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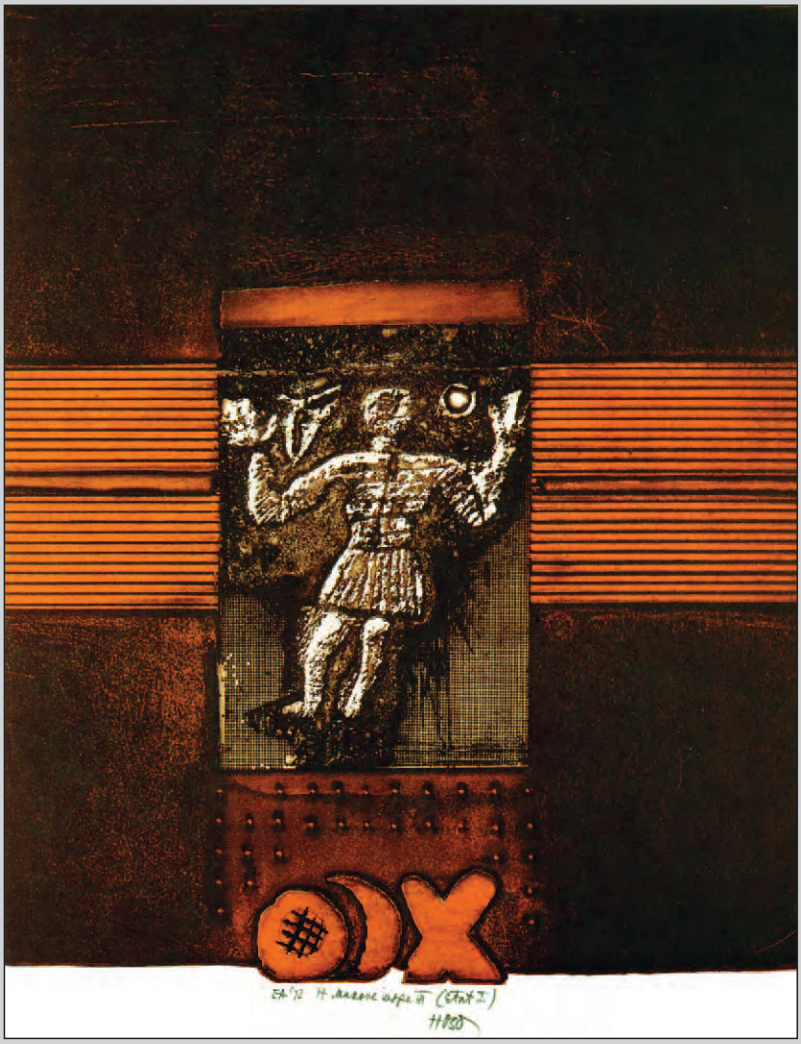

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Branch of the Cochrane Collaboration founded in Croatia

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At the end of 2006 the Croatian Medical Journal and the University of Split School of Medicine started a campaign to establish a Croatian entity of The Cochrane Collaboration. The reasons for founding a Cochrane entity in Croatia were the advancement of evidence-based medicine and multifold education of Croatian health care workers about The Cochrane Library and its importance for medicine. In 2008 the Croatian Branch of the Italian Cochrane Center was founded with the purpose of promoting evidence-based medicine, The Cochrane Collaboration and *The Cochrane Library* in Croatia, and to encourage Croatian healthcare workers to become authors of Cochrane systematic reviews. The Cochrane Collaboration prepares, maintains and promotes systematic reviews for the benefit of clinical medical practice. *The Cochrane Library* is a result of international effort, mostly based on the work of thousands of volunteers and the very few professional staff employed at Cochrane entities. Likewise, the Croatian Branch of the Italian Cochrane Center relies mostly on the volunteer work of multiple collaborators and has very few staff members in the central office. With the foundation of the Cochrane entity in Croatia, the evidence-based medicine movement in the whole region should be enhanced.

Key words: Systematic reviews, The Cochrane Collaboration, The Cochrane Library,

Introduction

In 2006 a small group of enthusiasts from the University of Split School of Medicine and Croatian Medical Journal were gathered around the ideas promoted by The Cochrane Collaboration. They saw the establishing of the Croatian Cochrane entity as an opportunity for enhancing medical practice and introducing Croatian healthcare workers to The Cochrane Library, as an important source of information for clinical decisions. As a result of their efforts and two-year preparations, the Croatian branch of the Italian Cochrane center (CBICC) was established in 2008. It immediately became an invaluable contribution to the evidence-based medicine (EBM) movement in Croa-

tia. CBICC is now one of the 26 international centers of The Cochrane collaboration.

The Cochrane Collaboration

The Cochrane collaboration is a unique, worldwide, non-profit organization that aims to help people make well-informed decisions about all forms of health care by preparing, maintaining and promoting the accessibility of systematic reviews of the effects of health care interventions (1).

A systematic review is a literature review focused on a single question, that tries to identify, appraise, select and synthesize all high quality research evidence relevant to that question. Systematic reviews of high-quality randomized controlled trials are crucial to evidence-based medicine. An understanding of systematic reviews and how to implement them in practice is becoming mandatory for all professionals involved in the delivery of health care. Systematic review may contain meta-analysis, a statistical method that combines results of several different studies, but there are also systematic reviews where meta analysis is not appropriate for various reasons (2).

Since systematic reviews are based on demanding methodology and they can be of variable quality and quickly become out of date, The Cochrane Collaboration is addressing all these issues by avoiding duplication, encouraging authors to update their reviews, promoting its resources and providing support to review authors.

The Cochrane collaboration was founded in 1993 and named after the British epidemiologist, Archie Cochrane. The members of The Cochrane Collaboration are organized into groups, known as 'entities', which include groups of health care workers, as well as groups of patients (3). Data from The Cochrane Library in 2004 show that there were more than 11,500 people working within The Cochrane Collaboration in over 90 countries, half of whom are authors of Cochrane Reviews. The number of Cochrane authors and collaborators has increased by about 20% every year for the last five years (3).

The Cochrane Library

The main product of The Cochrane collaboration is *The Cochrane Library*.

It is a collection of databases that contain high-quality, independent evidence to inform healthcare decision-making. Cochrane systematic reviews represent the highest level of evidence on which to base clinical treatment decisions. The Cochrane Library has multiple resources, among which the most popular and most used is the Cochrane Database of Systematic Reviews. Currently *The Cochrane Library* comprises 3916 complete reviews and 1905 protocols (4). Other than systematic reviews, The Cochrane Library provides other sources of reliable information: other systematic reviews abstracts, technology assessments, economic evaluations, and individual clinical trials – all the current evidence in one single environment (4).

The Cochrane Library is published by John Wiley and Sons Ltd., a commercial publisher. In this way The Cochrane Collaboration does not need to spend its scarce resources and staff on the process of publishing and advertising the reviews, and royalties that are earned through subscriptions are shared between publisher and The Cochrane Collaboration. On the other hand, the commercial publisher charges for the use of The Cochrane Library, so healthcare workers and users need to have either an institutional or a personal subscription. Multiple countries have purchased nationwide provision, which means that every computer in that country can access The Cochrane Library. Also, Wiley provides *Cochrane Library* access free-of-charge to the poorest countries. Third-party funds for ac-

cessing The Cochrane Library are limited to non-pharmaceutical sources of funding to prevent conflicts of interest.

The Croatian Branch of the Italian Cochrane Center and its business plan

The main goal of CBICC is knowledge translation, including continuing education and dissemination of information about available research evidence. Therefore, the First Croatian Cochrane Symposium was organized on June 27, 2009 at the University of Split School of Medicine. The Symposium was attended by people from Croatia but also by people from surrounding Eastern countries, especially Bosnia and Herzegovina. The participants were acquainted with the work of The Cochrane Collaboration and more specifically, the work of the CBICC and obtained other valuable information on how to use and possibly even publish in *The Cochrane Library.* Other CBICC activities include preparing free online continuous education courses about the preparation and maintenance of a Cochrane systematic review in the Croatian language, that will be available to Croatian healthcare workers, and for which they will be able to obtain continuous education credits from their respective professional associations. The CBICC would like to provide education about The Cochrane Collaboration and *The Cochrane Library* in the Croatian language to motivate Croatian healthcare workers and users to engage actively in evidence-based medicine by using its principles and creating the best evidence.

The CBICC has many more ambitious goals, for instance: securing funds for temporary scholarships that will provide full-time opportunities for creating systematic reviews; lobbying for nationwide access and establishing a Cochrane Review group in Croatia. But, to begin with, more effort is needed to raise awareness that The Cochrane Library exists, that it is available to biomedical consortia and that Croatian healthcare workers can become part of it. The past decades have seen The Cochrane Collaboration develop into a mature and internationally recognized organization that meets its goals (5). This is what CBICC hopes to accomplish.

Publishing a Cochrane systematic review

In order to write a Cochrane systematic review, the first step is to browse The Cochrane Library and to check whether a systematic review, protocol or title that are identical or similar to the chosen subject has already been published. If there is no such review, it is necessary to determine which Cochrane Review Group his or her area of interest belongs to. The next step is contacting the Review Group and suggesting a title for the review. To avoid duplication of effort within The Cochrane Collaboration, one title belongs to only one author or group of authors. Once the title is accepted, nobody else will be permitted to prepare a Cochrane systematic review with the same topic. After the title has been submitted, the Review group expects the author(s) to submit a protocol within 6 months. A protocol consists of an introduction - description of background knowledge about the subject, and a description of the methods that will be used, including details about search strategy, keywords for searching literature and databases that will be searched. When the protocol is submitted, the Review group evaluates it and then sends it to two peer-reviewers and sends feedback to authors. The protocol, when completed, is published in The Cochrane Library and then the authors may start to review literature based on the protocol and to prepare a full systematic review. Usually it takes up to 24 months for authors to prepare a review after the protocol is accepted.

The Cochrane Library is an international publication indexed in Current Contents and its impact factor for year 2008 is 5.1. This high impact factor serves not only as confirmation of the quality of a Cochrane publication, but also as a publication that can be used for academic advancement in Croatia. Preparation of a systematic review using the Cochrane methodology is challenging, but it is important to keep in mind that a Cochrane systematic review will be accepted for publication after it is completed, because a Cochrane Review Group helps the author(s) to prepare it and it is in their interest to have the review published. In other journals, the author sends completed manuscripts and can only hope that the manuscript will not be rejected.

In conclusion, The Cochrane Collaboration is an exciting organization. The CBICC has many plans for the future and would be happy to include neighboring countries in its activities. All potential authors, volunteers and organizations interested in Cochrane work are welcome to contact the CBICC to arrange future collaboration.

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Lymphocyte expression of CD4+CD25^{hi} and adhesion molecules in children with Atopic dermatitis: the effect of Levocetirizine treatment

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There is considerable evidence that several novel H1 antihistamines possess anti-allergic/ anti-inflammatory properties, through inhibition of leukocyte activation. Levocetirizin and other H1 antihistamines are considered central to the treatment of atopic dermatitis (AD) associated pruritis; however there is a lack of studies of possible anti-inflammatory effect of these drugs in children with AD. In this study, we investigated the lymphocyte sub-population profile in the peripheral blood of 15 children with AD at baseline and following two weeks of levocetirizine treatment. The clinical symptoms and flow cytometric analysis of the percentage expression of CD4+CD25+ subsets on T cells, as well as expression of the adhesion molecules; CD4+CD54+ (ICAM-I) and CD4+CD62+ (L-Selectin) on T cells were evaluated. The children exhibited a reduction in the percentages of the eosinophil count ($p<0.05$) as well as major clinical symptoms, itching/ scratching ($p<0.05$) and the subsequent bleeding of lesions ($p<0.05$); however the total symptom score was not significantly changed. A significant increase was observed in CD4+CD25^{hi} Treg cells while CD4+CD54+ (ICAM-I) cells were significantly decreased, and no significant change was observed in other populations. Reduction of CD4+CD54+ may be associated with suppression of IgE production and hence reduced mast cell recruitment into the inflammatory sites, on the other hand; expansion of CD4+CD25^{hi} indicates that Treg-mediated host immune defenses are augmented. Our study suggests the potential of the anti-inflammatory effects of levocetirizine in allergic inflammation.

Key words: Dermatitis, Treg cells, Adhesion molecules, Levocetirizine.

Introduction

Atopic dermatitis (AD) is a common chronic inflammatory skin disorder with intense pruritis and typical cutaneous symptoms, and is frequently seen in patients with a family history of atopy (1). Patients with AD are a heterogeneous group of whom about 80%

show immediate type skin reactions and elevated serum IgE levels (2). In most cases AD appears in early childhood, current lifetime prevalence is estimated to be between 10-20% in children and 1-3% in adults (3). For diagnosis, at least three of both the major and minor criteria (pruritis, typical morphology and distribution, chronic or relapsing skin lesions, personal or family history of atopy etc) should be present (4). The pathogenesis of AD involves a complex inflammatory process which is not yet fully understood and is constantly undergoing revision as more data become available (5, 6). Recent studies indicate that the marked elevation of IgE is the result of T-cell dysregulation in AD patients (7). Over the past few years it has become increasingly clear that T-cells contribute to the abnormal regulation of the immune response in atopic diseases. Th2-type CD4+ T cells appear to be crucial but still little is known about the contribution of other subsets of T cells (8).

In recent years, a specific subset of regulatory T cells bearing a CD4+CD25+ T-cell phenotype has been the focus of extensive investigation (9). These T cells, endowed with distinct immuno-modulatory properties, are important components of the homeostasis of the immune system, as impaired CD4+CD25+ T-cell activity can cause both autoimmune and allergic diseases (10, 11). There is evidence of the role of $CD4^{+}CD25^{+}$ regulatory T cells in suppressing T-cell responses to allergens (12, 13). Expression of the transcription factor, Foxp3, is critical to the development of $CD4^{+}CD25^{hi}$regulatory T cells with suppressor function. It was recently reported that human $CD4+CD25^{hi}$ T cells associated with inflammatory diseases such as AD may be a mixture of activated effector T cells and regulatory T cells, the two subtypes were identified on the basis of differential expression of the chemokine receptor CCR6 (14). Furthermore this study found that activated $CD25^{hi}$ T cells that lack expression of CCR6 promote TH2 responses.

Recent studies have demonstrated that several adhesion molecules play a critical role in the recruitment and migration of leucocytes to sites of inflammation in various diseases (15, 16). Important adhesion molecules expressed on leucocytes or endothelial cells include intercellular adhesion molecule-1 (ICAM-1) and L-selectin. The levels of adhesion molecules have been reported to increase in patients with allergic diseases (17-20). Higher levels of adhesion molecules in serum samples from atopic individuals may reflect the up-regulation of cell-surface ICAM-1 expression in allergic inflammation.

There is now considerable evidence from both in vitro and in vivo studies that several novel H1 antihistamines possess anti-allergic/ anti-inflammatory properties, through inhibition of leukocyte activation and reduction of ICAM1 expression on epithelial cells (21, 22, 23). Levocetirizine, as a R-entantiomer of Cetirizine dichloride having high bioviability and rapid onset of action, is effective for treatment of allergic rhinitis and chronic urticaria, showing several anti-inflammatory effects that are observed at clinically relevant concentrations that may enhance its therapeutic benefit. (21, 24). Levocetirizin and other H1 antihistamines are considered central to the treatment of AD associated pruritis and are widely used despite a lack of double blind randomized clinical trials (25). Also, there is lack of studies of the possible anti-inflammatory effect of H1 antihistamines in children with AD.

The aim of this study was to investigate the effect of levocetirizine on lymphocyte expression of $CD4+CD25^{hi}$ T cells and the adhesion molecules ICAM-I and L-selectin in children having a moderate – form of atopic dermatitis from early childhood.

Patients and Methods

Patients

The study included 15 atopic children; 9 females and 6 males with an age range of 7-14 years old (mean age 12.36 ± 0.9) diagnosed with moderate to severe atopic dermatitis. All patients were diagnosed from early childhood (before 5 years of age). The diagnosis of atopic dermatitis was based on a constellation of typical clinical features, such as extended eczematous lesions with pruritis and scratching of affected areas. Chronic or relapsing dermatitis was frequently associated with personal or family history of atopic disease. Atopy was confirmed by the increased level of specific IgE to one or more inhalant and /or food allergens (food allergy was implicated in approximately one third of our patients). Severity of the disease was assessed by a physician on the basis of skin condition experienced over the past 6 weeks, expressed as a total clinical symptoms score (TCSS) (1-12) which included the following: a) skin thickening: 1= mild, 2 = moderate, 3 = severe; b) skin itching/scratching: 1= mild, without significant changes in daily activities and without night sleep disturbance, 2 = moderate with occasional night sleep awakening, 3 = severe itching with frequent sleep disturbance; c) location of the conditions:1 = mild: flexuous side of arms and/or legs, 2 = moderate: +lesions on the neck and face, 3 = severe: +lesions on other part of the body with excessive dryness / scaling or blisters; d) number of times/ year that symptoms flare up: 1 = mild: 1-2 times , 2 = moderate: 3-5 times, 3 = severe: more than 6 times. Only children with moderate to severe dermatitis (clinical score ranged 8-12), were enrolled in the study. Patients on antihistamines and topical corticosteroids within the previous week were excluded. Fourteen healthy children with no history or sign of atopic diseases; 8 females and 6 males with an age range of 8- 15 years old (mean age 13.5 ± 0.6) served as a control group. Blood samples were collected from children with AD at baseline and following two weeks of treatment with levocetirizine (5 mg/ day); one blood sample was collected from each healthy child. All samples were collected in the early morning. Informed consent was obtained from the parents of the patients and the controls.

