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The Future of Academic Medicine

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Key words: Education, Medical; Schools, Medical; Biomedical Research; Evidence-Based Medicine; Bosnia-Herzegovina.

Academic medicine is usually defined as a triad of teaching, research and practice in medicine (1, 2). More broadly, it can be defined as the "capacity of the health-care system to think, study, research, discover, evaluate, innovate, teach, learn, and improve" (3). Although these attributes of academic medicine make it crucial for the improvement of health, there are many voices in the academic medical community that worry about the future of academic medicine (3). Many national and professional bodies in medicine discussed this problem and especially the many deterrents to pursuing a clinical academic career for young physicians. For example, the Academy of Medi-

cal Sciences in the UK, published in 2002 its report "Clinical Academic Medicine in Jeopardy: Recommendations for Change"; and the American Association of Medical Colleges released in 2004 their analysis of medical education system in the USA, entitled "Educating Doctors to Provide High Quality Medical Care: A Vision for Medical Education in the United States" (3).

Editors of medical journals, which are an important part of academic medicine, have also become aware of the problems in academic medicine, and decided to promote the discussion about the future of academic medicine at the beginning of the new millennium. *BMJ*, *Lancet*, and 40 other jour-

nals, including the *Croatian Medical Journal* (1, 4), in 2003 launched a global initiative to develop a new vision for the place of academic medicine in the global community. The first result of this initiative was the formation of the ICRAM – the International Campaign to Revitalize Academic Medicine (5, 6). The goal of this campaign was to offer young medical academics an opportunity to think about the future of academic medicine in a novel way and globally.

ICRAM is run by a working party of 20 medical academics (Table 1), nominated by colleagues from their academic communities. They represent 14 countries, half of the members are women, and half of them come from medium or low income countries. The ICRAM Working Party is chaired by the Leader of the Campaign, Prof. Peter Tugwell from the Centre for Global Health at the University of Ottawa, Canada; and the Campaign Coordinator is Dr Jocalyn Clark, Assistant Editor at the *BMJ*. (Table 1.)

As the contribution to the ICRAM, the *Croatian Medical Journal* published a series of 23 essays on academic medicine, both from the developed countries (7, 8) and developing and newly emerging countries (9-14). These challenging and thought-provoking articles from experts coming from 20 different countries all over the world were published as a separate book (15).

ICRAM Goals and Activities

The goal of the ICRAM Working Party was to produce a series of evidence-based recommendations for reform in global academic medicine by developing a vision and values of academic medicine, discuss strategies for building capacity of academic medicine, including better career paths, and proposing how academic medicine could improve its relationships with other stakeholders – patients, policy makers, funders, and health practitioners (5, 6). To reach these goals,

the ICRAM Working Party used different methodology: a) systematic reviews of the research on different aspects of academic medicine, b) regional meetings with academics from different areas to assess local specificities and needs, c) consultations with all stakeholders in academic medicine, and d) discussing and developing vision and values of academic medicine in future.

So far, four systematic reviews were conducted by the ICRAM members (Sharon Strauss from Canada, John Ioannidis from Greece, and I from Croatia) and their collaborators: 1) patient outcomes in academic vs. non-academic health institutions (16); 2) role of mentoring in academic medicine (17); 3) career choices in academic medicine (18); and 4) funding of clinical research (19).

To increase awareness of medical students and young physicians about possible careers in academic medicine, Gretchen Purcell, ICRAM Working Party member from the USA, published a series of articles in the *BMJ Career Focus* (<http://careerfocus.bmjournals.com/>), presenting exceptional individuals who chose careers in academic medicine and their advice to aspiring academics.

Members of the ICRAM Working Party also convened advisory groups of different stakeholders, as well as seven regional meetings, modeled after the regions of the World Health Organization.

Scenarios on the Future of Academic Medicine

Working on the visions and values of academic medicine, revealed many differences in opinions among the members of the Working Party (3). We could not agree on the values of academic medicine: was it to compete (to earn more) or to provide public service. We also disagreed on the role of the private sector, especially the pharmaceutical industry, and its importance for academic medicine of today and tomorrow – would

Table 1. Members of the International Campaign to Revitalize Academic Medicine, ICRAM (4)

