia-Pinero A, Alobid I *et al*. Persistent allergic rhinitis has a moderate impact on the sense of smell, depending on both nasal congestion and inflammation. *Laryngoscope* 2009; 119: 233-8.

65. Passali GC, Ralli M, Galli J *et al*. How relevant is the impairment of smell for the quality of life in allergic rhinitis? *Curr Opin Allergy Clin Immunol* 2008; 8: 238-42.

66. Bousquet J, Reid J, van Weel C *et al*. Allergic rhinitis management pocket reference 2008. *Allergy* 2008; 63: 990-6.

67. Baltas E, Nagy P, Bonis B *et al*. Repigmentation of localized vitiligo with the xenon chloride laser. *Br J Dermatol* 2001; 144: 1266-7.

68. Baltas E, Csoma Z, Ignacz F *et al*. Treatment of vitiligo with the 308-nm xenon chloride excimer laser. *Arch Dermatol* 2002; 138: 1619-20.

69. Baltas E, Csoma Z, Bodai L *et al*. Treatment of atopic dermatitis with the xenon chloride excimer laser. *J Eur Acad Dermatol Venereol* 2006; 20: 657-60.

70. Bachert C, Zhang N, Patou J *et al*. Role of staphylococcal superantigens in upper airway disease. *Curr Opin Allergy Clin Immunol* 2008; 8: 34-8.

71. Bachert C, Gevaert P, Holtappels G *et al*. Mediators in nasal polyposis. *Curr Allergy Asthma Rep* 2002; 2: 481-7.

72. Bachert C, Gevaert P, Holtappels G *et al*. Total and specific IgE in nasal polyps is related to local eosinophilic inflammation. *J Allergy Clin Immunol* 2001; 107: 607-14.

73. Ponikau JU, Sherris DA, Kern EB *et al*. The diagnosis and incidence of allergic fungal sinusitis. *Mayo Clin Proc* 1999; 74: 877-84.

74. Trehan M, Taylor CR. Low-dose excimer 308-nm laser for the treatment of oral lichen planus. *Arch Dermatol* 2004; 140: 415-20.

75. Weichenthal M, Schwarz T. Phototherapy: how does UV work? *Photodermatol Photoimmunol Photomed* 2005; 21: 260-6.

76. Novak Z, Bonis B, Baltas E *et al*. Xenon chloride ultraviolet B laser is more effective in treating psoriasis and in inducing T cell apoptosis than narrow-band ultraviolet B. *J Photochem Photobiol B* 2002; 67: 32-8.

77. Meltzer EO, Hamilos DL, Hadley JA *et al*. Rhinosinusitis: developing guidance for clinical trials. *J Allergy Clin Immunol* 2006; 118: S17-S61.

78. Stewart MG, Witsell DL, Smith TL *et al*. Development and validation of the Nasal Obstruction Symptom Evaluation (NOSE) scale. *Otolaryngol Head Neck Surg* 2004; 130: 157-63.

79. Alobid I, Benitez P, Valero A *et al*. The impact of atopy, sinus opacification, and nasal patency on quality of life in patients with severe nasal polyposis. *Otolaryngol Head Neck Surg* 2006; 134: 609-12.

80. Passali D, Bellussi L, Hassan HA *et al*. Consensus Conference on Nasal Polyposis. *Acta Otorhinolaryngol Ital* 2004; 24: 3-61.

81. Pawankar R. Nasal polyposis: an update: editorial review. *Curr Opin Allergy Clin Immunol* 2003; 3: 1-6.

82. Kankaanranta H, Moilanen E, Zhang X. Pharmacological regulation of human eosinophil apoptosis. *Curr Drug Targets Inflamm Allergy* 2005; 4: 433-45.

83. Dalziel K, Stein K, Round A *et al*. Endoscopic sinus surgery for the excision of nasal polyps: A systematic review of safety and effectiveness. *Am J Rhinol* 2006; 20: 506-19.

84. Watanabe K, Shirasaki H, Kanaizumi E *et al*. Effects of glucocorticoids on infiltrating cells and epithelial cells of nasal polyps. *Ann Otol Rhinol Laryngol* 2004; 113: 465-73.

85. Benson M. Pathophysiological effects of glucocorticoids on nasal polyps: an update. *Curr Opin Allergy Clin Immunol* 2005; 5: 31-5.

86. Hamilos DL, Thawley SE, Kramper MA *et al*. Effect of intranasal fluticasone on cellular infiltration, endothelial adhesion molecule expression, and proinflammatory cytokine mRNA in nasal polyp disease. *J Allergy Clin Immunol* 1999; 103: 79-87.

87. Bloom JW, Chacko J, Lohman IC *et al*. Differential control of eosinophil survival by glucocorticoids. *Apoptosis* 2004; 9: 97-104.

88. Bonfils P, Halimi P, Malinvaud D. Adrenal suppression and osteoporosis after treatment of nasal polyposis. *Acta Otolaryngol* 2006; 126: 1195-200.

89. Menter A, Gottlieb A, Feldman SR *et al*. Guidelines of care for the management of psoriasis and psoriatic arthritis: Section 1. Overview of psoriasis and guidelines of care for the treatment of psoriasis with biologics. *J Am Acad Dermatol* 2008; 58: 826-50.

90. Pathirana D, Ormerod AD, Saiag P *et al*. European S3-guidelines on the systemic treatment of psoriasis vulgaris. *J Eur Acad Dermatol Venereol* 2009; 23 Suppl 2: 1-70.

91. Lee E, Koo J, Berger T. UVB phototherapy and skin cancer risk: a review of the literature. *Int J Dermatol* 2005; 44: 355-60.

92. Grisendi S, Mecucci C, Falini B *et al*. Nucleophosmin and cancer. *Nat Rev Cancer* 2006; 6: 493-505.

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ATTACHEMENT: REPRINTS OF PUBLISHED PAPERS