Measurement of Lymphocyte subpopulations

Five ml of peripheral venous blood were collected from each subject in EDTA tubes and analyzed within 4-6 hours. Fifty µl of blood were incubated for 30 min at room temperature with 5 µl of flourescein-isothiocyanate (FITC), phycoerythrin (RD1) or PerCP (peridin chlorophyll protein) conjugated monoclonal antibodies (mAb), to surface markers of interest. The cells were then treated with Q-prep (Coulter Corporation, Hialeah, FL, USA) for hemolysis, stabilization and amplification of the antigen-antibody reaction and fixation with paraformaldehyde. Two and three color fluorescence analysis using an automated flow cytometer (Coulter Epics Altra) was performed. Positive analysis regions for cells expressing specific surface antigens were compared with isotypic controls and the specific binding of fluorophore-conjugated monoclonal antibodies was analyzed according to standard methods recommended by the manufacturer. Monoclonal antibodies specific for human CD4+CD54+ (ICAM1+ T cells), CD4+CD62+ (L-Selectin+ T cells) and CD4+ CD25+ (activated T cells – some with a regulatory phenotype) were used. All fluorophores were purchased from Immunotech, Beckman Coulter Corporation, Hialeah, FL, USA. Typical histogram data are depicted in Fig. 1, showing CD4+ and CD25+ subpopulations. The total population of CD4+ cells are mostly contained in areas B1 +M1 + M2, with CD4 + CD25+low cells represented in area M1;

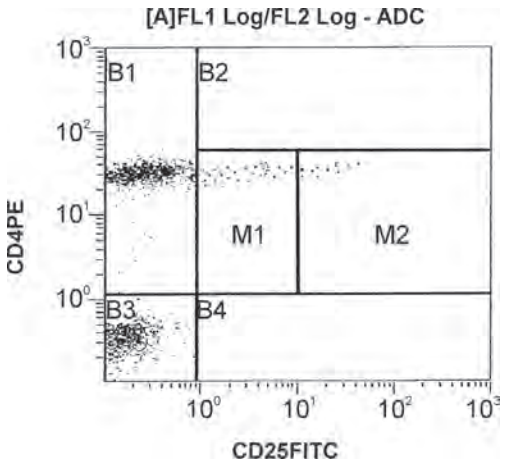

Figure 1 Expression of CD4+CD25+ subpopulations in one participant in the study. CD4+CD25+^{low} cells are represented in area M1; and CD4+CD25+^{hi} in area M2.

CD4 + CD25+hi in area M2 and CD4+ cells in area B1 considered to express negligible levels of CD25 (i.e. these are non-activated T cells). The frequency of CD4 + CD25+low was calculated as the frequency ratio for M1/B1 +M1 +M2 and the frequency of CD4 + CD25+hi as M2/B1 +M1 + M2. This analysis was used for cells taken from each participant in the study.

Statistical analysis

Data are presented as box plots displaying medians and interquartile ranges (IR) for the variables that exhibited statistically significant differences when compared between the study groups. As the variables evaluated were not distributed normally, the mean comparisons were done by non-parametric analysis (Kruskall–Wallis and, if significant, Mann–Whitney *U* test). All reported *p*-values represented two-tailed tests and $p \leq 0.05$ was considered statistically significant. Non-parametric Spearman correlations were performed to measure the association between variables. Statistical analyses were performed using the SPSS for Windows Program Version 14 (Norusis/SPSS Inc.).

Results

Levocetirizine treatment of AD patients had improved quality of life expressed as fewer disturbances of night sleep. The analysis of the clinical symptom score showed that levocetirizine had reduced the itching/scratching circle (p=0.011) as well as the bleeding of lesions (p=0.006) (Fig.2); how-

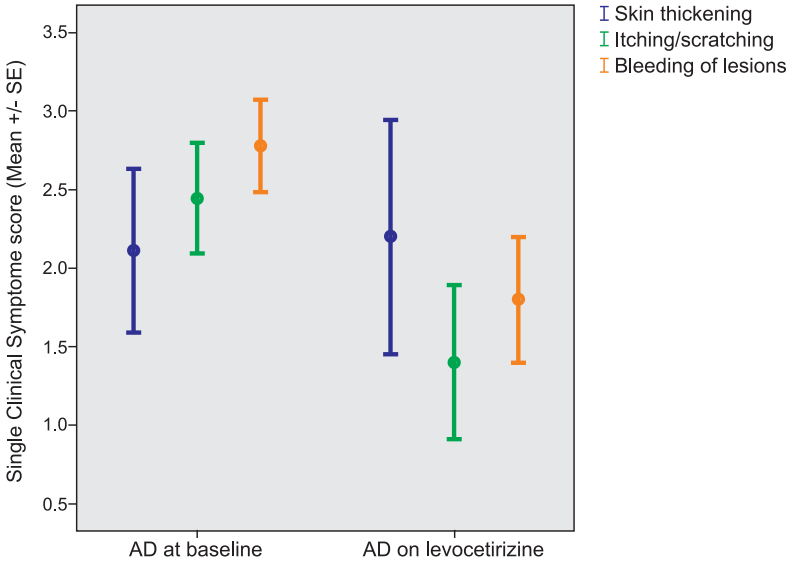

Figure 2 Box plot representation of the single symptoms score in AD patients. Comparisons were made between the groups studied: AD group at baseline (n = 15), AD following levocetirizine treatment and the control group (n =7). Statistical differences (*) were considered significant at p < 0.05.

Table 1 Median and IR (interquartil ranges) of eosinophils and T lymphocyte subpopulations in atopic dermatitis at baseline, following levocetirizine treatment and in controls

	Atopic Dermatitis at Baseline		Atopic Dermatitis on Levocetirizine		Control	
	Median	IR (75-25)	Median	IR (75-25)	Median	IR (75-25)
Eosinophils	7.85**	14.35-3.68	2.5ψ	6.2-1.93	2.2	3.2-1.2
CD4+CD25+ cells	2.97	4.45-2.0	1.93	2.47-1.3	2.4	2.3-0.5
$CD4+CD25^{hi}$	0.33*	0.56-0.3	0.99ψ	1.85-0.61	1.2	1.5-0.21
$CD4+CD25^{low}$	1.65	2.65-1.4	1.58	2.1-1.0	1.0	2.0-0.34
CD4+CD54+ cells	8.0**	9.2-6.1	2.5ψψ	2.9-1.99	2.3	5.5-1.7
CD4+CD62+ cells	8.1	17.8-6.5	10.95	15.5-10.9	3.9	19.3-2.7

Comparisons were made between eosinophil count and T lymphocyte expression of surface antigens in AD and control, statistical differences were considered significant at p<0.05.
**p<0.05, ** p<0.01 versus healthy control, ψ P<0.05, ψψ p<0.01 versus baseline*

ever the total symptom score was not significantly changed. As shown in Table 1, levocetirizine treatment significantly reduced the percentages of eosinophils (p= 0.027). Lymphocyte expression of CD4+CD25+ T cells with two subsets: $CD4+CD25^{hi}$ cells and $CD4+CD25^{low}$cells are shown. Following levocetirizine treatment, no significant change was observed in the percentage of CD4+CD25+ cells (median 1.93; IR: 2.47-1.3) versus baseline (median 2.97; IR: 4.45-2.0; p= 0.132), the percentage of $CD4+CD25^{hi}$ was significantly increased (median 0.99; IR: 1.85-0.61) versus baseline (median 0.33; IR: 0.56-0.3; p= 0.048), while the $CD4+CD25^{low}$ subset was not significantly changed (median 1.58; IR: 2.1-1.0) versus baseline (median 1.65; IR: 2.65-1.4; p= 0.295). CD4+CD54+ T cell subset (ICAM-I) was significantly reduced (median 2.5; IR 2.9-1.99) versus baseline (median 8.0; IR 9.2-6.1; p= 0.024) (Table 1 and Fig. 4), on the other hand CD4+CD62+ T cell subset (L-selectine) was not significantly changed (median 10.95; IR 15.5-10.9) versus baseline (median 8.1; IR 17.8-6.5; p= 0. 241).

Discussion

The management of AD is difficult due to the fact that its pathogenesis is still obscure. A major therapeutic challenge is to reduce the itching/ scratching circle, which could be achieved by controlling chronic allergic inflammation. H1-antihistamines are widely used in AD patients for the control of pruritis, despite the lack of double blind randomized clinical trials (25, 26). Antihistamine action in the treatment of allergic disease is the competitive antagonism of histamine binding to cellular receptors. Recently, many studies have shown that H1-antihistamines, beside their antihistaminic effects, have additional anti-inflammatory properties (5, 21, 22, 23). They are capable of inhibiting inflammatory cell migration and activation, and adhesion molecule expression in tissues affected by allergic inflammation (24, 27). Such effects are already known in the treatment of seasonal allergic rhinitis (28) and chronic urticaria (29) both in adults and children. However few studies have addressed the anti-inflammatory activities of H1- histamine antagonists in AD patients (30, 31). Levocetirizine, as an active enantiomer of cetirizine, is one of the most recent antihistamines and is indicated for symptomatic relief in different allergic diseases, with clear evidence of possessing anti-inflammatory activities (32), which could be useful in the treatment of AD patients.

We evaluated the efficacy of levocetirizine in fifteen AD patients to determine whether two weeks of treatment (5 mg once daily) would induce clinical improvement shown through CSS and changes in inflammatory parameters. As expected, our results were similar to the findings of other studies which showed significant improvement, expressed as reduction of CSS, particularly itching/ scratching (Fig. 2) and subsequent improvement in quality of life expressed as less disturbed night sleep.

Our understanding of the complex inflammatory process in AD is constantly undergoing revision as more data become available (26). It is already known that interaction among susceptible genes and environmental factors activate different immune cells and their products, leading to clinical manifestation. The phenotype of AD depends on factors which have an important impact on the severity of the disease. T cell-driven inflammation appears to be crucial in the pathogenesis, characterised by skin infiltration with migrating T lymphocytes. Although CD4+ T cells appear to be crucial in AD pathophysiology (8), little is known about the role of a specific subset of T cells bearing a CD4+CD25+ phenotype in AD patients (32, 33). As shown in our results, we have evaluated that a particular T cell subset in peripheral blood is a parameter of allergic inflammation. We found no significant difference in the percentages of these cells following levocetirizine treatment (Table 1). A particularly interesting outcome of treatment with this drug is the apparent induction of an expanded CD4+CD25+hi subpopulation ($p<0.05$) (Fig. 3) which suggests that it might augment Treg-mediated host immune defence. It has been shown that histamine stimulation of H4 receptors suppresses pathogenic processes and promotes expansion of peripheral blood Treg subpopulations (34). These findings raise the possibility that levocetirizine-H1 interaction may converge or reinforce histamine stimulation of H4 receptors.

Eosinophil participation in allergic inflammation depends on maintenance of cell viability and function. Eosinophils are recruited and activated at the site of inflammation, releasing a wide variety of mediators (33). Similar to the results shown by Segwik (27) that cetirizine is capable to affect eosinophil survival in patients with AD, our results showed a significant reduction in eosinophil count (Table 1).

Additionally, similar to other studies (6, 32, 35), our results confirmed the possible immune modulating role of levocetirizine, through reduction of adhesion molecule expression, especially ICAM-I (Fig. 4). Possibly, levocetirizine is capable of regulating the release of cytokines and chemokines and consequently reduces recruitment of the inflammatory cells (28). In contrast to ICAM-I molecules, we could not find significant reduction in L- selectin (Table 1). Such results could be a consequence of the fact that the selectin family mediates tethering and rolling of leukocytes while the Ig superfamily, including ICAM -1, is critical for the firm adhesion (6). It is also possible that ICAM –1 has closer cooperation with L-selectin, to mediate optimal leukocyte rolling (36), which we did not observe in this study. Reduction of expression of ICAM –1 and the selectin family may be responsible for suppression of IgE production, as explained by Shimada et al (6), which could reduce rapid mast cell recruitment into the inflammatory sites. Our results confirm previous analyses (28, 36) of the anti-inflammatory effects of levocetirizine in allergic inflammation. CONCLUSION: This study demonstrates that levocetirizine induces *in vivo* suppression of eosinophils as well as ICAM I expression on CD4+ T cell of AD patients, on the other hand, expansion of CD4+CD25+hi Treg cells was observed. These findings may indicate the important immunomodulatory

effects of this drug and suggest future investigation of the cellular and molecular mechanisms underlying the role of antihistamine in immunoregulation.

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Treatment at a day-care hospice of patients after mastectomy for breast cancer improves their physical and mental health

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Objective. The aim of this research was to establish whether three-month treatment by a multidisciplinary team at a day-care hospice improves the physical and mental health (PMH) of patients after mastectomy for breast tumours and after the completion of oncological therapy. **Patients and methods.** By a prospective study undertaken on the palliative care ward of the University Clinical Centre in Tuzla, Bosnia and Herzegovina from May 2006 to May 2007, 35 patients were surveyed who had undergone mastectomy for breast tumours and had completed specific oncological therapy. The treatment by the team at the day-care hospice lasted three months. For an assessment of PMH a SF-36 scale was used. In the statistics we used the even T-test and the Wilcox test. The difference was seen to be significant at $p < 0.05$. **Results.** The overall physical health of the patients examined after treatment at the day-care hospice was taken to be 0.55 (0.31 – 0.86) points and was statistically significantly better than the test before treatment at 0.42 (0.27- 0.83; $p < 0.0001$). Improvement was achieved in the sub-scales of general health and physical function. Treatment in the day-care hospice of the patients examined also led to improvement of their overall mental health, especially on the sub-scale of social functioning and mental health. **Conclusion.** The research established the improvement of all aspects of mental health and most aspects of physical health in the patients after three months' treatment by a multidisciplinary team at the day-care hospice.

Key words: Physical and mental health, Breast cancer, Hospice

Assessment of the state of the physical and mental health of patients suffering from malignant tumours should identify and describe the harmful effects of the disease and the therapy used, and create the possibility of choosing the most appropriate further therapy procedure. The old paradigm of treating oncological diseases based on the exclusive use of drugs, which most often do not treat the cause of the disease, and also introduce a large amount of interactions and side effects, requires thorough change. The

results of research into the inter-connection between spirituality, hope and social support for people with oncological diseases show their positive effect on physical and mental health (1). It is expected that about 32% of the population will suffer from some form of malignant tumour during their life, and almost 50% of patients require medical, social and financial support from the health care system, family and society (2).

Breast cancer is rare before 25 years of age. The highest incidence is between the ages of 45 and 55. The risk of breast tumours grows significantly with age so that almost ½ breast tumours occur in women after they are 65 and ¼ after 75 years of age (3). This is the most common form of malignant tumour in women, which develops quietly without any subjective difficulties. It is often seen by the patients and their environment as a hard, undeserved punishment, which after a great deal of suffering and trouble, leads to a fatal outcome. In patients with breast tumours other problems arise such as sexual function disturbance, difficulties accepting the loss of a body part and hair, but also problems related to the family such as "conflict" between the desire to protect the member who is sick and the desire for the children or the fragile patient to be spared stress. Precisely for these reasons there is a connection between emotional and psychosocial problems in both patients and members of their families, who also need support (4). The enormous growth of the number of sufferers from breast cancer demands a specific approach to this category of patients, an understanding of their problems, with the continuous education of the patients, but also their families and relatives. Most often the difficulties suffered by these patients may be divided into two groups: physical (weakness, pain, sleepiness, nausea, loss of appetite) and psychological (depression, anxiety, sadness, loss of concentration). These reactions may occur individually, several of them together or some may not occur at all. The intensity of reaction varies, but the psychological response of the patient is most expressed when the first signs of the illness are discovered, when they are informed of the diagnosis or immediately after that, on the first or repeated therapy, the end of the therapy and during the social reintegration of the patient. In patients with breast tumours the greatest need for group psycho-social and spiritual support occurs immediately after the diagnosis, or during active treatment. These groups should be homogenous (only patients with breast tumours and not other tumours) making it possible to pass on experience intensively between patients and for medical staff to offer information about further treatment and the prognosis of the disease (5). The first day-care hospice, opened in 1999 at St. Christopher's Hospice in London, set as its primary goal the improvement of the quality of life of patients, that is, giving a new dimension to the program of care already established for patients and their families. The aim of that project was to help people to get out of their "four walls", to rest from their illness, to socialize and feel supported, all as part of a wide variety of activities (6).