Peter Tugwell, <i>Leader of the Campaign</i>	University of Ottawa, Institute of Population Health, Ottawa, Canada
Jocalyn Clark, <i>Campaign Coordinator</i>	Assistant Editor, BMJ, London, UK
<i>ICRAM Working Party Members:</i>	
Tahmeed Ahmed	Clinical Sciences Division, ICDDR, Dhaka, Bangladesh
Shally Awasthi	Department of Paediatrics, King George's Medical University Lucknow, India
Mark Clarfield	Department of Geriatrics, Soroko Hospital, Ben Gurion University, Beersheva, Israel
Lalit Dandona	Centre for Public Health Research, Administrative Staff College, Hyderabad, India
Amanda Howe	School of Medicine, University of East Anglia, Norfolk, UK
John Ioannidis	Department of Hygiene and Epidemiology, University of Ioannina, Ioannina, Greece
Edwin Jesudason	Department of Child Health, University of Liverpool, Liverpool, UK
Juan Manuel Lozano	Department of Pediatrics and Clinical Epidemiology, Javeriana University, Bogota, Colombia
Youping Li	Chinese Cochrane Centre, West China Hospital, Chengdu, China
Ana Marušić	Department of Anatomy and Croatian Medical Journal, Zagreb University School of Medicine, Zagreb, Croatia
Idris Mohammed	Department of Immunology and Infectious Diseases, University of Maiduguri, Maiduguri, Nigeria
Gretchen Purcell	Department of Surgery, Pittsburgh Children's Hospital, Pittsburgh, IL, USA
Karen Sliwa-Hähnle	CH Baragwanath Hospital, University of Witwatersrand, Johannesburg, South Africa
Sharon Straus	Department of Medicine, Toronto General Hospital, Toronto, Canada
Tessa Tan-Torres Edejer	Department of Health Systems Financing, Expenditure and Resource Allocation, WHO, Geneva, Switzerland
Tim Underwood	Cancer Sciences Division, University of Southampton, Southampton, UK
Robyn Ward	Department of Medical Oncology, St Vincent's Hospital, Darlinghurst, Australia
Michael Wilkes	School of Medicine, University of California Davis, Davis, CA, USA
David Wilkinson	Mayne Medical School, University of Queensland, Brisbane, Australia

business interests threaten or save academic medicine?

In such a deadlock, we decided to use the methodology of scenario building, which is often used by commercial companies for short-term decision making and long-term strategic planning. In the process of scenario building, alternative futures are developed as credible stories which are not predictive but are plausible. Building such scenarios helps to stretch thinking about the future, allows richer conversations, and addresses conflicts, disagreements, dilemmas and divergent opinions.

Scenario building has proven useful not only for planning business strategies but also in different situations and settings, such as the Mont Fleur scenarios undertaken in South Africa during the post apartheid tur-

moil about the Future of South Africa (16), the vision of AIDS problem in 2025 by UN-AIDS (17), or the future of patient-centered care in 2015 by Picker Institute (18).

When several ICRAM Working Party members met in London on the 7th to the 9th February 2005 to work on the scenarios, I thought that we would do our job very quickly – we would sit down and write how we imagine the future. However, we soon learned that scenario building is structured process, requiring team work and exploring different perspectives, including the considerations of instabilities of the present and the drivers for the future. Among the main instabilities we identified in the present academic medicine (3, 23), the most prominent were the unsustainability of the “teaching-research-practice” triad of academic medi-

cine; “brain drain”; poor career incentives; poor translation of research, both from basic science into clinical studies and from clinical studies into medical practice and health decisions; and poor relationships with stakeholders. Globalization and feminization of medicine, as well as new internet and communication technologies were identified as important drivers for the future.

We then imagined the future after a span of 20 years and wrote up five scenarios, some less and some more futuristic from the perspective of 2025 (3, 23).

First Scenario: Academic Inc.

In this scenario, academic medicine flourished in the private sector (3): “Slowly but surely the public sector around the world realized that it could not support the costs of academic medicine. Medical students had high earnings during a professional lifetime: why shouldn’t they pay for their education? And if researchers were doing something valuable then shouldn’t they be able to find a market for their product – accepting that sometimes payment would come from the public sector?”

Medical schools became private, with deans acting rather like Chief Executive Officers (CEO) of a company than academics. Many schools targeted specific populations and provided niche training, such as for older students, or in community medicine, or surgery. Schools asked high fees from students, and used the resources for high staff salaries as well as for purchase of cutting edge facilities and technology. There was intense competition and pressure to reduce costs and improve quality, and research took place in range of private companies. Successful companies-medical schools were responsive to the needs of their customers – governments, researchers, or patients. Academic medicine became a great market field, where innovative, flexible, responsive, and cost-conscious

and cost-reducing companies flourished, and those less competitive failed. With such competitiveness, overall efficiency and effectiveness of academic medicine improved, but equity suffered – with the rich easily creating careers in academic medicine and the poor becoming increasingly disadvantaged to enter the profession. Also, “brain drain” accelerated, and innovation in research often suffered because the shareholders were more interested in financial outcomes than in exploring novel but high-risk ideas.

Second Scenario: Reformation

In this scenario, all members of academic medicine teach, learn, research, and improve, following “the death of academic medicine” (3): “There was increasing concern about the gap between academic medicine and practice with important research results not being implemented, too much irrelevant research, bored students, and practitioners who stopped learning. The response was not to try and strengthen academic medicine but to abolish it and instead to bring the processes of teaching, learning, and researching into the main stream of health care. This innovative—though not initially welcomed—response proved to be highly successful and was copied everywhere. A century of separation of academic medicine was ended. Professors disappeared. The entity “academic medicine” was dead. It was akin to the destruction of the monasteries and so became known as the reformation of academic medicine.”

In this scenario, medical schools ceased to exist as entities, and education, research, and quality improvement took place in the practice setting. The triad of teaching, research and practice was not any more a requirement for an academic – team approach was adopted, supported by advanced learning and communication technologies. Team members were health practitioners, stu-

dents, researchers in basic or clinical medicine, and patients. Research questions arose in professional-patient interactions and special national services provided evidence based responses. Leadership came from diverse specialist societies, which joined together in an international academy with great social and political influence. Medical students spent the first six months learning how to learn, then learned by working in a team, starting with a round in general practice. Some students specialized early, some becoming competent specialists within five years, as there was no distinction among undergraduate, specialist, and continuing education.

Team work fostered learning, but the failings of this approach were in the fact that not all teams held shared values, which threatened stability, consensus, and decision making. Team structure also often prevented brilliant individuals to shine as leaders.

Third Scenario: In the Public Eye

In this scenario, success in academic medicine comes from delighting patients and the public, and using the media (3): “Academic medicine was slow to recognize the rise of global media, “celebrity culture,” and the use of public relations (or spin) to drive the political process, but once it did it responded dramatically. Whereas it had once been suspicious of the media and public appeal and rather patronizing to patients, academic medicine realized that to succeed it must delight patients and the public and learn to use the media. The most successful academics became those who were very responsive to patients and the public, capturing their imaginations, and appearing regularly on their television screens. Some medical academics became as well known as film and rock stars and were feted by politicians.”

In this scenario, the patients and the public became the center force of academic

medicine. The most important department at medical schools became that for public and media relations because the school’s priorities and activities became dominated by the public, i.e. the patients. Students received most of their training not from medical academics but from expert patients. There was a great diversity in the form and size of institutions, and the competition was intense for the best teachers and researchers. Academic institutions had strong links with consumer movements and local non-governmental organizations. Financial support for research came from media “interest”, similar to TV games and reality shows. The downsides of this scenario were manifold, including increased anxiety of academics about their job security and ability to succeed, because scientific advances and clinical practice were shaped by popular appeal, so subject to fashion and not evidence. Also, there was very little regulation of health information.

Fourth scenario: Global Academic Partnerships

This scenario described how academic medicine contributed to global health equity (3): “The world began to find the growing gap between the rich and poor unacceptable. The concern was driven partly by the media and global travel bringing the plight of the poor in front of the eyes of the rich, but it was also driven by anxieties over global security. Terrorism was recognized to be fuelled by the obscene disparities between rich and poor. Global policy makers also understood better that investment in health produced some of the richest returns in economic and social development. Health care was a “must have” not a “nice to have.”

The primary concern and goals of academic medicine were to improve global health. A global health focus offered academics intellectual stimulation and prestige, and Academics championed human rights,

economics, and the environment as key determinants of health. Basic science remained important because of emerging global diseases. The richest (G8) governments signed an accord that prohibited recruitment of academic health professionals from developing countries, thus alleviating brain drain from these academic settings. Universities in the North committed 10% of faculty time to the South; North-South and South-South academic partnerships and networks were established and worked to the benefit of all partners.

The 90:10 gap between the developed and developing nations narrowed rapidly. GAP was idealistic and suffered because political will and global cooperation were often lacking.

Fifth Scenario: Fully Engaged

In this scenario, academic medicine engaged energetically with all stakeholders (3): “Academic medicine realized that its relationships with its stakeholders were mostly poor. The public had little or no understanding of what academic medicine was or why it mattered. Its very name implied irrelevance to many. Patients often felt patronized by academics, and many practitioners—including doctors—were unconvinced of the value of academic medicine. Policy makers found that academics didn’t understand their problems and that the studies they produced came too late to be useful. Some leading academics did have good relationships with politicians, who recognized that biotechnology might be very important in future wealth creation, but the public profile of academic medicine was both low and clouded.”

Medical academics worried that they were misunderstood, underappreciated, and seen as irrelevant by the public. The main goal of academic medicine became to engage fully with the stakeholders of academic medicine—patients, practitioners, policy makers, and the public. New organizations were cre-

ated, and existing ones were reshaped, embracing openness, and the media were used to interact with the public. Governance of academic medicine involved all stakeholders, so that the leading figures in academic institutions could be patients, journalists, or leaders in the community. Medical students were not any more simple consumers of academic medicine, but they shaped and drove medical education. The downfall of the such fully engaged academic medicine was that it got too “popular” and perhaps “dumbed down”, and academic medicine had to struggle to stay truly original and independent.

Lessons from the Scenarios

What we learned from these scenarios was that none of them would predict the future with certainty, but that the future would have elements of each of the five scenarios. There were also some common features to all scenarios. Firstly, academic medicine will need to relate better to other stakeholders and learn how to use media to relate its importance to the public. It will also have to become more business-like in the modern world, as the competition will increase in the global society. Globalization will stimulate academic medicine to be more and more globally minded and embrace new technologies. Medical academics cannot be any more “jack of all trades” and will have to give up the whole triad of research-teaching-practice to the teams of professionals. The emphasis in academic medicine will be teaching and lifelong learning, both in clinical and non-clinical areas. Academic institutions will also diversify, offering specialized expertise. Quality improvement will have to be combined with basic and applied research. Academic medicine will have to accept a broader thinking and skill sets, intensively collaborating with other research and professional fields, such as economics, ecology, law, and humanities. We will also

have to learn more about developing leadership skills. And we will have to think more about the future, starting with the decisions do we need to take now to achieve the desired future.

After the publication of five scenarios, BMJ asked its readers to judge which scenarios they thought likely and desirable. According to the poll (24), the most desirable scenario was “Global Academic Partnerships”, but it was also judged as the least likely scenario. In contrast, the most likely scenario was “Academic Inc.,” where academic medicine becomes a full business enterprise, although it was judged least desirable.