The day-care hospice has a regular and structured program of activities, and patients can visit it on the days that best suit them. They usually take 10-15 patients a day, enabling them to be away from home longer than would otherwise be possible. They find new meaning and purpose in life there. Gentle exercises in the physiotherapy department to relaxing music are part of the regular program, which all promotes their mobility, improves their mood and reduces anxiety and stress (7). Occupational therapy is intended to stimulate the patients, enrich their lives and give them back a feeling of their own worth (8), and the friendships made help to restore self-respect and self-confidence, which also creates better rela-

tionships with family members. It is necessary to gain the patients' full confidence, and open room for communication in once completely isolated and withdrawn patients. This is best illustrated by the patients in their own words, "Some patients get stuck on the surface level of their experience and they need help to move from a superficial reaction to something deeper during treatment" (9).

The aim of the research was to establish whether three-month treatment by a multidisciplinary team at a day-care hospice improves the physical and mental health of patients after mastectomy for breast tumours and after completion of specific oncological therapy.

Patients and methods

The prospective study was conducted on the palliative care ward of the University Clinical Centre in Tuzla from May 2006 to May 2007. 35 patients were surveyed of an average age of 59.85 ± 10.37 years who had undergone mastectomy for breast tumours, and who had subsequently completed specific oncological therapy (chemotherapy, radio therapy). Treatment at the day-care hospice with palliative care lasted at least 12 weeks. The basic criteria which needed to be met for joining the research were that the subjects had had a confirmed patho-histological diagnosis of breast tumour, that after that they had undergone the surgical procedure of mastectomy, and that according to the findings by the oncologist they had completed specific oncological treatment. All the patients were previously acquainted with the goals, nature and methods of the research, and they gave their signed consent to participating in the research.

Physical and psycho-social support for patients at a day-care hospice assumes a structured program and the involvement of a multidisciplinary team. On each visit a doctor monitors the patient's condition, assesses the need for additional diagnostic or therapeutic procedures and, together with the nurse, plans the activities of the other members of the team. Individual, group or combined treatment by a psychotherapist is aimed at removing the psychological barriers caused by the illness and reducing the level of anxiety and depression. The activities of the occupational therapist are aimed at encouraging creativity through making simple objects, the patient performing simple tasks or activities in the art room. Physical therapy treatment consists of group or individual, passive or active kinetic therapy exercises to improve mobility and physical activity in the patients and to treat lymphostasis which is a frequent complication of breast tumours. Advice and specific activities by the social worker help the patients to realize their material and other social welfare rights prescribed by law. Treatment also includes transport, lunch and the all-day stay of the patients in specially adapted facilities.

To assess physical and mental health, we used the SF-36 scale (10, 11) which has three levels consisting of 36 questions (level 1), grouped in 8 scales (level 2), and the third level, with four subscales each, consists of two collective assessments on the basis of which an assessment is made of overall physical and mental health. Physical function is assessed on the basis of 10 questions relating to performance of everyday physical activities, whilst four questions relate to physical, and the following three to mental problems which limit the patients' activities. Physical pain and general health are assessed by two questions each, and mental health with five questions, which give an assessment of feelings of tension, despair, peace, disappointment, or happiness. Socializing with friends and family is assessed using two questions within the social function scale, whilst four questions related to feelings of being full of energy for life or a feeling of being worn out are part of the scale of vitality. A number expressing a value up to 0.25 points shows

a poor result, from 0.26 to 0.50 a moderate result, from 0.51 to 0.75 points is good, and over 0.76 points is an excellent result for each scale.

Statistical analysis was performed using the biomedical software known as MedCalc for Windows, version 9.4.2.0. For testing the repeated measurements of dependent samples, depending on the distribution of the variables, we used the even T-test and the Wilcox test. Statistical hypotheses were tested at the level of significance of $\alpha = 0.05$, that is, the difference between the samples was considered significant if $P < 0.05$.

Results

In the surveyed patients on their first testing, their overall physical health was assessed as moderate (0.42 points), whilst when tested after three months their overall physical health was assessed as good at 0.55 points, which is statistically significantly better in relation to the first test. The greatest progress was seen in the subscale of physical functioning and general health (Table 1).

After three months' treatment at the day-care hospice there was a significant rise in the number of patients, from 6 to 17, who assessed their overall physical health as good, whilst at the same time there was a fall in the number of patients, from 25 to 15, with the assessment moderate (Figure 1).

The results of the second test, after the patients had been treated for 3 months at the day-care hospice, were better in the subscales of physical functioning, general health and thereby overall physical health.

Table 1 Physical health of surveyed patients before and after treatment

Scales	Examined patients (n = 35)		
	Starter testing	Testing after three months	P
Physical functioning*	0.49 ± 0.18	0.58 ± 0.17	0.0002
Role–Physical**	0.25 (0.25 - 0.25)	0.25 (0.25 - 0.50)	0.004
Bodily Pain**	0.74 (0.74 - 0.84)	0.84 (0.74 - 0.90)	0.01
General Health**	0.20 (0.15 - 0.30)	0.42 (0.37 - 0.54)	< 0.0001
Physical Component Summary**	0.42 (0.36 - 0.50)[I]	0.55 (0.48 - 0.60)[J]	< 0.0001

Presented as *mean ± SD and **Median (Interquartile range)

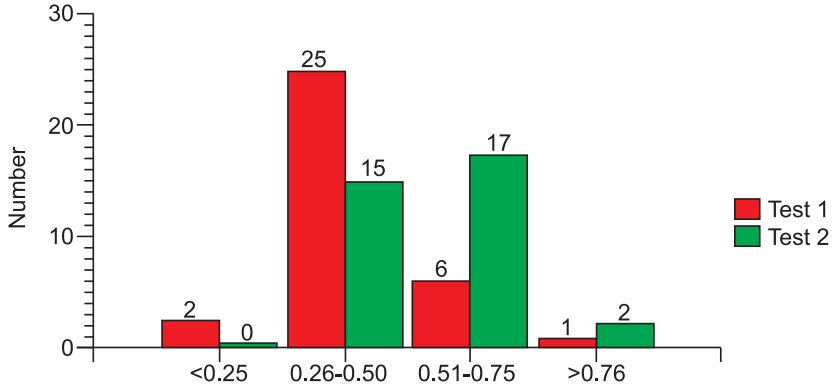

Figure 1 Overall physical health of surveyed patients before and after treatment. Test 1 – the number of patients before treatment; Test 2 – the number of patients after treatment.

On the first visit to the day-care hospice, the overall mental health of the surveyed patients was assessed as moderate at 0.26 points. When tested after three months, the overall mental health was assessed as good at 0.65 points, which was statistically significantly better than on the first test. Obvious progress was made on all sub-scales of overall mental health (Table 2).

The number of patients was significantly reduced who assessed their mental health as poor on the first test (from 18 to 2), or moderate (from 15 to 1), and the number of patients increased whose assessment was good (from 2 to 27) or excellent (from 0 to 5) (Figure 2).

The results of the test after three months' treatment by the multidisciplinary team at the day-care hospice in the surveyed patients show significant improvement in all subscales of overall mental health.

Discussion

The results of our research show that physical treatment given at the day-care hospice, which assumes professionally run active group exercises and individual treatment adjusted to each patient, leads to improvement of the overall physical health in the subscales of physical functioning and general health. The study by Turner et al (7), as a pilot study on 10 patients who had completed therapy for breast cancer, showed that suitably structured exercises of moderate intensity help to reduce the feeling of fatigue, improve mood and improve physical functioning and general health. The importance is mentioned of

Table 2 Mental health of surveyed patients before and after treatment

Scales	Examined patients (n = 35)		
	Starter testing	Testing after three months	P
Mental Health	0.24 (0.20 - 0.31)	0.72 (0.65 - 0.76)	< 0.0001
Role - Emotional	0.33 (0.33 - 0.33)	0.66 (0.33 - 0.66)	0.0001
Social Functioning	0.25 (0.25 - 0.25)	0.75 (0.62 - 0.75)	< 0.0001
Vitality	0.25 (0.20 - 0.35)	0.65 (0.60 - 0.70)	< 0.0001
Mental Component Summary	0.26 (0.23 - 0.30)	0.65 (0.59 - 0.70)	< 0.0001

Presented as Median (Interquartile range)

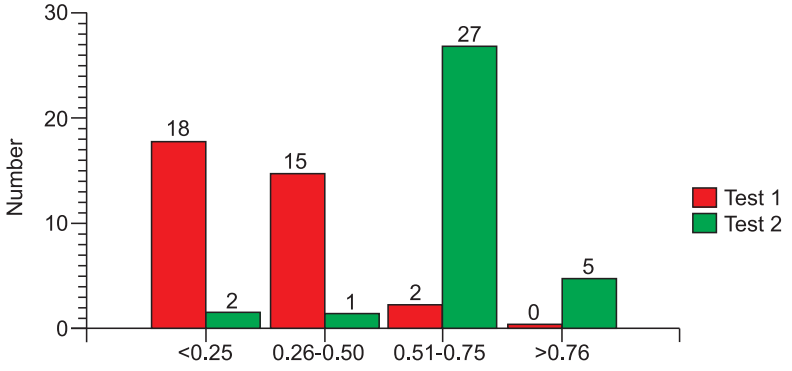

Figure 2 Overall mental health of surveyed patients before and after treatment. Test 1 – the number of patients before treatment; Test 2 – the number of patients after treatment

medical professionals, but also the mutual support given by to each other during the exercises, which improves mutual communication and some scales of mental health.

From the results of our study, the conclusion arises that continuous and suitably chosen physical treatment at a day-care hospice brings a statistically significant improvement in overall physical health. In the research undertaken by Sheree et al. (12) physical activity was monitored in 287 patients with breast cancer for 6, 12 and 18 months after the end of oncological therapy. About 80% of the patients went through three phases of testing, but it was shown that only one third of patients, who had continuity and were led by professionals in appropriate group physical therapy, showed improvement in their general physical health. The study also suggests the existence of a large number of patients who were insufficiently physically active or were not led by professionals in their physical activities.

The results of our study also showed that patients who were treated in group and individual psychotherapy exercises, stimulated by occupational therapy and medical staff, with the support of other patients, had better results for overall mental health, with clear improvement in the sub-scales of mental health, social functioning and vitality. The testing conducted by Plass and Koch (13) on 132 patients with breast cancer at the oncology clinic of the University Hospital in Hamburg showed that 37 patients who took part in sessions of group psycho-social support and in self-help groups run by other patients had much better results in the sub-scales of mental health, social functioning and vitality. The other 95 patients mentioned as the main reason for not taking part insufficient support from their family and friends, but also their doctors, who did not advise them or refer them to psychotherapy, and they also had poorer results in all subscales of mental health.

In our study overall physical health in two patients, on the second test after three months of treatment at the day-care hospice, was assessed as excellent, with more than 0.76 points, whilst in 5 patients on the second test their overall mental health was also assessed as excellent. Analysis shows that these were younger patients (42 to 45 years of age), who had undergone minimal surgical intervention (segmentectomy or tumerectomy) and did not receive chemotherapy. The study by Ganca et al. (14) which compared physical and mental health in older (more than 65 years) and younger patients with breast cancer, showed better results for physical and mental health in younger patients. Similar to our study, Deborah et al. (15) showed that younger patients who underwent a partial mastectomy and who did not receive chemotherapy, had significantly better results for mental and physical health than older patients with total mastectomies and repeated chemotherapy.

Limitations of the research

In the study design used there is a time shift of 3 months between the 2 assessments, which means that unknown disturbing variables could not be controlled.

Conclusion

The severity of mental and physical problems which come with the diagnosis and treatment of breast cancer, damaged body image and psychosocial disturbances, are easier to overcome if there is adequate and professional support.

This study confirms this unambiguously, showing improvements in all aspects of mental health and most aspects of physical health in patients who received that support through treatment by a multidisciplinary team at a day-care hospice.

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Clinicals skills training: two generations and two worlds apart
Part One

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Objective. Here, we compare clinical skills training in the 20th and 21st centuries in two different countries, in order to underline advancements and principal obstacles. **Methods.** The clinical training of medical students in the nineteen-sixties at the Sarajevo School of Medicine, Yugoslavia, and contemporary training at one of Europe's prestigious medical schools at Heidelberg University, Germany were analyzed with respect to the organization of training, teaching tools, methods, and staff. Several issues were defined as unimproved over the course of time, and we suggest that they present the core of the current problem. **Results.** Considerable advances have been made in teaching methodologies, tools and assessment of students. The major remaining obstacles are the institutional value system, poor motivation of teaching staff, curriculum structure, timing, and placement of training in the curriculum, as well as the patients' attitude towards participation in the training. **Conclusions**. In the process of bettering the existing training models we suggest acting along several lines. Increased institutional awareness of obstacles, as well as willingness to develop the ways and means to increase the motivation of the faculty, is imperative. Furthermore, it is necessary to introduce changes in the structure and timing of training and to complement it with a Catalogue, Practicum and Portfolio of Clinical Skills. We believe that recognizing the impediments and employing the proposed solutions could significantly improve the quality of clinical skills training.

Key words: Clinical skills, Medical education, Curriculum reform, Catalogue, Portfolio.

Introduction

The majority of authors dealing with curriculum reform in medicine agree that substantial progress in the training of undergraduates in clinical skills is still to be achieved. In spite of radical changes and improvements in medical education, in-hospital hands-on training remains the weakest link in many curricula (1-6). In comparison with clinical medicine and biomedical research, which have both made unbelievable progress over

the past several decades, the progress made in training of medical students seems to lag behind. Teaching proper is still to be recognized as a complex art, which should be learned and permanently improved, alongside with clinical competencies and research activities. From a financial standpoint, teaching is in most cases an orphan, usually supported by the teacher's primary activities as a clinician or investigator (7, 8).

Although medical students reported many examples of positive role models as well as effective and approachable teachers, they also described a hierarchical and competitive atmosphere in the medical school, in which haphazard instruction and teaching by humiliation occur, especially during the clinical training years (9,10). Today, mastering of a significant number of clinical skills cannot be achieved during undergraduate training; this task has shifted to residency programs, which is certainly not the preferable option.

There are many tangible and less tangible reasons for the suboptimal condition of clinical skills training: conceptual, cultural, financial, legal, and prejudicial in nature. Here we have attempted to identify the prominent problems by comparing the distinct features of clinical skills training over a 40 – year span.

Methods

We opted for an approach based on the personal experiences of both authors, who underwent medical training over a span of forty years, in two different countries. We recalled the essential features of clinical training in the nineteen-sixties at the Medical School in Sarajevo, ex-Yugoslavia, and a contemporary one, 40-years later, at Heidelberg Medical School, Germany.

We anticipated that by comparing two approaches and two models we would be able to identify major changes, improvements or standstills. Presumably, if the same obstacles are present and unresolved after 40 years, they could easily be the root of the problem.

Training of Clinical Skills Forty Years Ago, Sarajevo, ex-Yugoslavia

The setting

The senior author (VJS) started his clinical training at the University Hospital in Sarajevo, the principal health facility in Bosnia-Herzegovina (BH). This hospital, a complex built at the end of the 19th century, with pavilions for each clinical discipline, was surrounded by parks, lawns and trees. It was constructed in accordance with the Austro-Hungarian understanding of good hospital design and patient care, which relied primarily on sunshine, fresh air and repose. Rooms for patients were large, sometimes accommodating 20-plus patients; a room with eight patient beds was frowned upon as a luxurious commodity.

Teaching Staff and Teaching Methodologies

Introducing us to the secrets of medicine was the responsibility of *professors,* who were assisted by a number of *assistants.* The professors, who headed the departments as well, were mostly educated at the beginning of the 20th century in Vienna and German was still the *lingua franca* in our part of the world. Information from the West was sparse (rarely anybody spoke English), information from the East (Russia) was frowned upon. The medical community in BH enjoyed its blessed seclusion and ignorance, mostly unaware of developments in the outside world. Patients were discussed rarely, if ever: there was no need to plan different diagnostic or treatment strategies, because the Professors had a ready answer for any kind of medical mystery. Seeking a second opinion was un-

thinkable; confrontation of Herr Professor's *Dictum* unheard of.

The clinical skills teaching was organized in three forms: in classical *ex cathedra* lectures, clinical rounds and through *clinical* exercises, practical clinical training under assistants' guidance.

Grand rounds (also known as the Professor's visit) were the highlight both of the hospital routine and of teaching, held once a week. On this holy day, patients were attacked in their rooms before sunrise by a battalion of junior nurses and cleaning ladies with an important task - to shine and polish the floors, night stands, chairs and beds; even the chamber pots had to shine with a pleasant glow. The principal duty of senior nurses was to adjust the bed clothes, which had to be spotless, snow white and starched stiff. At that time the concept of quality assurance was not as ubiquitous as today and the whiteness of sheets, along with the amount of applied starch, reflected the quality of nursing care.

The show used to start at eight sharp; leading the cortege first came *Herr Professor*, half a step behind his *Matron*, carrying soap and a towel, followed by a fair number of staff: docents, assistants, residents and interns. Ward nurses were expected to stand by the head of the patients' beds, in the posture of the guard of honor at Lenin's Mausoleum. If the reader adds ten or fifteen students to this scene, the picture is complete.