Current Activities on Restructuring of High Education in Bosnia and Herzegovina

In Europe, the future of academic medicine is related to the ongoing restructuring and harmonization of higher education, defined in the Bologna Joint Declaration of the European Ministers of Education in 1999. Bosnia and Herzegovina, like other Central and Eastern European countries in post-communist transition, have specific problems related to the political and socioeconomic framework in which their medical curricula have been shaped in the past (25). Bosnia and Herzegovina has the additional heavy burden of immense war destruction and population migration, which also affected medical education (26, 27).

Despite these disadvantages and exceptionally complex political, ethnic and religious situation in the country, medical schools in Bosnia and Herzegovina were pioneers in revitalization of academic medicine, not only in the region but in the global context (27, 28). The schools functioned normally

during and after the war, and established fruitful collaboration in many areas (29), contributing to the peace process in the country (30), and confirming positive experiences from other political conflicts (31). All five medical schools in Bosnia and Herzegovina joined together to work on medical curriculum reform under the framework of the Trans-European Programme for Co-operation in Higher Education in Central and Eastern Europe (Tempus project “Design of an Integral Curriculum to Undergraduate Medical Education in Bosnia and Herzegovina – DICTUM”) (25, 27, 28). The European partners in the project were medical schools from Austria, Belgium, Denmark, and Germany. Within the framework of the DICTUM program, all five medical schools performed a structured and well planned internal and external assessment of the medical curricula (25), as an exercise in generating an objective insight and generate ideas for institutional development and joint curriculum reform.

In fact, the activities currently under way at the medical schools in Bosnia and Herzegovina have many elements of the most desirable scenario for global future academic medicine – global academic partnership. Actually, researchers within the framework of the DICTUM project were the first to propose dual commitments of academics from developed and developing academic communities as a possible solution to bridging academics and learning from each other (29).

With a new journal dedicated to academic medicine, *Acta Medica Academica*, the academic community in Bosnia and Herzegovina is on the best way to make “Global Academic Partnerships” not only most desirable, but also very likely future of academic medicine!

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Antimicrobial Activity of Different Extracts from Rhizome and Root of *Potentilla erecta* L. Raeuschel and *Potentilla alba* L. Rosaceae*

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Introduction

Following the previous research on the antimicrobial activity of plant sorts *Potentilla*,

The tested plant material of rhizome with roots of *Potentilla erecta* (L.) Raeuschel and *Potentilla alba* L. was collected in 2003. Determination of the total phenolics content, non-tannin phenols was conducted by applying the method of Folin-Ciocalteu reagent and proanthocyanidin content by Porter. The method used for determination of the antibiotic activity was used in accordance with the European Pharmacopoeia (procedure 2.7.2.) on medium A and bacteria *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739 and *Candida albicans* ATCC 10231. The water, ethyl acetate, acetone and ethanol extracts, prepared earlier, were tested. Medium A was used for testing *Staphylococcus aureus* and *Escherichia coli* while Medium F was used for testing *Candida albicans*.

The values obtained after testing phenol compounds (% on dry plant material) are as follows: the value for *Potentilla erecta*: total phenolics 16.90%, Non-tannin phenolics 0.09%, Proanthocyanidins 2.70% while the value for *Potentilla alba*: total phenolics 11.74%, Non-tannin phenolics 0.71%, Proanthocyanidins 2.73%. The ethanol and acetone extracts have the antimicrobial effect on *Escherichia coli*, ethyl acetate extract of rhizome *Potentilla erecta*, while water extracts of both tested species in dissolution 1:20 have the antimicrobial effect on *Staphylococcus aureus*. The tested species have not had any effect on *Candida albicans* fungus.

Key words: Plants, Medicinal; *Potentilla*; Rosaceae; Anti-Bacterial Agents; Microbial Sensitivity Tests .

belonging to the family of Rosaceae, the present study is a continuation of further examination of the antimicrobial activity of those plants (1, 2). The testing of the antimi-

crobial activity of the higher plants is topical both in respect of finding a rational replacement for the existing antibiotics but also due to a resistance of pathogen bacteria to antibiotics after a long usage (2, 3, 4).

Material and methods

The plant material (rhizome and root) was collected in Bosnia and Herzegovina during September 2003 (in the vicinity of small towns of Olovo and Han Pijesak). The picked plants were cleaned, washed and dried in thin layers protected from the direct sun light. The dried plant material was kept in paper containers. Prior to the experiment the material was cut and pulverized.

Extracts preparation

Acetone extract and ethanol extract: The extraction of the fresh pulverized rhizome and roots (*Potentilla erecta* and *Potentilla alba*) was done by using the 70 % acetone or by

70% ethanol in the course of 24 hours at the temperature + 4 °C with periodical mixing. One part of rhizoma and root was extracted with 10 parts of the solvent. After extraction the material was separated from the extract by filtration and rinsed by a double quantity of the solvent. The obtained extract was evaporated to dryness at a lowered pressure and temperature below 35 °C. If necessary, it was kept in the inert atmosphere until usage. Prior to examination the extracts were dissolved in dimethyl sulphoxide p.a. in the same volume as the initial plant material mass. The antimicrobial activity of the dimethyl sulphoxide on the examined bacterial strains was not noticed.