Herr Professor was an undisputable authority, and when he showed interest in a patient who happened to have, e.g., stenosis of the mitral valve, the poor '*stenosis*' (patients were invariably referred to by the diagnosis, never by name) was, without delay, positioned in a sitting position, the responsible nurse tearing off his (or better to say its) pajama top and the stethoscope's bell glued on '*stenosis*' chest. After listening for a minute or less the great man washed his hands using the soap and towel provided by his Matron, and exclaimed, with the finality of a lynch judge handling a case of horse theft, "This is a classic example of mid-diastolic rumbling murmur". Next came the *Herr Dozent* with the remark, "The first heart sound is beautifully accentuated" and another overambitious doctor added, "What a terrific textbook example of a snapping sound." The majority of the entourage preferred to hold back, still and quiet. In short, everybody was attuned and enchanted with such a clear demonstration of the highest possible diagnostic clinical skills, only '*the mitral valve stenosis*' and we, students, were not able to understand what was so beautifully accentuated and why all of this was so terrific. Occasionally, some deflections from this straight and well-paved road occurred. "This is a classic case of liver cirrhosis in an alcoholic. It is a terrible burden for our society and economy, that so many of our people in Bosnia drink," declared *Professor* who, besides medicine, had a broad understanding of philosophy, sociology, economics and the fine arts. Everybody around him was nodding sadly, deeply disappointed with such unreasonable habits and behavior by the common Bosnian people. The mutiny erupted when least expected: "I am not an alcoholic," *the cirrhosis* declared resolutely, "I am a devoted Muslim faithful and I never drunk a drop of alcohol in my life!" Everybody was stunned with such insubordination: the ultimate authority had given the verdict and some half-literate '*cirrhosis*' questioned it. The *Matron* was the first to start the salvage operation: "Well, my dear, but from time to time, just a little. Even I drink a little wine for Christmas." However, *'the cirrhosis'* kept his ground, supported by the highest spiritual authority, "Never, never and never have I touched the substance, I swear to Allah the Merciful." What a stubborn man, still so persistent, even when all pieces of the diagnostic puzzle fitted so well.

The teaching assistants were supposed, during '*the clinical exercises',* to teach us to

take histories, physical examination methods, and to allow us to feel, touch, palpate, percuss and auscultate whatever happened to be on the menu for the day. Assistants were junior doctors at the very beginning of their academic career, but not necessarily: among them one could find less ambitious individuals close to retirement age, who spent their entire working lives in such honorable positions. There was not much glory and money in this position, just a vague promise and hope that, if one remained quiet, obedient and patient over many years, one could, far away in the future, progress and be promoted to *Dozent*. As a consequence, their enthusiasm and motivation for teaching was close to non-existent. When the absence of any control of their performance or results is added, it becomes easy to imagine the quality of teaching they delivered.

In addition, everybody at the hospital held students to be a nuisance and burden, and everyone looked upon us as the lowest form of living creatures in the hospital hierarchy. We used to attack the wards in groups of 10, 15 or 20 students, depending on the number of *assistants* at our disposal. Often, when an assistant forgot his obligation, or was occupied with some real or invented emergency, two groups were joined together. Cleaning ladies cursed us because they had to clean up after us, nurses frowned and swore because they could not approach the patients and do their job. The patients did not complain aloud, they tried to run away and hide in bathrooms or similar hideaways instead.

If they showed up, the assistants were rarely on time, and usually half of our time scheduled for practice passed in waiting. When we finally located and surrounded our *assistant*, they would roll their eyes up to Heavens looking for rescue, and, as the Heavens usually remained silent, took us to patients' room, where only the immobile and terminal were still lying, abandoning themselves to their inevitable fate.

"Take a detailed history of this fellow's illness, and I will come back in half an hour," instructed our tutor, disappearing at the speed of light. We were on our own again, with the patient who also looked to the Heavens in quest of mercy, uttering: "Please, I explained ten times so far, I have never had a Penicillin shock nor a sexually transmitted disease in all my life. I am a respectable married man with six children; I have no time for that." And so, time went by. On rare occasions, some very young and still ambitious assistant was willing to show us something tangible. "Here, my dear colleagues, in this patient you will find, if you listen carefully, the classic sound of succussion, first described by the great Hippocrates, father of the medicine."

We approached, shook vigorously the poor '*classical example*' and confirmed (what else could one do) that this is indeed a beautiful example of a clear sound of succussion. At the same time, in the vicinity, the cleaning ladies operated the vacuum cleaner at full blast, nurses yelled at disobedient patients, patients were discussing last Sunday's football match and hospital food, and less enthusiastic students were flirting with shy female colleagues. I doubt that even our father, the great Hippocrates, would have been able to recognize his own sign under such circumstances.

Teaching tools

Textbooks were rare, expensive and of poor quality. Even if the student had the means to buy the textbook (which only a few did), its procurement was as complicated as procurement of plastic explosives. Rare decent books I remember from this period of my education were the translation of Guyton's "Medical physiology" and an old, well-used 25th edition of Gray's Anatomy, published in

1948 and supplemented by a Hungarian anatomical atlas. In the given conditions certainly nobody bothered to introduce the students to the universe of medical journals.

To enliven their teaching, the professors had mainly two tools. Anatomists used to bring a box of chalks in different colors and drew anatomical schemes on the blackboard during the lectures. Why they did that remains unclear to this day – decent anatomical atlases were available even then. Technically more advanced teachers used slide projectors to present transparencies, which were the last word in modern technology, and many years were to pass before we were introduced to overhead projectors. And that was it, if we overlook the human bones held in the anatomy department crypts and other hardly recognizable human organs held in jars filled with formalin. I prefer to skip the gruesome collection of nooses, knives, axes and all other imaginable tools of human destruction and self-destruction, in the possession of the Department for Forensic Medicine.

Training of Clinical Skills Forty Years Later, Heidelberg, Germany

The setting

The medical campus of Neuenheimer Feld in Heidelberg, Germany, where the junior author (FS) underwent his clinical education, is a vibrant place where the basic sciences and clinical practice are intertwined in a motivating manner. The recent award of the 2008 Nobel Prize to Prof. Harald zur Hausen of the German Cancer Research Institute in Heidelberg could serve to illustrate the productivity of this environment. At this point, most hospitals are modern functional facilities, geared toward the fast and high turnover-driven health care of the 21st century.

Teaching Staff and Teaching Methodologies

The hospital military-like hierarchy is one of the many persisting and culturally transcending aspects of medicine, at least in the Old World. Professors are also heads of departments and masters to be respected and not to be questioned too often. The several Docents (clinicians with academic appointments), their second-in-commands, were followed by senior doctors and specialists, and lastly by assistants (or residents). The clinical hierarchy was reflected in the teaching tasks: the Professors and Docents gave lectures, other senior clinicians were responsible for seminars, and residents were responsible for clinical skills training.

The nature of lectures has not changed much, apart from the introduction of multimedia projectors and power-point presentations. Seminars, as opposed to lectures, were based on interaction of the specialist-presenter with students, which attended in smaller groups.

The variety of our time spent together with assistants increased significantly over the last forty years through the introduction of various novel forms of teaching, most notably: problem based learning (PBL) sessions, demonstrations in the clinical skills lab, standardized patients sessions, bedside teaching and clerkship. Only the latter three deal with clinical skills *per se* and they will be discussed in this text, whereas PBL teaching has been extensively discussed elsewhere (11).

Learning in the **Clinical skills lab** in Heidelberg comprised of practicing, roughly speaking, various manual techniques on mannequin bodies or body parts. Models were subject to great diversity. At one end of the scale were true pieces of art, which simulated complex clinical situations and enabled the students to do much more for the '*patient*' than they would have dared to in a real setting. At the other end one could find, for example, unimaginative pieces of

rubber resembling an extremity, with a thick blue line denoting a vein. Exercises with the former type of sophisticated equipment lend us greater confidence through affirmation of our skills or through underlining of our faults, while the latter type of contraptions were, as one can imagine, difficult to profit from.

Models and mannequins are a useful novelty, but one that needs to be enjoyed with a grain of salt. They are excellent for introducing students to the respective technique (e.g. blood sampling, palpation of the prostate, chest auscultation, palpation of the breast) but they can take a student only half way to mastering a skill. Palpating an extended liver on a plastic model of the abdomen is like regarding a map of high waters without ever setting sail – you will never feel the scent of the salty wind.

In the exercises involving **standardized patients**, students were confronted with actors which were trained to present a set of complaints pertinent to common conditions. Our task was to take a complete medical history and to conclude with a number of differential diagnoses and a sketch of a diagnostic and therapeutic protocol. Some actors were professionals and they truly did an amazing job in mimicking homeless alcoholics, concerned mothers or irritated businessman who wanted to get treated and leave as soon as possible, as well as a whole array of other characters. The interviews were sometimes videotaped and examined at a later point by the whole group, led by a psychologist and a doctor who would then comment on our performance from a medical and psychological (or 'communication skills') viewpoint. In our opinion, the usefulness of the standardized patients is similar to that of mannequins in the clinical skills lab. They are useful at the beginning of clinical education, when the junior students are confronted with history taking for the first time.

Last but under no circumstances least; we finally set sail in the so-called **bedside teaching** sessions. In spite of the modern name, they were dauntingly similar to '*clinical exercises*', as described in the previous part of this article. A significant difference was (due probably more to the different mentalities of Germanic and southeast European peoples than to a concrete improvement in teaching) that our assistant almost never failed to show up for the exercises. Clinical routine is by its nature unpredictable and we would sometimes have to wait for our teacher, but generally we were treated with reasonable respect and teaching was done according to schedule.

Apart from that little had changed. Teaching students remained a tedious and unrewarding task for the resident who had to bear the functioning of the ward on his shoulders. Some possessed an innate love for teaching, and some enjoyed displaying their battle-tested competence and knowledge, but there were also those who were simply apathetic, and our time together on the ward was spent in an atmosphere of fake politeness and barely concealable impatience. I recall a neurology resident, whom we physically had to shake back to receptiveness from a daydreaming haze.

Almost invariably our collective task was to take a patient's history and to examine him, with the goal of '*presenting the case*' to the clinical instructor. In spite of the obvious inconsequentiality of our endeavor, patients were in most cases receptive and talkative, eager to share their story with us. It was possible to practice history taking and examination, and a willing resident was able to do a good job in connecting what we experienced with theoretical background. Sometimes, if the moment was right, we could do a minor procedure under guidance. In conclusion, this form of teaching had fabulous and useful moments, but only when both,

the instructor and the students, happened to be eager and enthusiastic.

During the three years of the clinical curriculum, we were expected to complete four months of **clerkship** (or *famulatur*, in German) in the departments of our choice. This was the least structured form of clinical training I received, and by far the most rewarding. The idea was that a student (alone, without a group of peers) joins the dynamics of a ward on a full time basis, and helps his seniors by conducting certain basic tasks, such as taking histories at patient admissions, drawing blood, placing i.v. lines or assisting surgery. In addition, the student was welcome on departmental rounds, conferences and seminars with the clinical staff. Often someone would find time to discuss a case or a technical or theoretical detail with me. Many times I earned the trust of junior doctors who showed me a more advanced procedure such as lumbar puncture or wound suturing, which I would next time conduct under their supervision and assistance. For me it was important to feel that I was actually contributing, even with my modest skills and knowledge, to the functioning of a real ward, instead of playing games in virtual reality with mannequins or hired actors.

Teaching tools

Teaching resources, as everything else at the present time, are over-abundant, which could have negative connotations; the inability to choose between various books for the same subject frequently leads to frustration. Also the variety of the learning materials has greatly expanded to include the Internet and various kinds of interactive software. A student thirsty for knowledge and willing to learn can nowadays really complain about only one thing: lack of time to digest all that is at his disposal. In addition, two more skills are necessary: to surf cyber space and to critically appraise the thousands of *hits*.

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Effects of oral antidiabetic drugs over lipid parameters in Turkish type 2 diabetes patients

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Objective. We examined the effects of oral antidiabetics and insulin/insulin analogs over lipid parameters for the first time in Turkish type 2 diabetic (T2DM) patients. **Methods**. A total of 312 T2DM subjects were included within 4 study groups (sulphonylurea, biguanide, insulin/insulin analogs, sulphonylurea+biguanide) in this retrospective study. The demographic, biochemical and clinical data of the patients were evaluated and the study groups were compared for all the variables. The biochemical and lipid parameters were examined in pairs for their correlations for each study group. **Results**. Body mass index (BMI) was found to be lower in the insulin/insulin analogs group in comparison to the biguanide and sulphonylurea+biguanide groups ($p < 0.01$); systolic blood pressure (SBP) was found to be lower in the insulin/insulin analog group in comparison to the sulphonylurea, sulphonylurea+biguanide and biguanide groups ($p < 0.05$); diastolic blood pressure (DBP) was found to be lower in the insulin/insulin analog group in comparison to the biguanide group ($p<0.05$). No difference was found for total-cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol among the study groups ($p>0.05$). Fasting blood glucose and HbA1c levels were found to be lower in the biguanide group when compared to the sulphonylurea and insulin/insulin analogs ($p<0.001$). In all study groups a positive correlation was found between blood glucose and HbA1c levels ($p<0.001$). A weak positive correlation was observed between blood glucose and triglyceride ($p<0.05$) and HbA1c and LDL-cholesterol ($p<0.05$). **Conclusion**. Although no difference persists between the treatment groups for total-cholesterol, triglycerides, HDL-cholesterol, and LDL-cholesterol, the insulin/insulin analogs seem to lower serum lipids most effectively, which may help prevent coronary events in T2DM patients.

Key Words: Type 2 diabetes, Sulphonylureas, Biguanides, Insulin and analogs, Serum lipids

Introduction

Diabetes is one of the leading causes of morbidity and mortality throughout the world. Approximately 2.2-3% of the world's population suffers from Type 2 diabetes (T2DM) (1). The prevalence of T2DM in Turkish adults was estimated as 2.89 million (11.0%) of the population aged ≥35 years) (2). In T2DM, disturbances of lipid profiles and especially increased susceptibility to lipid peroxidation is observed (3). An increased oxidative stress has been observed in diabetic patients as indicated by high free radical production (4). Although the pathophysiological mechanism of atherosclerosis in diabetic patients has not yet been fully understood, it is thought that hyperlipidemia, increased oxidation of low-density lipoproteins (LDL) and impaired vascular function promote atherogenesis in diabetic patients (5). Glucose deficiency in adipose tissue induces metabolic compensation, leading to the hydrolysis of triglycerides and release of fatty acids, which are oxidized by the liver and transformed to ketonic derivatives (6). In patients with T2DM, besides controlling blood pressure and lipid levels, the major therapeutic goal is to optimize glycaemic control in order to reduce the development and/or severity of long-term diabetic complications (7). Antidiabetic drugs control blood sugar levels in individuals with T2DM (8).

Although oral antidiabetic agents may initially control hyperglycemia, most patients with T2DM will ultimately require insülin therapy, as β-cell function progressively declines (9, 10). Antidiabetic drugs may be subdivided into six groups: sulphonylureas, alpha-glucosidase inhibitors, biguanides, meglitinides, insulin and thiazolidinediones. Sulphonylurea derivatives are class of antidiabetic drugs used in the management of T2DM ("adult-onset"). Biguanides and sulphonylureas are widely used for the treatment of NIDDM and have been used for the prevention of diabetes in non-diabetic patients (11, 12). They act by increasing insulin release from the beta cells in the pancreas. Sulphonylureas [Acetohexamide, Chlorpropamide, Tolbutamide, Tolazamide (tolinase), glipizide (glucotrol), gliclazide, Glibenclamide (glyburide), Gliquidone] act by increasing release from the beta cells. Glimepiride (amaryl) a member of sulphonylurea class, appears to have a useful secondary action in increasing insulin sensitivity in peripheral cells (13). Alpha-glucosidase inhibitors are oral antidiabetic drugs used for T2DM treatment, acting by preventing the digestion of carbohydrates. Alpha-glucosidase inhibitors acarbose (precase), miglitol (glyset), and Voglibase do not enhance insulin secretion but rather inhibit the conversion of disaccharides and complex carbohydrates to glucose. Alpha-glucosidase inhibitor drugs are useful for either monotherapy or in combination therapy with sulphonylureas or other hypoglicemic agents (8, 13). Biguanides form a class of oral hypoglycemic drugs used for diabetes mellitus or prediabetes treatment. Metformin (glucophage, phenformin, buformin) is the only avaliable member of the Biguanide class. Metformin decreases hepatic (liver) glucose production, decreases intestinal absorption of glucose and increases peripheral glucose uptake and use. Metformin may be used as monotherapy (alone) monotherapy, or in combination therapy with a sulphonylurea (8, 13). The meglitinide class of drugs treat T2DM by blocking the potassium channels in beta cells, which closes the ATP-dependent potassium channels and opens the cells calcium channels. The resulting calcium influx causes the cells to secrete insulin. There are two members of the meglitinide class: repaglinide (prandin) and nateglitinide (starlix). The mechanism of the action of the meglitinides is to stimulate insulin production. This activity is both dose dependent and dependent on the presence of low blood

glucose levels. The meglitinides may be used alone or in combination with metformin. The manufacturer warns that nateglinitide should not be used in combination with other drugs that enhance insulin secretion (8, 13). Insulin and insulin analogs (Humulin, Novolin) are responsible for glucose utilization. It is effective in both types of diabetes, since even in insulin resistance, some sensitivity remains and the condition can be treated with larger doses of insulin. Most insulins are now produced by recombinant DNA techniques, and are chemically identical to natural human insulin. Isophane insulin suspension, insulin zinc suspension and other formulations are intended to extend the duration of insulin action and permit glucose control over longer periods of time. An insulin analog is an altered insulin, different from the insulin secreted by the human pancreas, but still avaliable to the human body for performing the same action as human insulin These modifications have been used to create two types of insulin analogs: those that are more readily absorbed from the injection site and therefore act faster than natural insulin, intended to supply the bolus level of insulin needed after a meal: and those that are released slowly over a period of between 8 and 24 hours, intended to supply the basal level of insulin for the day (8, 13). The medication class of thiazolidinedione was introduced in the late 1990s as an adjunctive therapy for T2DM and releated diseases. Rosiglitazone (Avandia) and Pioglitazone (Actos) are members of the thiazolidinediones class. They act by both reducing glucose production in the liver, and increasing insulin dependent glucose uptake in muscle cells.They do not increase insulin production.These drugs may be used in combination with metformin and sulphonylurea.(8, 13).

Material and methods

In this retrospective study the files from between 2003 and 2007 of type 2 diabetic patients were evaluated. An equal number of patients was selected for the study. A total of 2500 files were examined. The metabolic variable levels were recorded from the current treatment, which lasted for at least 6 months. Patients using lipid lowering drugs were excluded from the study. Other blood glucose lowering drugs users (69 patients) such as acarbose, nateglinide or combination uses of these drugs were not included in the study. A total of 312 subjects were included in the study. The 4 study groups were composed of oral antidiabetic sulphonylurea, biguanide users, insulin/insulin analog users and combined oral antidiabetic (sulpho nylurea+biguanide) users. The demographic characteristics (age, gender, height, weight, BMI) were analysed. The biochemical analyses included determination of fasting serum glucose, HbA1c, triglyceride, HDL-cholesterol, LDL-cholesterol, total-cholesterol. The clinical data of the patients, (such as systolic blood pressure, diastolic blood pressure, obesity, hypertension, pulmonary disease, coronary events, family history of diabetes) were also evaluated. All the patients were under hypertensive treatment. The non-hypertensive patients with elevated macroalbuminiria, ACE inhibitors were used to prevent nephropathy.

Statistical analysis

Statistical analyses were conducted using Unistat 5.1 software. All numerical values are reported as means ± SE. A comparison of variables between the four groups was performed using one-way ANOVA. Gender, obesity, hypertension, family history, coronary events and pulmonary disease were estimated by chi-square test. Pearson's correlation coefficient (r) was determined where appropriate. p- values less than 0.05 were considered significant.

Results

The study group comparisons (sulfonlyurea, biguanide, insulin/insulin analogs, sulfonlyurea in combination with biguanide) for each demographic characteristic are shown in Table 1. No significant difference was observed for hypertension, obesity, family history of diabetes, pulmonary disease, and coronary event when the study groups were compared. Body mass index (BMI) was found to be lower in insulin/insulin analogs group in comparison to biguanide and sulphonylurea+biguanide groups ($p < 0.01$); systolic blood pressure (SBP) was found to be lower in insulin/insulin analog group in comparison to sulphonylurea, biguanide and sulphonylurea+biguanide groups ($p < 0.05$); diastolic blood pressure (DBP) was found to be lower in insulin/insulin analog group in comparison to biguanide group ($p < 0.05$) (Table 1). The biochemical characteristics were compared between all the study groups in Table 2. No difference was found for total-cholesterol, triglyceride, HDL-cholesterol and LDL-cholesterol among the study groups ($p>0.05$). Fasting blood glucose levels were found to be lower in the biguanide group when compared to sulphonylurea and insulin/insulin analogs ($p < 0.001$). HbA1c levels were found to be lower in the biguanide group when compared to sulphonylurea, sulphonylurea+biguanide and insulin/insulin analogs ($p < 0.001$) (Table 2). Correlations between variable pairs as a function of therapy groups are presented in Table 3. In all study groups a positive correlation was found between fasting blood glucose and HbA1c levels ($p < 0.001$). A weak positive correlation was observed between blood glucose and triglyceride ($p < 0.05$) and HbA1c and LDL-cholesterol ($p < 0.05$) (Table 3).

Table 1 Demographic characteristics comparison of the therapeutic study groups

Demographic characteristics	Sulphonylurea (n)	Biguanide (n)	Insulin and analogs (n)	Sulphonylurea+_ Biguanide (n)	ANOVA p
Age (years)	58.6 ± 1.3 (90)	54.8±1.2 (70)	49.2 ± 1.8^{AD} (70)	57.6±1.1 (77)	< 0.001
Gender (M/F)	39/52	44/27	49/39	34/44	< 0.05
Height (cm)	161.0±1.1 (87)	165.1 ± 1.3^{A} (69)	164.8 ± 1.0 (68)	161.7±1.0 (76)	< 0.05
Weight (kg)	78.0 ±1.7 (87)	86.2±2.1^{ACD} (69)	75.4 ±2.0 (68)	79.2 ±1.5 (76)	< 0.01
BMI (kg/m^2)	30.1 ± 0.6 (87)	31.7 ± 0.7 (69)	27.9 ± 0.7^{BD} (68)	30.3 ±0.5 (76)	< 0.01
Hypertension (No/Yes)	18/35	18/27	10/23	18/34	> 0.05
Obesity (No/Yes)	16/29	12/33	8/22	12/28	> 0.05
Systolic blood pressure (mm Hg)	144.7 ± 3.0 (83)	144.5±2.9 (60)	133.3±2.8^{ABD} (63)	145.2±2.5 (75)	< 0.05
Diastolic blood pressure (mm Hg)	83.6 ± 1.5 (82)	85 ±1.3 (60)	79.1 ±1.4^{B} (63)	83.9 ±1.4 (75)	< 0.05
Family history DM (No/Yes)	13/53	15/42	4/43	8/53	> 0.05
Coronary events (No/Yes)	24/5	29/7	24/2	26/7	> 0.05
Pulmonary Disease (No/Yes)	23/6	30/6	24/1	27/3	> 0.05

$^{A}p < 0.05$ in comparison to sulphonylurea group; $^{B}p < 0.05$ in comparison to biguanide group; $^{C}p<0.05$ in comparison to insulin and analogs group; ^{D} $p<0.05$ in comparison to Sulphonylurea + Biguanide group.

Table 2 Biochemical characteristics comparison of therapeutic study groups

Biochemical characteristics	Sulphonylurea (n)	Biguanide (n)	Insulin and analogs (n)	Sulphonylurea+ Biguanide (n)	ANOVA p
Glucose (mg/dl)	186.2±7.0 (88)	153.45±6.7[AC] (66)	201.87±10.8 (66)	176.21±5.27 (78)	< 0.001
HbA1c %	8.5 ±0.2 (90)	7.01 ±0.2[ACD] (67)	8.60 ±0.3 (68)	8.63 ±0.21 (75)	< 0.001
Total -Cholesterol (mg/dl)	220.1±6.2 (69)	212.1±5.9 (61)	198.2±6.6 (47)	216.48±5.44 (67)	> 0.05
Triglycerides (mg/dl)	206.3±16.7 (67)	195.0±14.4 (60)	152.6±16.0 (44)	179.77±10.23 (64)	> 0.05
HDL-Cholesterol (mg/dl)	48.3 ±1.7 (59)	47.0±1.5 (57)	43.5±1.8 (42)	47.98±1.6 (62)	> 0.05
LDL-Cholesterol (mg/dl)	132.1±5.2 (46)	133.9±6.1 (41)	125.0±6.7 (28)	137.48±5.28 (42)	> 0.05

[A]p < 0.05 in comparison to sulphonylurea group; [B]p < 0.05 in comparison to biguanide group [C]p < 0.05 in comparison to insulin and analogs group [D]p < 0.05 in comparison to sulphonylurea + biguanide group.

Table 3 Pearson's correlation test applied to pairs of variables

Pairs of variables	Sulphonylurea (n)		Biguanide (n)		Insulin and analogs (n)		Sulphonylurea+ Biguanide (n)	
	r	p	r	p	r	p	r	p
Glucose-HbA1c	0.64	< 0.001	0.80	< 0.001	0.56	<0.001	0.52	< 0.001
Glucose- Triglycerides	0.31	< 0.05	-0.04	> 0.05	- 0.14	> 0.05	- 0.12	> 0.05
HbA1c- HDL-Cholesterol	- 0.06	> 0.05	0.26	> 0.05	0.007	> 0.05	- 0.05	> 0.05
HbA1c- LDL-Cholesterol	0.03	> 0.05	0.16	> 0.05	0.47	> 0.05	0.07	> 0.05

Discussion

The purpose of this study was to compare the effects of two oral antidiabetic drug groups, sulphonylureas and biguanides together with insulin and insulin analogs on serum lipid levels in Turkish patients with T2DM. The major therapeutic goal in patients with T2DM is to optimize glycaemic control by controlling blood pressure and lipid levels, in order to reduce the development and/or the severity of long term diabetic complications. The severity of diabetes by the number of oral antidiabetic agents required prior to inclusion and by the large range of HbA1c allowed at inclusion (7). The fasting blood glucose and HbA1c levels of our study groups were above the normal range, demonstrating insufficient glycaemic control (1). The lipid parameters were within the normal range only in the patients using insulin/insulin analogs. In type 2 diabetic groups using oral antidiabetic drugs the lipid levels were higher than normal, which shows that when glycaemic control cannot be reached lipid levels are elevated. Sulphonylureas reduce blood glucose levels by stimulating pancreatic beta cells to secrete insulin, which results in an elevated plasma insulin concentration. A secondary action is the improvement in hepatic and peripheral insulin sensitivity. This effect may be related to hyperglycemia-induced insulin resistance, often referred to as "glucose toxicity," which decreases as the sulphonylureas lower blood glucose levels. Sulphonylureas

alone initially control blood glucose levels in about 50 percent of patients. Sulphonylureas produce a reduction in HbA1c of 1.5 to 2.0 percentage points Their effectiveness declines as the failure of beta cell function progresses, because sulphonylureas are effective only in the presence of a significant residual insulin secretory function. Karlander and collleagues have compared the long-term effect of combined treatment with insulin and glyburide versus insulin alone on serum lipid levels in non-insulin-dependent diabetic (NIDDM) patients with secondary failure to sulphonylurea therapy and found an 11% decrease in HbA1c levels (14). In both groups, there was an increase in high-density lipoprotein cholesterol of approximately 20% lasting throughout the study. There was a decrease in serum cholesterol ($p < 0.05$) and serum triglycerides ($p < 0.05$) in both groups. All changes in lipid variables were comparable in magnitude and duration in both treatments with insulin, and glyburide in NIDDM patients with secondary sulphonylurea failure improves lipid metabolism to a similar degree as insulin therapy alone. In concordance with Karlander et al. (14), the HbA1c levels were not found to differ in the sulphonylurea treated group to that of the other groups in our study. Whereas, although not statistically significant, total-cholesterol, triglyceride, HDL-cholesterol and LDL cholesterol levels were found to be lower in the insulin treated group in comparison to other treatment groups. Decreased levels of plasma glucose due to successful sulphonylurea treatment improve fasting and postprandial hypertriglyceridemia, reduce the number of abnormally small low-density lipoprotein (LDL) particles, and tend to return decreased high-density lipoprotein (HDL) levels to normal (15). This effect of sulphonylureas appears to be secondary to the glucose-lowering effect. Failure to reach target lipid levels would indicate a need for specific pharmacotherapy.

By lowering the daily insulin dose, sulphonylurea drugs appear to improve the sensitivity of exogenous insulin in subjects with type 2 diabetes mellitus manifesting a lapse of glycemic control. Moreover, glimepiride appears to possess a greater insulin-sparing property than other sulphonylureas (16). Drzewoski et al reported a positive correlation between fasting blood glucose and HbA1c (17). Similar to the results of Drzewoski et al, we also demonstrated positive associations between fasting blood glucose and HbA1c in all of the treatment groups analyzed.

In the present study, we observed a weak positive association between fasting blood glucose and triglyceride in the sulphonylurea group, but a weak negative association between fasting blood glucose and HDL-cholesterol. In the insulin/insulin analog treated group a weak positive association between HbA1c and LDL-cholesterol was found. Pasquali et al have shown that metformin can lead to waist circumference reduction (18). The improvement of HbA1c levels in the sample was independent of waist circumference reduction, indicating that metformin improves sensitivity to the action of insulin by mechanisms already described, such as inhibition of hepatic gluconeogenesis (19). Although over half the patients reached HbA1c levels below 8%, only 14% reached ideal metabolic control (HbA1c up to 7%), and 47% kept their HbA1c above 8%. DeWitt et al. have hypothesised that more intensive insulin therapy with fast-acting insulin and self-monitoring might have led to better results (20). The mean fasting blood glucose and HbA1c values of our patients were similar to those reported in other studies (21, 22).

The literature shows discrepant results about the influence of metformin on lipid profile (23). Some studies, in agreement with ours, reported reduction only in TC levels (24, 25), while others reported reduction of TC and TG with an increase of HDL-C

(26, 27). Still other studies showed no changes in lipid profile (28, 29). Another investigation showed an association of metformin with an improvement in the lipid profile even in non-diabetic patients (30). New studies are needed to clarify this issue, since TG and HDL-C are very important parameters for the evaluation of metabolic syndrome.

Yamanouchi et al. compared the metabolic effects of pioglitazone, metformin, and glimepiride in the treatment of Japanese patients with newly diagnosed Type 2 diabetes (31). They reported that the rate of reduction of HbA1c was fastest in patients receiving glimepiride and slowest in patients receiving pioglitazone. Although there were no significant differences among the three groups in HbA1c levels at the end of the study, patients taking pioglitazone had relatively lower fasting plasma glucose levels than patients taking the other two drugs. Our results are not in agreement to Yamanouchi et al since we found the lowest rate of HbA1c in the biguanide treated type 2 diabetic patients .

Long term prospective and randomised studies of the cardiovascular effects of antidiabetic agents in non-diabetic individuals with insulin resistance are lacking. The effect of metformin on systolic and diastolic blood pressures in non-diabetic patients with systemic hypertension is controversial and the long term effect of metformin or glipizide on blood pressure in non-diabetic patients with normal blood pressure is not well known (32, 33). In the present study, subjects taking antihypertensive medication were excluded before entry into the study, and all subjects had arterial blood pressure over normal limits. The sulphonylurea in combination with the biguanide treatment group had the highest level of systolic blood pressure levels, whereas the diastolic blood pressure was found to be the highest in the biguanide group. The systolic blood pressure levels were found to differ in all groups (sulphonylurea, biguanide, sulphonylurea+biguanide) to that of insulin/insulin analogs which also had the lowest level of SBP. Additionally, the DBP was found to differ significantly in the insulin/insulin analogs treated group to that of the biguanide treated group. Individuals in the metformin group had a mild but significant decrease in systolic and diastolic blood pressure during the follow up period. Subjects in the glipizide group also had a non-significant reduction in blood pressure. It is known that glipizide has no effect on lipid profile, whereas metformin may have a favourable effect on plasma cholesterol concentrations (34, 35, 36, 37).

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Amputations in a tertiary care hospital

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Objective. This study was conducted to highlight the pattern of amputations and their outcome in a tertiary care hospital over a period of 16 months. **Material and Methods.** This retrospective study of medical records was conducted at the Al-Noor Specialist Hospital, Makkah, Saudi Arabia from 04-03-2004 to 18-06-2005. The subjects' files were extracted by using ICD-10 codes for amputations from the medical records department by the authors of the Health Research Center. The files were reviewed for surgeons' clinical notes as well as nurses' notes. Operating theatre notes were also reviewed in detail. Certain variables were documented, including demography, clinical aspects and outcomes of amputations. **Results.** Of 50 study subjects males accounted for 72% and 84% were Saudis. Fifty percent were middle aged (range 45-64yrs). The range of stay was 3-48 days and 44% patients stayed for 3-7 days. There were 43 (86%) amputations due to diabetes with peripheral circulatory disorder. Forty two (84%) of patients improved and 12% were discharged against medical advice (DAMA) after amputation whereas 6% died. Forty-four percent of patients were admitted once, while 2% of patients were readmitted 9 times. The most amputations were at the level of the toes (54%), followed by 17 around the knee (34%). Nineteen (38%) patients underwent amputation under general anesthesia. The re-amputation rate was 10%. **Conclusion.** Diabetes with peripheral vascular disease and neuropathy was the main cause of amputations in our hospital.

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Introduction

Amputation is a global problem. It is a procedure by which the removal of a limb or other appendages or outgrowth of the body is performed e.g. below knee amputation. In one study in UK hospitals, approximately 5500 amputations are performed each year in England. The number increases as population age increases. Seventy-five percent of patients are >60 years of age and 65% are men. Major limb amputations are rarely required, 3% of totals. The indications for amputations are usually vascular diseases, diabetes (85%), trauma (10%) and tumors (3%). Major upper limb amputations are rarely required (1).

The epidemiological impact of diabetes is evidenced by the growing morbidity and

mortality rates, and by the fact that it causes permanent disabilities such as blindness, diabetic retinopathy, end stage renal failure and lower extremity amputations. Among the risk factors in lower extremity amputation in diabetes mellitus patients are: long duration of the disease, prolonged hyperglycemia, dyslipidemia, smoking and drinking, neuropathy, peripheral vascular disease, and prior ulcers (2).