Ethyl acetate extract: One part of the pulverized rhizome and root was poured over by ten parts of water at the room temperature and extracted in the ultrasonic mixer in 30 minutes. The powder was separated from the extract by filtration and rinsed with one part of the water. The obtained water extract was extracted three times by the equal vol-



Figure 1. *Potentilla erecta* (L.) Rauschel (5)

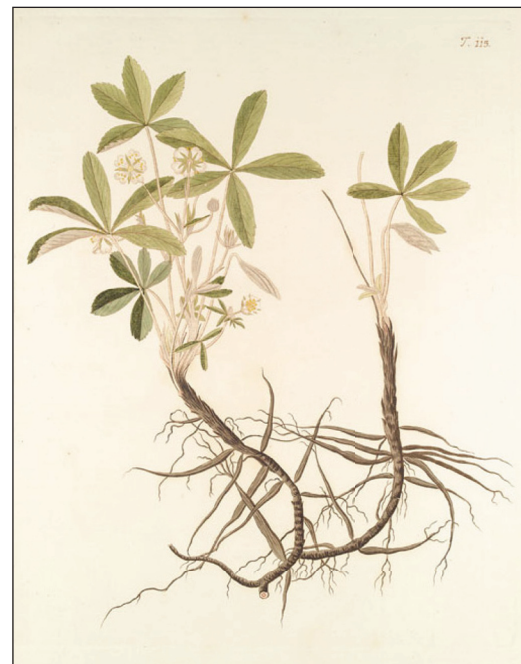


Figure 2. *Potentilla alba* L. (6).

ume of ethyl acetate. The ethyl acetate extracts were joined while water was removed by filtration over anhydrous sodium sulfate. The ethyl acetate extract was evaporated at a lower pressure in rotavapour at the temperature up to 40 °C. After that, the dry extract was suspended in the same water volume as the initial pulverized plant material mass in order to be deposited on the microbiological base.

Antimicrobial activity examination

A diffusion method according to the European Pharmacopoeia edition 5 (Ph. Eur. ed 5) was used as a method for the examination of antibiotic activities (7). The choice of the method was based on its simplicity and widespread application, but also because it enables us to compare the obtained results with others.

The culture media for examination of *Bacillus subtilis* ATCC 6632 was of the following composition: Peptone 5 g, Meat extract 2,4 g, Agar 15 g, Purified water up to 1000 g (8).

The culture media for the examination of *Staphylococcus aureus* ATCC6538, *Staphylococcus epidermidis* ATCC 122228 and *Escherichia coli* ATCC 8739 was the culture media A for the examination of antibiotics by a diffusion method (8).

Density of inoculums:

Staphylococcus aureus ATCC6538 T= 80%

Escherichia coli ATCC 8739 T= 60%

Bacillus subtilis ATCC 6633 T=30%

Candida albicans ATCC 10231 T=80%

Spectrophotometric determination of phenolics

The phenolic analysis started with the plant material crushing and its extraction (9). In case when phenolic compounds were difficult to solve, the examined material was treated by hydrolysis during the process of

extraction in order to get soluble compounds which were analyzed as derivatives.

To examine this group of phenolic compounds the spectrophotometric method was used with Folin- Ciocalteu reagent because of the simplicity and selectivity of reagent to the phenol group (9). The obtained results of the examination with Folin- Ciocalteu can be termed as the tannin index because tannin is a standard in this kind of examination.

In the present study we used the method of total phenolics determination in the plant material, phenolics determination in detanninized extract after removing tannin with polyvinyl polypyrrolidone (PVPP). This method was accepted by FAO organization in 2000 as a standard method for tannin determination (7, 9).

Sample preparation: Extraction solvent: acetone diluted with purified water to 70% (v/v) concentration. After grinding, the plant material (IKA Universal mule M 20) was removed into a flask and poured over by the solvent with periodical stirring. The material was left to stay at the temperature of 4 °C for 24 hours.

Afterward, further material processing for the purpose of analysis was carried out.

This method for tannin determination can be used with the insoluble matrix, polyvinyl polypyrrolidone (PVPP, binding tannin) (9). The obtained values can be expressed as a tannin equivalent. The nature of commercial tannin differs from sample to sample. In our examination we used Acidum tannicum p.a. Kemika, Zagreb.

Determination of the proanthocyanidin content

Determination of the proanthocyanidin content was done according to Porter (by vanillin method) in the following way: 1 ml of the water extract (one part of the plant material and 20 parts of purified water) was mixed with 2 ml of the freshly prepared vanillin

solution (1 g vanillin/ 100 ml 70% H₂SO₄) and kept for 15 minutes at the temperature of 20 °C (10). Absorption was measured at 500 nm (10).

Sample preparation: The powdered rhizoma and root (0.500 g) was poured over by 10 ml of water and extracted in 30 minutes in the ultrasonic mixer. After extraction, the plant material was separated from the extract and water was added up to 10.00 ml. The quantity of the 0.20 ml water extract was diluted to 1.00 ml with water. This was made in order to enable the measurement reading of absorption.

Standard preparation: 2, 50 mg of the catechin is dissolved in water and water is added up to 10 ml.