Although amputee limb salvage rates for patients with peripheral vascular diseases have improved substantially, amputation may be the only practiced treatment for a limb severely affected by trauma, infection, tumor, or the end stages of ischemia. Unfortunately, vascular surgeons have traditionally viewed amputations as manifestations of failure – failure to comprehend or control the disease process, failure of the referring physician or patient to seek help in a timely fashion, or failure of the vascular surgeon to perform successful revisualization. The immediate aims of amputations are 1) removal of diseased tissues; 2) relief of pain; 3) primary healing of the amputation at the level chosen; and 4) construction of a stump and provision of a prosthesis that will permit useful function (3).

In view of these facts, we undertook this study to discover the clinical profile audit of amputation at the Al-Noor Specialist Hospital, Makkah, Saudi Arabia.

Material and Methods

This retrospective case series study was carried out over a period of 16 months from 4-3-2004 to 18-6-2005 at Al-Noor Specialist Hospital, Makkah, Saudi Arabia. The subjects' files were extracted by using ICD-10 codes (4) for amputations from the medical records department by the authors of the Health Research Center. The files were reviewed for surgeons' clinical notes as well as nurses' notes. Operating theatre notes were also reviewed in detail. Certain variables were documented, including demography, clinical aspects and outcomes of amputations. The data were categorized into age groups, i.e. 1-14, 15-24, 25-44, 45-64, >65, gender, i.e. male and female; nationality, i.e. nationals and non-nationals; duration of stay, i.e. 3-7 days, 8-14 days & >15 days; frequency of admission, i.e. single, 2-3 times, >3 times; level of amputation, i.e. lower limb and upper limb, and anesthesia given, i.e. general and other type of anesthesia. The patients who were kept in different wards due to the shortage of beds in surgical wards or transferred to other wards due to changes to the patients' disease category or level of severity were also considered. The final outcome of patients was illustrated as improved, death and discharge against medical advice (DAMA).

Institutional review board of Alnoor Specialist Hospital granted permission to conduct this study.

Al-Noor Specialist Hospital is a 550-bedded referral teaching hospital providing tertiary care throughout the Makkah region of Saudi Arabia for more than 18 years.

The data were analyzed by the Statistical Programme for Social Sciences (SPSS) 16.0 version. Numerical data were subject to descriptive analysis that is mean+standard deviation (SD) and range. Categorical data were analyzed as frequency, percentage. Parametric data were analyzed by the Student T-Test. The two tailed p-value was considered significant if <0.05.

Results

Fifty patients had amputations performed. These amputations were of part of either the upper or lower limbs. The male to female ratio was 2.5:1. Saudis were dominant, 42 (84%), while the majority of non-Saudis were Nigerians. Only 1 (2%) was from Mali. The age range was 1-84 years with a mean age of 53 years. The mean ages of diabetics and non-diabetics were 58.1 and 21.3 years, respectively ($p <0.005$). The peak incidence of amputations was observed in the 45-65 yrs age group. Personal history highlighted

that 5 (10%) were smokers. Two (4%) were students, 2 (4%) were government employees and for 32 (64%) their occupation was not documented (Table 1).

The duration of stay was between 3 to 48 days, with a mean stay of 13.3 ±10.9 days. Most of the patients, 41 (82%), were referred to the general surgery ward. Diabetic amputations 43 (86%) accounted for most cases. The readmission rate was 56%, which was mainly due to poor follow- up and home care 15 (55.6%), followed by poor compliance with medication 7 (25.9%). Remaining cases were readmitted due to other reasons, i.e. hypertension (HTN), ischemic heart disease (IHD) and chronic renal failure(CRF) (Table 2).

Table 1 Socio-demographic data of amputees

Variables			(n=50)	%
Sex	Males		36	72
	Females		14	28
Nationality	Nationals		42	84
	Non-nationals		8	16
Age(years)	1-14		4	8
	15-24		2	4
	25-44		5	10
	45-64		25	50
	>65		14	28
Personal history	Smoking	Smoker	5	10
		Non-smoker	7	14
		Ex-smoker	3	6
		Not documented	35	70

Table 2 Clinical Data of amputees

	Variables			(n = 50)	%
Duration of stay in hospital Range (3-48 days)	3-7			22	44
	8-14			16	32
	> 15			12	24
Service units (Wards)	General surgical ward			41	82
	Orthopedic ward			5	10
	Male vascular surgical ward			1	2
	Male Urology Plastic Vascular Ward			1	2
	Female Medical ward			1	2
	Female urology plastic surgery ward			1	2
Associated illnesses	None			7	14
	DM alone or with complication			28	56
	DM + HTN* + IHD† + CRF††			15	30
Final diagnoses	ICD-10 Codes	E14.5	Unspecified DM with peripheral circ. disorder	43	86
		S88.1	Traumatic amputation	6	12
		S 81	Open wound infection	1	2
Outcomes	Improved			42	84
	DAMA (after amputation)			5	10
	Death			3	6
Admissions	Single admission			22	44
	2-3 Times			21	42
	> 3 Times			7	14

*HTN = Hypertension, †IHD = Ischemic heart disease, ††CRF = Chronic renal failure

Table 3 Amputations and anesthesia

Features regarding amputations				(n = 50)	%
Level of amputations	Lower limb	Toes	Digits	20	40
			Ray	7	14
		Syme		1	2
		Below Knee (transtibial)		13	26
		Above Knee (transfemoral)		4	8
	Upper limb	Fingers (digits)		4	8
		Elbow disarticulation		1	2
> One amputation				5	10
Anesthesia given	General Anesthesia			19	38
	Local Anesthesia			15	30
	Spinal anesthesia			7	14
	Lumber Nerve block			1	2
	Ankle block			4	8
	Not documented			4	8

There were 27 (54%) patients who underwent amputation at the level of the toes, followed by, 17 (34%) around the knee. We found that lower limb amputations were predominant 42 (97.7%) in diabetics while no difference was found among non-diabetics, i.e. upper limb 4 (57.1%) versus lower limb 3 (42.9). Only 5 (10%) cases had more than one amputation on the same limb. Nineteen (38%) patients underwent amputation under general anesthesia. Five (10%) patients underwent amputation more than once (Table 3).

Discussion

Our study allowed us to list all the cases of amputations carried out in this tertiary care referral teaching hospital. Males were more dominant as compared to females, showing correlation to the studies done by Al-Turaiki (5), Trautner (6), Dangelser (7), Agarwal (8), Leung (9) and Mohamed (10), but contrary to Johannesson (11). Diabetes was the most common cause of amputation in our study and it correlated with the studies of Trautner (6) and Dangelser (7). Presently more than two thirds of amputations on civilians in western society are performed for peripheral vascular diseases (12, 13, 14, 15), and performed for four main categories of vascular diseases (1) arteriosclerosis obliterans (2) arteriosclerosis obliterans with diabetes (3) thromboangiitis obliterans and (4) miscellaneous conditions such as embolic occlusion, peripheral aneurysm, vascular trauma, and venous obstruction (12). Diabetes is an etiological factor in one quarter of patients requiring lower limb amputation (12). Likewise; diabetes is the cause of up to 70% of all the non-traumatic amputations in the world. This can be explained by the fact that the number of persons with diabetes is increasing rapidly (16). The peak age for amputation was 45-65 years in our study contrary to the peak values at ages between 65-74 yrs in diabetics and 55-64 years in non-diabetics in the study by Nazim (17) and also the peak incidence of amputations was observed in the decade from 67 to 76 years in both age groups (18). The mean age of amputees was 64.7 years according to the studies by Trautner (6), Leung (10), Laaperi (13), Eskelinen (15), and Nazim (17). On the other hand,

our study showed a mean age of 53 years. Mohamed (10) and Ebskov (19) found the mean age less than in our study. Likewise 5-72 years was the range of ages of the amputees according to Mohamed (10), while ours had wider age range. AL-Turaiki (5), Agarwal (8), Mohamed (10), and Stinus (14) found more traumatic amputations than us. The risk of lower extremity amputation was 15 times higher in diabetics than in age matched non-diabetics (20) but AL-Turaiki (5) found more traumatic amputations. Regarding the level of amputations in our study, below knee amputations were more frequent than above knee, showing consistency with Mohamed, but contrary to the studies of both Laaperi (13) and Eskelinen (15). Similarly according to Hussein (16), 24% of diabetic amputations were of toes, 5.8% were mid-foot, 38% were below the knee, and 21.4% were above knee; the remaining 10% included other sites. We found a majority of lower limb amputations in diabetics. The most likely reason in such cases was peripheral sensory loss with the development of foot ulcers, as also mentioned by Birke (21).

Moreover, we found a readmission rate of about 56%, which was mainly due to poor follow up, poor home care and poor compliance with medication intake. The other reasons included complications of IHD, CRF and HTN.

Conclusion

Diabetes was the major factor in amputations in this study. Most patients were between 45-65 years of age. Lower limb, especially foot amputations were predominant. Congenital, carcinoma or other diseases of bone were not noticed. Peripheral vascular disease and neuropathy were the main causes of amputations. Poor home care, follow up and compliance with medication were the main reasons among the readmitted patients which demonstrates the immense need for a multidisciplinary team approach with an objective to reduce the amputation rate, especially in diabetics.

Recommendations

1 – Special emphasis should be paid to foot care services as well as patient education.

2 – Prevention of complications, i.e. peripheral vascular disease and neuropathy would be warranted to prevent amputations and subsequent high disability.

3 – To reduce the re-amputations, surgeons should be more involved in long-term evaluation of functional outcome in such patients and modify their technique for future procedures.

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Biliary atresia

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Biliary atresia (BA) remains a puzzling medico-surgical condition more than 100 years after its original description. It is the most important disease in paediatric hepatology and the most frequent indication for liver transplantation in children and young adults. BA occurs sporadically in around 1/17,000 live births in Europe and North America and there is no risk of recurrence within the same family. In patho-anatomical terms, BA is characterised by a progressive ascending obstruction of the biliary tree which evolves into biliary cirrhosis and end stage chronic liver disease within infancy. Corrective surgery – portoenterostomy or the "Kasai operation" can be effective in up to 50-60% of timely operated children, whilst the others should be considered for early liver transplantation. Medical treatment is important to provide optimal nutrition during chronic cholestasis and ensure the normal physical and neurological development of the affected infants. Early and precise diagnosis is of critical importance since delaying surgery may reduce the chances of a successful outcome, while unnecessary surgery for unrecognised other infantile cholestatic conditions is deleterious. Diagnostic algorithms vary depending on the local expertise, but most centres use expert ultrasonography and percutaneous liver biopsy, as clinical examination and biochemical findings are non-specific. Most children with prolonged neonatal conjugated hyperbilirubinaemia will have pale stools, dark urine, a degree of soft hepatosplenomegaly and mildly elevated transaminases. Acholic stools point strongly in the direction of a surgical problem. In ambiguous cases, direct cholangiography, preferably endoscopic retrograde cholangiopancreatography (ERCP), performed at specialized centres, will be diagnostic and spare unnecessary laparotomy, if not indicated. The aetiology of BA remains uncertain. Its inflammatory nature is undoubted and many centres use steroids routinely post-Kasai portoenterostomy in the hope of reducing the inflammatory component and minimising ensuing fibrosis and progression to cirrhosis. The scientific evidence for the benefit of steroids is lacking and there is a lively ongoing research debate about further means of improving the postoperative outcome including the use of steroids at different doses and regimens, and proinflammatory cytokine-blocking agents in the future.

Key words: Neonatal cholestasis, Biliary atresia, Kasai portoenterostomy, Liver transplantation.

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Biliary atresia (BA) is a progressive life-threatening obliterative cholangiopathy presenting within the first few weeks of life (1). It occurs sporadically and has no gender preponderance. Its aetiology is unknown and incidence estimates vary from 1/8,000 live births in the Far-East Asia and Oceania to 1/17,000 live births in Europe and North America (2, 3). Untreated BA evolves to end-stage biliary cirrhosis with its complications, leading to death, typically within the first two years of life. Therefore, BA is by far the most frequent indication for liver transplantation (LT) in paediatric medicine (4).

BA is the commonest chronic cholestatic condition in children and typically presents with prolonged neonatal conjugated jaundice, dark urine and acholic stools. These clinical signs are non-specific and could also be noted in some other liver conditions at this age, including non-specific giant-cell hepatitis, PiZ alpha-1-antitrypsin deficiency, Alagille syndrome, cystic fibrosis, bile salt export pump deficiency or septo-optic dysplasia. The key clinical signs are acholic stools, which could initially contain variable amounts of pigment, particularly in the non-syndromic forms of BA, with presumed perinatal or postnatal onset (see later). The variation in this clinical sign, secondary to progressive yet incomplete obstruction of the biliary tree, often with good clinical condition, lack of feeding difficulties and appropriate weight gain, may lead to false reassurance and late diagnosis. Surgical correction of BA has better prognosis early, when the evolving patho-anatomic process is less advanced. Thus, the need for early diagnosis of BA cannot be overemphasized amongst health professionals, including midwives, family doctors and health visitors as their exposure to such a rare condition is likely to be limited during their careers.

Abnormality of biochemical markers in BA reflects non-specific cholestasis. Further diagnostic pathways vary depending on the expertise available locally, but the majority of the centres combine ultrasound, liver biopsy and direct cholangiography for confirmation of BA (4). Percutaneous liver biopsy, performed under local anaesthetic, is diagnostic in around 80% of patients, providing sufficient information to proceed to laparotomy, intraoperative cholangiography and corrective surgery, if required (5). Typical histological features include oedematous portal tracts, with reduplication of the smaller bile ducts and panlobular and intraportal cholestasis with variable degrees of giant cell transformation and early fibrosis (Figure 1). Neonatal sclerosing cholangitis (NSC) could present with the identical histological features, but these children have pigmented stools.

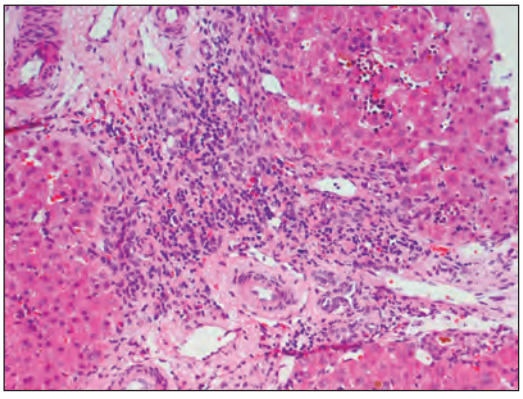

Figure 1 Histopathological findings in the liver biopsy of a seven week old child suggestive of biliary atresia: expanded portal tracts with loose connective tissue and intraportal bile plugs indicative of cholestasis with some portal and interface inflammation (haematoxylin & eosin, x 250)

Some studies have suggested that expert ultrasound scanning could provide important diagnostic indicators either by demonstrating the abnormal outline or absence of the gallbladder (6), or a "triangular" sign, suggestive of portal fibrosis (7). In ambiguous cases, direct radiological methods such as endoscopic retrograde cholangio-pancreatography (ERCP), percutaneous transhepatic cholangiography or cholecystogra-

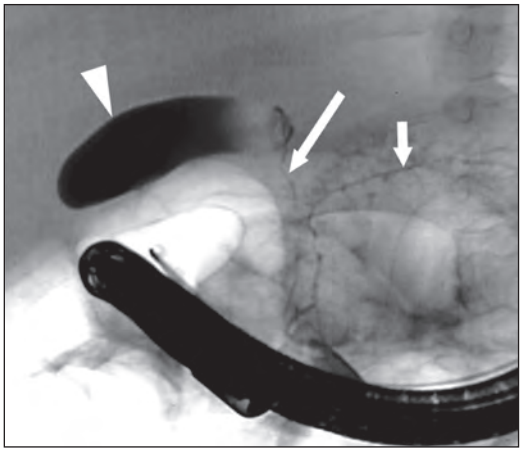

Figure 2 Endoscopic retrograde cholangiopancreatography demonstrating opacification of the pancreatic and common bile duct and the gallbladder, but no visible common hepatic duct and its proximal branches. Biliary atresia type III was confirmed on laparotomy

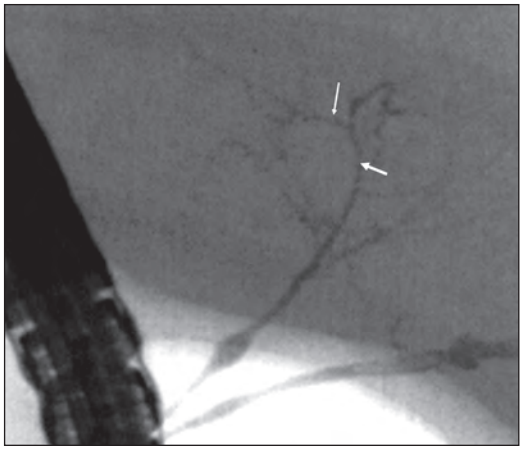

Figure 3 Endoscopic retrograde cholangio-pancreatography in a ten week old infant demonstrating the abnormal appearances of the extrahepatic duct, common hepatic duct and intrahepatic branches of the bile ducts, suggestive of chronic cholangiopathy

phy (PTC) could be considered as they offer the possibility of direct visualisation of the biliary system. Furthermore, during ERCP presence of bile in the duodenum can be documented, excluding BA. Although requiring certain technical skills for this age group, this technique is also the best method to diagnose NSC (8) (Figure 2 and 3).

Clinical variants

The predominant type of BA, seen in 85-90% infants, is a non-syndromic one, with presumed perinatal or early postnatal onset. The remaining 10-15% children have the syndromic (embryonal) variant, associated with splenic malformations (asplenia, polysplenia), midline defects (situs inversus, intestinal malrotation, cardiac isomerism), absence of the inferior vena cava, pre-duodenal portal vein, pancreatic abnormalities, bronchial ciliary dyskinesia and cardiac malformations, such as atrial or ventricular septal defects (9). It is usually referred to as BA-splenic malformation (BASM) syndrome (9, 10). There are also some sporadic associations with BA, such as with intestinal atresia, Cat eye syndrome, renal abnormalities, but different from the laterality anomalies spectrum described for the BASM syndrome (10). A higher incidence of maternal gestational diabetes and female gender has been observed in the BASM syndrome (9). Post-LT prognosis of children with BASM syndrome has often been described as inferior (11, 12, 13), with anatomical variability, vascular inconsistencies and possibly inferior immune responses due to the splenic involvement as a possible partial explanation (14). The BASM subgroup appears to benefit more from surgery at an earlier age than the non-syndromic one (13).

The Japanese Society of Paediatric Surgeons has suggested macroscopical classification of BA: type I - atresia of the common bile duct with patent proximal ducts, type II - atresia of the hepatic duct with patent proximal ducts, and type III - atresia involving the left and right hepatic ducts at porta. The first two types, much rarer and which affect distal parts of the biliary tree, have been referred to as "correctable", while type III

BA, seen in around 88% of the infants (15) is termed "non-correctable", as it requires more radical biliary surgery, involving intrahepatic resection deep into the portal plate.

The Japanese surgeon, Morio Kasai, introduced a novel method of corrective biliary surgery, now named after him, in the 1960s (15). His radical operation involves excision of the atretic biliary system and fashioning the Roux-en-Y loop from the patient's own jejunum (15, 16). Kasai portoenterostomy (KPE) achieves clearance of jaundice in 50-60% of operated children (5, 12) and some 11% have no clinical signs of liver disease after 10-year follow up (17). Nevertheless, one third of all children with BA require LT by 2 years of age, while one more third may need it by adolescence (5, 12). Kasai's pioneering work was first to recognize the critical role of the patient's age at surgery, which has been repeatedly observed since worldwide (16, 18). It is conceivable that timely surgery captures BA at earlier stages, when the pathophysiological process has not yet advanced to the proximal intrahepatic biliary radicles, and when re-establishment of the portal flow may still be achievable. Overall, the positive prognostic factors include young age at diagnosis and surgery, experience of the surgical team and specialized long-term medical care (2, 3, 5, 18).

The KPE could also be considered in infants coming to surgery late (i.e. >100 days old), at least as palliation, as long as their chronic liver disease remains clinically compensated, but the outcome success rates decline sharply (19). After the KPE, cirrhotic children often have refractory ascites due to the presence of portal hypertension, peritonitis and ascending cholangitis. In reality, exceptional children presenting with BA at the age of >120 days are usually directly referred for primary LT.

The surgical or short term success of KPE is conventionally defined by normalisation of serum bilirubin by 6 months of age. Recently, one study suggested that good hepatic excretion of isotope on HIDA scanning at that age could predict better outcome at 2 years (20). However, radionuclide studies always need to be clinically interpreted, with preserved hepatic uptake and adequate gut excretion of the isotope not always indicating lack of advanced chronic liver disease and clinical complications.

Pathogenesis

The pathophysiology of BA remains enigmatic, despite its initial description by the Scottish surgeon Thomson more than one hundred years ago (1). Thus far all attempts to explain the aetiology and pathogenesis of BA have remained incomplete (4).

BA is arguably a final clinical phenotype of combined environmental and host pathogenic factors, including defective early morphogenesis or embryonal vascular supply, toxic or infectious triggers, immunogenetic background and/or the aberrant immune reactivity of the young infant (4) (Figure 4).

It is biologically conceivable that a potential cholangiotropic infection could trigger an attack on the bile duct when the adaptive immune responses of the young infant are still defective, and in a susceptible host this could progress to unabated "autoimmune" liver injury. This hypothesis does not account for the occasionally observed twin or even triplet pregnancies with only one baby affected with BA. It was hoped that these rare case reports, antenatally suspected BA (on ultrasound scanning) or the BASM subgroup could shed more light, but unfortunately there is no convincing or definite information yet about the genetic predisposition (4, 9, 21).

Host factors such as HLA phenotype (22, 23, 24) and mutations in *CFC1* or *inv* genes, implicated in determining left-right asymmetry during embryonal development (25, 26) have been suspected to play a role in the

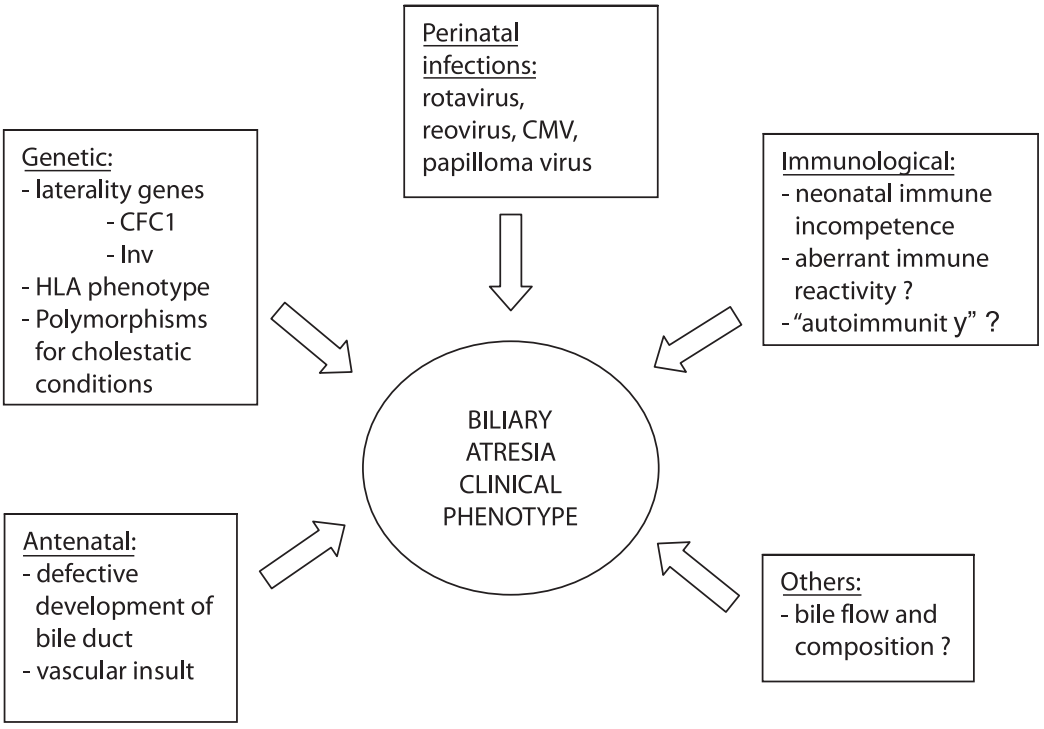

Figure 4 Multifactorial aetiology of biliary atresia

aetiology of BA. Our group, using PCR-based technology, failed to identify any HLA phenotype and cytokine gene polymorphism associations in a multiethnic group of British BA children (22), while two more recent smaller studies, using less sensitive lymphocytotoxicity assays suggested increased prevalence of B8 and DR3 in Egyptian and of HLA-DR2, A24 and B52 in Japanese BA infants (23, 24).

A heterozygous transition c.433G>A in exon 5 of the *CFC1* gene, encoding the CRYPTIC protein, and causing amino acid substitution Ala145Thr has recently been identified in 5 of 10 patients with BASM syndrome, but not all of those 5 had the same clinical phenotype, as illustrated by different types of congenital heart disease (25). As this missense mutation is one of many involved in the laterality defects and was also present in 12.5% of the controls, the authors concluded that it could represent only a co-factor for genetic predisposition to BA (25). Several infectious pathogens have been proposed to be implicated in animal models and in human BA, including rota virus, reovirus, human papilloma, and herpes group viruses, but their presence was not universally demonstrated (4). Recently, a German group studied the presence of the DNA or RNA of common community viruses in the stored biliary remnants of operated BA children, using a range of PCR-based assays, and found that 21/64 samples were positive for reovirus and 8/74 for cytomegalovirus (27). The study had no control group and the viral prevalence increased with the age of the children, suggesting increased environmental exposure, preventing the authors from interpreting their findings as significant (27).

Inflammation in biliary atresia

BA is a chronic inflammatory process and up-regulation of $CD4^+$ and $CD8^+$ lympho-

cytes (28, 29), and several proinflammatory cytokines including interferon-gamma, tumour necrosis factor-alpha, interleukin-2 and interleukin-18 was demonstrated both in humans and in rodent models (30, 31, 32). Immunohistochemical staining can demonstrate a significant periportal reactive inflammatory component in the excised biliary remnants (33, 34, 35). Davenport et al. have shown that HLA-DR is aberrantly expressed in the biliary epithelium in BA, with predominantly $CD4^+$, natural killer ($CD56^+$) T cells and macrophages ($CD68^+$) in the inflammatory infiltrate (33). Their increased presence was associated with the poorer outcome in this study (33).

One available animal model for BA is infection of newborn Balb/c mice with a Rhesus group A rotavirus (RRV) strain, which leads to jaundice, progressive inflammation, biliary obstruction and end-stage liver failure within the first month of life (33). The inflammatory cytokines display a Th1 profile, with elevated levels of interferon-gamma and tumour necrosis factor alpha (36). Shivakumar et al. reported the absence of progressive biliary injury in an interferon-gamma knockout Balb/c mouse model following neonatal RRV infection, which would have led to BA in wild type mice. Similarly, $CD8^+$ T cell depletion in the same experimental model ameliorates the severity of bile duct obstruction and liver injury (37). Taken together, these observations suggest that targeting interferon-gamma mediated differentiation of $CD8^+$ T cells could reduce biliary obstruction in experimental BA, and by analogy could have a similar effect on the evolution of BA in humans (36, 37).

Mack et al. demonstrated oligoclonal expansion of $CD8^+$ T cells in the livers and bile duct remnants of children with BA (35). The predominantly Th1 cytokine "signature" of the immune response in the excised BA remnants at KPE was also suggested, using the gene array chip technique (36). Recently, the same group extended their observations in a rhesus rotavirus (RRV) infected neonatal Balb/c murine model of BA, suggesting that IFN-gamma is the key cytokine mediator and $CD8^+$ T cells the main effectors of the inflammatory process in experimental BA (37, 38). Finally, Mack et al. have recently demonstrated that the target of autoreactive cells in the experimental model of BA are bile duct epithelial cells, suggesting the concept of "autoimmune" bile duct injury, based on several pathophysiological similarities between BA and autoimmune disorders (39).

The described experimental data support the concept that immune manipulation could play some role in medical management post-KPE. For many years many centres have been using empirical anti-inflammatory treatment with steroids (40). Several small retrospective and non-randomized studies have reported the beneficial effect of adjuvant treatment with high-dose steroids in the early postoperative period after KPE (40, 41). However, when tested in a randomised placebo-controlled trial, low dose steroids (2 mg/kg/d) failed to influence the short-term

Table 1 Published controlled clinical studies on use of steroids after Kasai portoenterostomy

Design	Dose	Number of patients	Measures of outcome	Result	Author
Randomised Placebo-controlled Double-blind Two-centre	2 mg/kg D7-21 1 mg/kg D22-28	38 38 controls	Bilirubin at 6 and 12 m Native liver at 6 and 12 m	NS	Davenport et al. 2007
Open label Single-centre	10 mg/kg D1-5 1 mg/kg D6-28	20 29 controls	Bilirubin at 6 and 12 m Native liver at 6 and 24 m	NS	Petersen et al. 2007

outcome in children with BA (42). Their effect on reducing serum bilirubin, however, appeared to be present in younger infants, possibly related to their immature enzymatic pathways in the liver (42). Another prospective, but small and non-randomised open-label study, using an initial dose of 10 mg/kg/d of prednisolone from D1-D5 post KPE, followed by 1 mg/kg/d from D6-D28, has recently also failed to demonstrate the benefits of steroids for the medium-term outcome (43). Both studies reported that the postoperative use of steroids at these doses was not associated with short-term side effects. Undoubtedly, more controlled studies of the use of anti-inflammatory treatment after KPE are still required.

Medical management

Medical management of children with BA is limited by our incomplete knowledge about its pathogenesis. Table 1 provides the basic guidelines about treatment (Table 1).

Aggressive nutritional support is a principal component of medical management (44). Suboptimal bile flow in children with BA, even after "successful" KPE, secondary to the ongoing fibro-obliterative process in the bile ducts, removed gallbladder and development of chronic liver disease, leads to malabsorption of several nutrients. This pathophysiological problem particularly affects metabolism of the long chain triglycerides and fat-soluble vitamins (44). To improve choleresis, many centres routinely supplement their patients with ursodeoxycholic acid. A recent small French study demonstrated the beneficial effect of this medication after KPE (45).

Milk formulas for cholestatic children should be enriched by medium chain triglycerides (MCT), as they do not require micellar formation for fat solubilization and thus are directly absorbed into portal circulation. Breast feeding can be combined with MCT-formulas, but this will only be exceptionally sufficient to meet the increased energy requirements of an infant with chronic liver disease, which often range between 120-150 calories/kg/day.

In infants with chronic liver disease, if oral feeding fails to achieve the nutritional requirements, further options are nasogastric bolus and/or continuous/overnight feeding. Small calibre soft nasogastric tubes are well tolerated and generally only minimally increase the bleeding risks from oesophageal varices. In portal hypertension gastrostomy feeding is rarely recommended due to the likelihood of developing further complications such as development of peristomal varices, interference with the existing gastric varices and ultimately complicating surgical aspects of eventual LT. Oral feeding of children with chronic liver disease should be actively encouraged even for a small volume throughout infancy, since after protracted periods on exclusive nasogastric feeding children could develop resistant nutritional, psychological and speech complications, commonly manifesting only after successful LT.

Ascending cholangitis is common after KPE due to the abnormal biliary anatomy, including the absence of papilla Vateri, absent peristalsis in the anastomosis and reduced bile flow. This often facilitates proximal translocation of bacteria from the gut with invasion of the biliary system and the liver, where local defence forces, such as Kupffer cells, may be impaired. The biliary infection could generalize to septicaemia, but could also aggravate the existing liver injury. The initial clinical signs of ascending cholangitis can be very subtle and jaundice may appear late. Therefore, the prompt use of intravenous antibiotics is recommended in children with a history of BA who develop fever in the absence of localising features, lasting for more than 24 hours. Blood and liver biopsy cultures rarely provide positive

identification. One retrospective study suggested the increased prevalence of gram-negative enteric bacteria including E.coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Bacteroides and Acinetobacter Baumanni (46). For repeated or resistant infections extended courses of rotating antibiotics (third generation cephalosporins, co-trimoxazole, ciprofloxacin) could be given.

Children with any chronic liver disease, including BA, are at risk of developing neoplastic changes within the liver. Their follow up must include monitoring of serum alpha-fetoprotein and ultrasound surveillance for focal lesions on a six-monthly basis. Double-phase computerised tomography is often required to assess the vascularity of the suspected lesions, distinguishing malignant changes from more commonly observed regenerative nodules or focal nodular hyperplasia (FNH). Microbubble contrast-assisted ultrasonography is an important new technique for their differentiation, with potential application in the paediatric age group.

Generally, medical management of BA is relatively limited and includes short postoperative oral antibiotic prophylaxis, prolonged fat-soluble vitamin supplementation, ursodeoxycholic acid and optimal nutritional support. Some centres use phenobarbitone and cholestyramine, hoping that microsomal enzyme induction in the hepatocytes and interruption of enterohepatic circulation, respectively, could ameliorate postoperative cholestasis, although there is no formal proof of their benefits (Table 2).

All children with BA should be considered as potential liver transplant recipients from their initial diagnosis. Therefore, in anticipation of long term post-LT immunosupression, they should undergo early routine immunisation, supplemented with hepatitis A and B, pneumococcal, varicella and meningococcal vaccines. The regular boosters are also recommended, although some reports have observed inferior immune responses in children with BA, possibly explained by the severity of their liver disease (47).