Results

The results of the spectrophotometric determination of total phenolics (Table 1) and proanthocyanidins (Table 2) have shown the similarity in respect of contents.

The results of the antimicrobial activity are presented in Tables 3, 4 and 5.

Table 1. Total phenolics content in the examined *Potentilla* calculated on dry material

Rhizome and root	Total phenolics (%) acetone extraction	Total phenolics (%) ethanol extraction	Phenolics after tannin removal (%)
<i>Potentilla erecta</i>	17.56	16.90	0.09
<i>Potentilla alba</i>	14.10	11.74	0.71

Table 2. Proanthocyanidin content in the examined *Potentilla* species

Rhizome and root of	% Catechin
<i>Potentilla erecta</i>	2.70
<i>Potentilla alba</i>	2.73

Table 3. Results of antimicrobial activity examination of water extracts

Plant material	Concentration of water root and rhizome extract	Zone of inhibition in mm		
		<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6633	<i>Candida albicans</i> ATCC 10231
<i>Potentilla erecta</i> L.	1:10	10.7	0	0
<i>Potentilla erecta</i> L.	1:20	8.3	0	0
<i>Potentilla erecta</i> L.	1:30	0	0	0
<i>Potentilla alba</i> L.	1:10	11.0	0	0
<i>Potentilla alba</i> L.	1:20	8.6	0	0
<i>Potentilla alba</i> L.	1:30	0	0	0
Neomycin sulfate	20µg/ml	20	20	0

Legend: 0 = no activity

Table 4. Results of antimicrobial activity examination of ethyl acetate extracts.

Plant material	Concentration of water extract	Zone of inhibition in mm	
		<i>Staphylococcus aureus</i> ATCC 6538	<i>Bacillus subtilis</i> ATCC 6633
<i>Potentilla erecta</i>	1:1 ethyl acetate extract	12.6	0
<i>Potentilla alba</i>	1:1 ethyl acetate extract	0	0
Acidum tannicum	2% solution	12.6	0

Legend: 0 = no activity

Table 5. Results of antimicrobial activity examination of ethanol and acetone extracts

Plant material	Concentration of extract	Zone of inhibition in mm	
		<i>Staphylococcus aureus</i> ATCC 6538	<i>Escherichia coli</i> ATCC 8739
<i>Potentilla erecta</i>	1:1 acetone extract	*	18.3
<i>Potentilla erecta</i>	1:1 ethanol extract	*	17.7
<i>Potentilla alba</i>	1:1 acetone extract	13.4	15.9
<i>Potentilla alba</i>	1:1 ethanol extract	*	14.7
<i>Acidum tannicum</i>	2% solution	12.6	18.9

Legend: * = no examination performed

Discussion

The obtained results show that total phenolics content is different in the examined species (11). The antimicrobial activity examined on agar by applying a diffusion method has shown that acetone and ethanol extracts differ with regard to the strength of the microbiological response while the extraction of phenolic compounds is better (more quantitative) if the 70% concentration acetone is used instead of ethanol of the same concentration. This fact has been confirmed by the results obtained in other researches on the extraction of phenolics in different plant materials (12, 13, 14, 15 and 16).

The obtained results of those examinations have contributed to the knowledge of the analytics of the *Potentilla* plant sorts, as well as to our knowledge of their antimicrobial activity.

Conclusions

The antimicrobial activity of the examined samples of the plant sorts *Potentilla erecta* and *Potentilla alba* is similar. The choice of solvents such as the 70% acetone has confirmed the predictions in respect of quantitative extraction of the active constituents (phenolics) and a slightly stronger antimicrobial activity in relation to ethanol extract. The methods used have proven to be suitable and they have given reproducible results.

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Comparison of Nitrazepam Tablets Release Profiles

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Introduction

Nitrazepam is a psychotropic drug, benzodiazepine derivative. Its basic effect is hypnotic, and it also has anxiolytic, anticonvulsive and myorelaxant properties. All the effects of this drug are a result of potentiating of the gamma-aminobutyric acid (GABA) activity in the central nervous system. Following oral application, nitrazepam is well and rapidly absorbed from the gastrointestinal system. Peak plasma concentration is achieved in ap-

The amount of bioavailable active substance is essential in demonstrating therapeutic drug efficacy. *In vitro* technique regarding the comparison of released quantity of active substance from drug can be a relevant anticipation test for *in vivo* drug characteristics. Comparison is usually conducted between test generic drug and a referent, innovative product (1). This comparative study shows the release of nitrazepam content from preparations Trazem® tablets 5 mg (Bosnalijek - test product) and Mogadon® tablets 5 mg (ICN Iberica S.A., Barcelona, Spain - referent product).

Based on the results obtained from the analysis, similarity factor f_2 may be calculated. It is a parameter that, according to FDA guidelines (US Food and Drug Administration), measures similarity of dissolution profiles between two preparations. When two dissolution profiles are identical, $f_2 = 100$. In case when f_2 value ranges between 50 and 100, FDA defines that such two dissolution profiles may be considered similar (2).

Key words: Nitrazepam; Tablets; Solubility; Chemistry, Pharmaceutical; Dissolution.

proximately 90 minutes. By most of its part, the drug binds to plasma proteins. Nitrazepam is characterized by a long elimination half-life that ranges between 24 and 29 hours.