Table 2 Medical management after Kasai portoenterostomy

Prevention of infection
– Antibiotics (cephalosporins, ciprofloxacin, co-trimoxazole) – Standard and additional immunisations (DTP, polio, BCG, HiB, meningitis C, hepatitis A and B, varicella-zoster, pneumococcus, MMR)
Improvement of cholestasis
– Choleretics (Ursodeoxycholic acid, 20-30 mg/kg/d) – Enzyme inducers (Phenobarbitone, 5 mg/kg/d) – Interruption of entero-hepatic recirculation (Cholestyramine, 2-4 sachets/d)
Control of pruritus
– Rifampicin (5-10 mg/kg/d) – Naltrexone – Ondansentrone
Nutritional support
– MCT-based formula milks – Calorific additives – Fat soluble vitamin supplements – Vitamin K (1 mg/d) – Vitamin D preparations (2000 IU/day or 60,000 IU/month) – Vitamin E (100 mg/d) – Vitamin A (2500 IU/d or 20,000 IU/month

Biliary atresia and liver transplantation

Overall at least about two thirds of children with BA will eventually require LT (48, 49). Indications for LT in children with BA have evolved over the years and are based not only on the major complications of chronic liver disease, such as gastrointestinal bleeding or severe malnutrition, but also the suboptimal quality of life and unsatisfactory academic progress. (50). The majority of experienced centres report one-year patient and graft survival rates in excess of 90% for elective LT (51, 52). Chances of successful LT appear to be increased in older, better nourished and immunologically more mature transplant recipients (11, 51). Primary LT is thus performed only exceptionally for very late presentations of BA, due to the perceived benefits of postponed transplant surgery.

Palliative options in treating portal hypertension secondary to BA, such as transjugular intrahepatic portosystemic or conventional surgical shunts, carry significant risks for worsening encephalopathy and should be discouraged. Complications of BA, prompting consideration for LT, include persistent jaundice with intractable pruritus, advanced portal hypertension with uncontrolled gastrointestinal bleeding or development of hepatopulmonary syndrome, severe malnutrition with failure to thrive and metabolic complications, recurrent life-threatening ascending cholangitis, delayed neurodevelopment and chronic encephalopathy and development of hepatocellular malignancies (12, 50). In the specialised centres, sequential treatment of children with BA, including combination of KPE and timely LT, provide long-term survival of >95% with a normal quality of life (51).

It still remains unclear what proportion of patients with BA will require LT during their lifetime. The results of the longest European studies show that 23% of patients are alive with their native livers 20 years after KPE (48), while Japanese data are more encouraging, suggesting a dramatic improvement in survival rates over the last 20 years: 10 year survival with native liver in around 66% (49), with satisfactory quality of life and education patterns (52). Successful pregnancies have been reported in long-term survivors of BA, where the compensated biliary disease, undoubtedly present in all, has not been an early obstetric concern (52). The Sendai group reported that out of 14 pregnancies in their 11 medium-term survivors (>16 years post-KPE) there were 2 last-trimester foetal losses (53). It is of note that in 2 patients the pregnancy triggered decompensation, necessitating liver transplantation (53). Therefore, much closer follow up BA patients is required both during the pregnancy and in the early postpartal period, since the enlarged uterus could complicate pre-existing portal hypertension, while the pregnancy-associated increased hormonal burden could endanger hepatic synthetic function. Therefore, it is prudent to perform an elective upper gastrointestinal endoscopy at around four months' pregnancy to assess the severity of portal hypertension and the risk for bleeding.

Conclusions

More than 100 years after its original description, BA remains a puzzling medical condition. The most important part of its management is early detection. This is based on the premise that the shorter postnatal duration of cholangiopathy reduces histological injury and allows an expert surgeon to identify small intrahepatic biliary radicals, increasing the chances of effective re-establishment of the bile flow (18). The relative rarity of the condition makes screening programmes difficult to implement, but the recent successful campaign of distributing stool colour charts to all families of newborns in Taiwanese hospitals, where 90% of infants

with BA were diagnosed within the desired age of <60 days (54), appears affordable and promising. Another positive prognostic factor for BA is management in a centralised tertiary facility, with well-documented benefits demonstrated by studies from the UK and France, where the low-volume centres, performing less than 5 KPEs per year, were found to have much inferior results (2, 3).

Management of BA with KPE as primary corrective surgery, centralized expert medical care and LT as salvage therapy has been well established and provides a satisfactory clinical algorithm in the countries where LT is feasible (50, 51). However, further improvement in non-LT management options, which appear to have been maximally developed already, will not happen until we better understand the pathogenesis of this intriguing condition.

Future research into the basic and clinical aspects of BA must be multicentre and collaborative due to its sporadic nature and relatively low incidence. There are ongoing international initiatives both in Europe and North America trying to address this. The potential areas of the highest interest are: a) introduction of realistic neonatal screening methods, b) host susceptibility studies, where the BASM forms of the condition offer the obvious target, c) evaluation of minimally invasive corrective options, such as laparoscopic surgery against conventional KPE, and d) further testing of different modes of immune manipulation for control of inflammatory response, including use of different steroid regimens or selective anticytokine therapies, preferably in a prospective and randomised manner.

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Jela Grujić-Vasić
(1923-2009)

Academic Jela Grujić-Vasić left us quietly on 6th October 2009. We had not seen her much in the Academy over the last months because she was not in good health. Nevertheless, both the members of the Academy and the staff felt her presence as she always warmly and humanly shared in the good, and not so good, moments in their everyday work and life. This relationship remained the same from the moment she was elected to the Academy of Sciences and Arts of Bosnia and Herzegovina, at first as a corresponding member (1984), and then as a full member in 1995.

We must mention here her many sided engagement during the most difficult time in the life of the highest scientific institution in Bosnia and Herzegovina – the war period 1992-1995 – when she and other members of the Academy did their best to sustain the Academy's activity. To describe the personality and work of Academician Jela Grujuć-Vasić is not an easy task, having in mind her large scientific opus and work, and the well-known fact of the energy, enthusiasm, and feeling she had for all achievements in the field of pharmaceuticals which she adopted and went forward to face new scientific challenges. Academic Jela Grujić-Vasić was born in 1923 in Foča. She lived in Sarajevo from 1930. She attended elementary school and high school in Sarajevo, and the Pharmaceutical Faculty in Zagreb (1947).

Her university career started there:

1949 – Assistant in Medical Chemistry at the Medical Faculty in Sarajevo;

1960 – PhD at the Medical Faculty of the University of Sarajevo

After that, she was elected to be Assistant professor, and then Associate professor of Medical chemistry at the Medical Faculty in Sarajevo;

1982 – Full professor of Medical chemistry at the Medical Faculty of the University of Sarajevo

1976-79 - President of the Institute for biology, chemistry and specific pharmaceutical topics.

- She founded the Department of pharmacognosy and drug chemistry in 1975, and ran it successfully.

- 1974, when the Pharmaceutical Faculty was established, she was elected as full professor in pharmacognosy and drug chemistry (areas in which she was deeply engaged until the end of her life). She was also the Dean of the Faculty for some time.

- 2004, she attained the title of Professor emeritus.

Academic Jela Grujić-Vasić demonstrated and shared her knowledge at a number of universities in Europe, as visiting professor or as part of exchanges – Lyon, Paris, Bordeaux, Moscow, and Piatigorsk. In Prague, she took part in a seminar at the Institute of polariography; in Saarbrucken in a seminar on chromatography and phyto-chemistry; as part of UNESCO's fellowship program, from 1959-60 she lived in Vienna and Bonn working at their famous institutes with distinguished professors. Due to her participation in congresses, conferences, and visits to institutions around Europe, the academic Jela Grujić-Vasić made a number of acquaintances with eminent scientists in the field of pharmacology through which she had the chance to obtain relevant information and exchange views and knowledge with her colleagues. She was also engaged in the activities of professional associations: she was a Member of the Pharmacopoeia Commission of SFRJ; President of the Section for medicinal plants of the Association of pharmaceutical societies of Yugoslavia; Member of the Medical Plant Research Society; member of the USA Pharmacognosy Society; expert in the Research Fund of B&H; consultant to the "Bosnalijek" pharmaceutical company.

For her scientific and professional work, Academic Jela Grujić-Vasić received many awards and recognitions, such as:

– "Veselin Masleša" Award 1984,
– Acknowledgment for her engagement in the Winter Olympic Games in Sarajevo 1984,
– "27th July Award" for achievements in the pharmaceutical field 1990
– Acknowledgment of the Pharmaceutical Faculty in Sarajevo for her contribution and achievements (30 years) 2005.

The research and scientific work of the Academic Jela Grujić-Vasić was mainly focused on the study of the physiology and pharmacology of the active natural products of plants. Her work, besides abundant experimental data, demonstrates an excellent understanding of related problems, and use of advanced research methodology. The Academic Jela Grujić-Vasić made a particular contribution in the study of pharmacologically active substances of plants: vitamin C, coumarin and its glycosides, saponins and saponin drugs, phenol compounds, especial phenol acid and tannins, and the anti-microbial activity of some drugs. Also, she has investigated human substances, particularly cholinesterase. What made the Academician Jela Grujić-Vasić special was her obvious ability to collaborate with other researchers and to include in her research a large number of associates. In her research, she always used the latest methodologies. She presented her research findings at conferences or published them in domestic and foreign periodicals, so that her results were always evaluated in the most qualitative way. The results of her research activity are: 6 books, 200 published works in journals, 116 papers from scientific and professional conferences, as well as engagement and collaboration in 15 scientific projects.

The Academic Jela Grujić-Vasić made a major contribution to science at University institutions, but also to the work of the Department of medical research of the Academy of Sciences and Arts of Bosnia and Herzegovina, and especially, as the president, to the activities of the Board for drugs. Within the Academy, she was engaged in spreading knowledge of science and any activities to promote the Academy as the highest scientific institution in our country. In the work of the Department for medical sciences, and especially on the Board for drugs, she insisted on the dissemination of scientific information in the chain of preparation, production, promotion, distribution, legislation and registration of medications. Working within this Board, the Academician Jela Grujić-Vasić initiated all vital issues in the field of pharmacology and pharmacy, from laws on medication, pharmacopoeia, European drug standardization, and

pharmacovigilance, including in the discussions at the Board meetings all participants and relevant professionals and experts in this field.

One of the most important contributions of the Board for drugs and the Academic Jela Grujić-Vasić was the organization of the seminars on pharmacovigilance. Academician Jela Grujić-Vasić left us quietly, in the same way as she lived her life. There was always a smile on her face, her patience, and gentleness will stay in our memory. The loss of Academic Jela Grujić-Vasić will deeplyaffect her family, the Academy and its members, former students, and medical and pharmaceutical professionals. We will keep the memory of her in our hearts, but, we will also be proud that such a great woman, eminent scientist, pedagogue and good friend was part of our institution.

Slobodan Loga

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Other Published Material

Newspaper article:
Tynan T. Medical improvements lower homicide rate: study sees drop in assault rate. The Washington Post. 2002 Aug 12;Sect. A:2 (col. 4).

Dictionary and similar references:
Dorland's illustrated medical dictionary. 29th ed. Philadelphia: W.B. Saunders; 2000. Filamin; p. 675.

Electronic Material

CD-ROM:
Anderson SC, Poulsen KB. Anderson's electronic atlas of hematology [CD-ROM]. Philadelphia: Lippincott Williams & Wilkins; 2002.

Audiovisual material:
Chason KW, Sallustio S. Hospital preparedness for bioterrorism [videocassette]. Secaucus (NJ): Network for Continuing Medical Education; 2002.

Journal article on the Internet:
Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Monograph on the Internet:
Foley KM, Gelband H, editors. Improving palliative care for cancer [monograph on the Internet]. Washington: National Academy Press; 2001 [cited 2002 Jul 9]. Available from: http://www.nap.edu/books/0309074029/html/.

Homepage/Web site:
Cancer-Pain.org [homepage on the Internet]. New York: Association of Cancer Online Resources, Inc.; c2000-01 [updated 2002 May 16; cited 2002 Jul 9]. Available from: http://www.cancer-pain.org/.

Part of a homepage/Web site:
American Medical Association [homepage on the Internet]. Chicago: The Association; c1995-2002 [updated 2001 Aug 23; cited 2002 Aug 12]. AMA Office of Group Practice Liaison; [about 2 screens]. Available from: http://www.ama-assn.org/ama/pub/category/1736.html

Database on the Internet:
Who's Certified [database on the Internet]. Evanston (IL): The American Board of Medical Specialists. c2000 – [cited 2001 Mar 8]. Available from: http://www.abms.org/newsearch.asp

Tables

Tables should be embedded in the text of your article. The preferred software for tables is Microsoft Excel (MS Word is acceptable).

Number tables consecutively in the order of their first citation in the text. Use Arabic numerals. Cite each table at the end of the sentence which is relevant to the table(s). Supply an explanatory title for each.

The title should be placed above the table. Give each column a short or abbreviated heading. Authors should place explanatory matter in footnotes, not in the heading. Explain in footnotes of the table all nonstandard abbreviations. For footnotes use the following symbols, in sequence: *, †, ‡, §, ||, ¶, **, ††, ‡‡. Identify statistical measures of variations, such as standard deviation and standard error of the mean. *Be sure that each table is cited in the text.* If you use data from another published or unpublished source, obtain permission and acknowledge them fully.

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Supply a legend for each figure. Titles and detailed explanations belong in the legends, however, not on the figures themselves. Figures should be made as self-explanatory as possible. Letters, numbers, and symbols on figures should therefore be clear and even throughout, and of sufficient size that when reduced for publication each item will still be legible. Photomicrographs should have internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background.

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Type legends below each figure or on a separate page – immediately following the references. Type or print out legends using double spacing.

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Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples. Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

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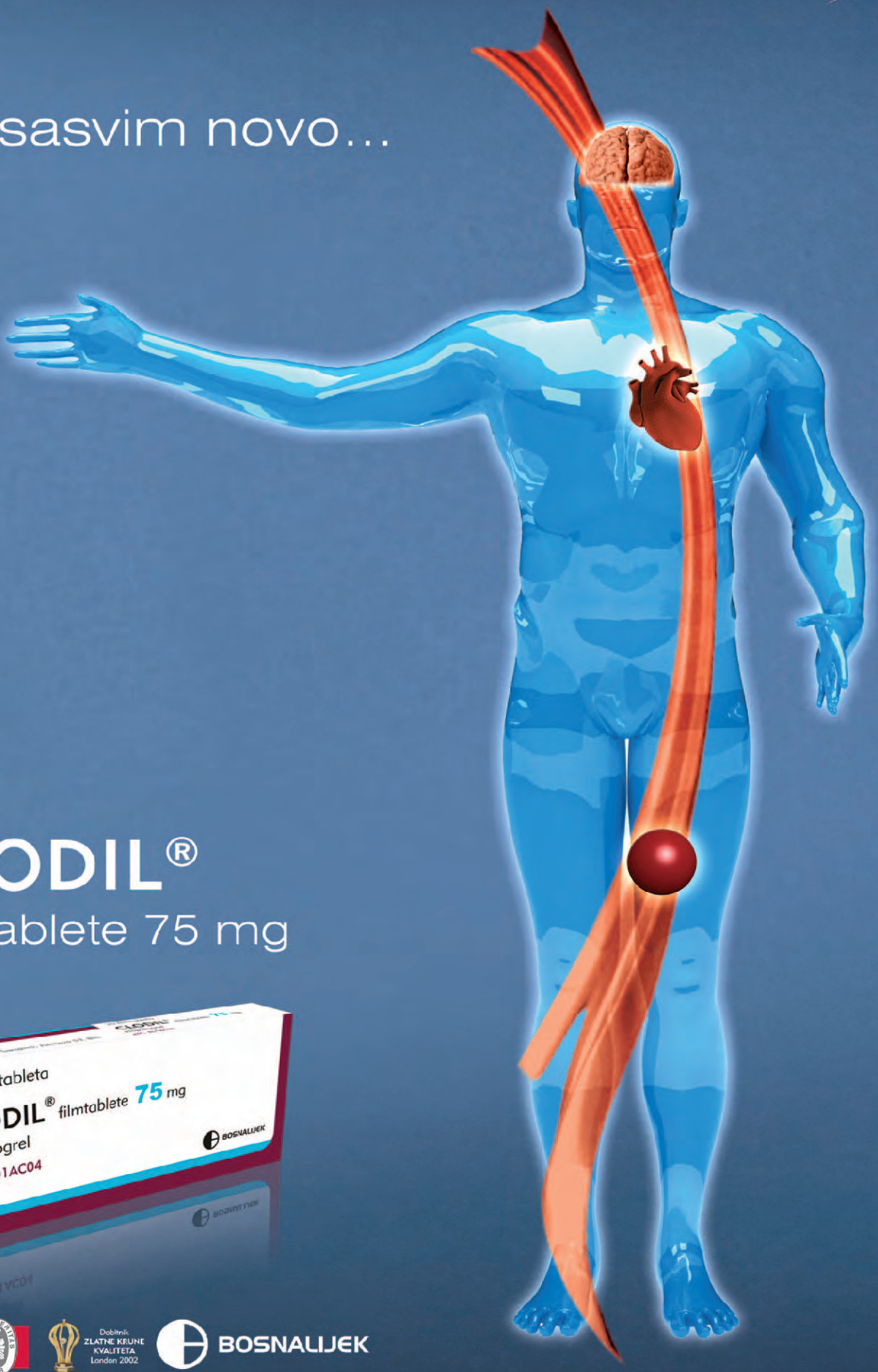

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