Experimental part

In vitro dissolution (content release) of nitrazepam was performed according to general procedure USP<711> apparatus 2, Method of rotating paddle. Use 0.1 mol/l of hydrochloro-

ric acid, 900 ml as a medium, at temperature of $37 \text{ }^{\circ}\text{C} \pm 0.5 \text{ }^{\circ}\text{C}$, with mixing speed 50 rpm. Sample medium was taken every 5 minutes during 30 minutes, and nitrazepam assay was determined by spectrophotometric method.

Principle:

Spectrophotometric method

Reagents:

Standard solution

Weigh 12.5 mg of Nitrazepam standard into 50 ml volumetric flask, dissolve in 0.5% v/v solution of hydrochloric acid in methanol and dilute with the same to volume.

Pipette 1 ml of this solution into 50 ml volumetric flask, dilute with 0.1 mol/l hydrochloric acid to volume.

Test solution

Test is to be performed on 12 tablets. The solution from each vessel is to be taken every 5 minutes.

Procedure:

Measure the absorbance of the test solution in 1 centimeter flow cell and the absor-

bance of the standard solution at 280 nm in a 1 centimeter cell, using 0.1 mol/l hydrochloric acid as blank (Table 1, 2).

Table 1. Test parameters

Apparatus 2	Method of rotating paddle
Medium	Hydrochloric acid 0.1 mol/l, 900 ml
Temperature	$37 \text{ }^{\circ}\text{C} \pm 0.5 \text{ }^{\circ}\text{C}$
Mixing Speed	50 rpm
Number of Tested Tablets	12
Testing Cycle	5 min
Total Time	30 min

Table 2. Reagents and Standards

Reagents	All the reagents have analytical purity
Standard Substance	Nitrazepam working standard Assay of Nitrazepam 100.3 %

The following systems for dissolution were used: Erweka DT 80 and peristaltic pump IPC 80. The spectrophotometric analysis was performed using UV-VIS spectrophotometer Shimadzu UV-1601.

Table 3. Review of comparison of dissolution profile of TRAZEM® tablets 5 mg with reference to MOGADON® tablets 5 mg

Time (min)	Tab	MOGADON® tablets 5 mg					TRAZEM® tablets 5 mg						
		5	10	15	20	25	30	5	10	15	20	25	30
Amount of nitrazepam Dissolved (%)	1	70.97	78.35	79.11	79.11	78.53	78.54	78.92	82.89	82.33	81.76	81.00	80.05
	2	78.54	82.70	82.51	82.14	81.57	81.00	77.22	82.14	82.14	81.38	80.62	79.87
	3	65.29	72.48	74.00	74.94	75.13	75.32	78.73	81.76	81.38	80.62	80.05	79.11
	4	69.08	76.46	78.16	78.92	79.11	78.92	70.02	83.46	83.65	83.08	82.33	81.38
	5	71.16	79.30	79.68	79.68	79.30	78.92	64.54	77.97	78.92	78.54	77.78	77.22
	6	72.48	78.73	80.43	81.00	81.00	81.19	67.56	83.65	85.16	84.79	84.03	83.46
	7	78.92	82.14	82.14	81.57	80.81	80.24	88.19	94.16	93.49	92.73	91.79	91.03
	8	75.89	80.62	80.62	80.05	79.49	78.92	81.76	88.19	88.38	87.62	86.87	85.92
	9	65.48	77.03	78.92	79.11	78.73	78.35	81.57	90.27	89.52	88.57	87.81	86.87
	10	59.99	75.89	77.59	77.40	76.84	76.27	78.92	88.19	88.19	87.25	86.49	85.73
	11	77.40	79.11	78.54	78.92	77.97	76.84	81.38	87.25	86.68	86.11	85.16	84.60
	12	66.81	78.73	81.19	81.57	81.38	81.00	81.38	92.55	91.79	91.03	90.27	89.52
Mean		71.00	78.46	79.41	79.53	79.17	78.79	77.52	86.03	85.97	85.29	84.52	83.73
SD		5.97	2.79	2.30	2.01	1.92	1.91	6.79	4.83	4.46	4.36	4.31	4.31
RSD		8.41	3.56	2.89	2.52	2.42	2.42	8.76	5.61	5.19	5.11	5.10	5.14

f_2

67.4

SD - Standard deviation, RSD - Relative standard deviation, f_2 - Factor of similarity

Results

The results of examination are given in tables and graphs (Table 3; Figure 1, 2, 3).

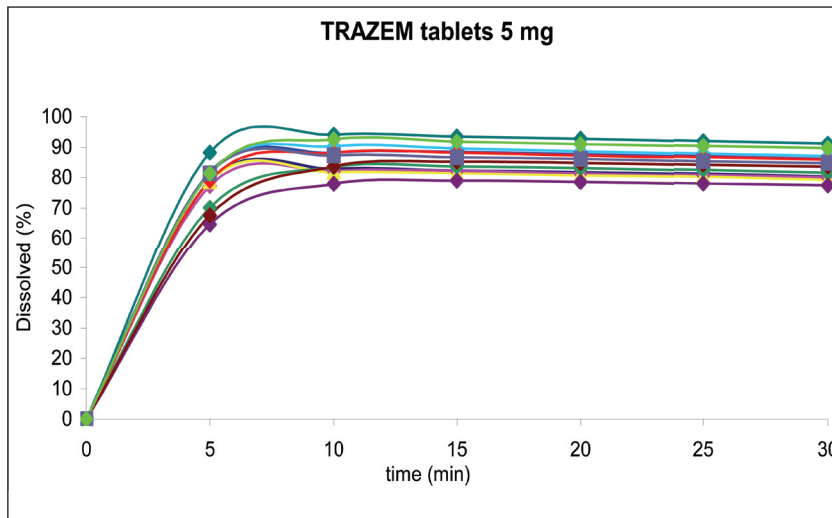


Figure 1. Graph review of dissolution profile of nitrazepam for TRAZEM® tablets 5 mg

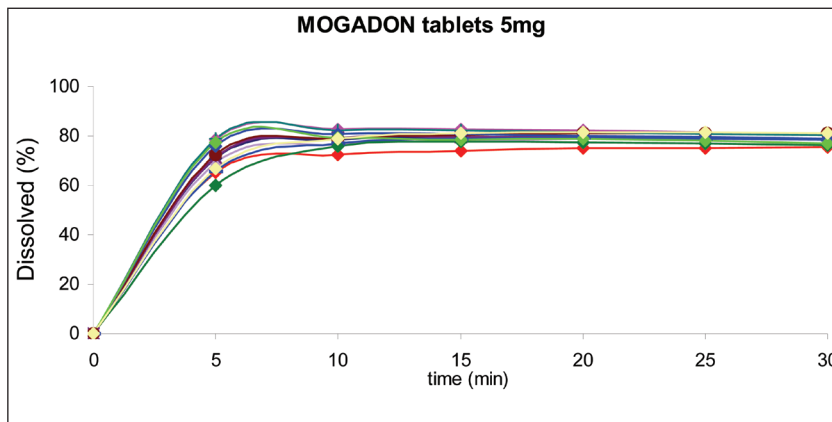


Figure 2. Graph review of dissolution profile of nitrazepam for MOGADON® tablets 5 mg

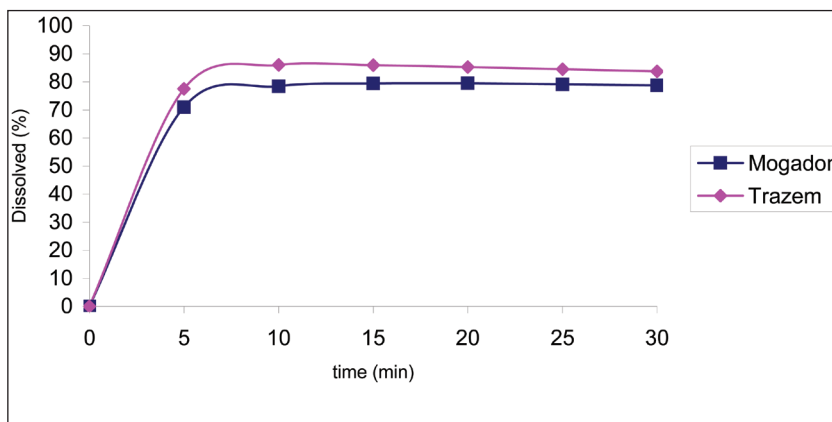


Figure 3. Graph review of average value of dissolution profile of nitrazepam for TRAZEM® tablets 5 mg (◆) and MOGADON® tablets 5 mg (■)

Figure 1 represents graphical display of nitrazepam release from Trazem® tablets (12 samples of tablets) in the sampling cycle of every five minutes for the period of 30 minutes.

Figure 2 represents graphical display of nitrazepam release from Mogadon® tablets (12 samples of tablets) in the sampling cycle of every five minutes for the period of 30 minutes.

Figure 3 represents graphical display of mean values of nitrazepam release profile for Trazem® tablets 5 mg (◆) and Mogadon® tablets 5 mg (■).

In view of the results, factor of similarity f_2 , which is a measure of similarity of dissolution profile for two preparations, can be calculated.

For calculating f_2 the following formula is used:

$$f_2 = 50 \cdot \log_{10} \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^n (R_t - T_t)^2 \right]^{-0.5} \cdot 100 \right\}$$

If f_2 value is between 50 and 100, the two dissolution profiles are to be considered similar (3).

The results showed the factor of similarity between TRAZEM® tablets 5 mg/ and MOGADON® tablets 5 mg to be 67.4.

Conclusion

Based on the conducted comparative analysis regarding the release of nitrazepam content from said preparations, the following has been established:

1. After 30 minutes of testing, 78.79% of nitrazepam from Mogadon® tablets and 83.73% of nitrazepam from Trazem® tablets were released (Table 3).

2. Similarity factor of release profiles was 67.4, which points that those two products have similar dissolution profiles.

Based on the presented obtained results, it can be concluded that the tested preparations are similar.

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Antimicrobial Activity of Some Essential Oils and Major Constituents of Essential Oils

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The screening of the antimicrobial activity of sage, rosemary, eucalyptus, melissa, lavender and thyme essential oils and active compounds 1.8-cineole, citral, linalyl acetate and thymol was conducted by a diffusion test against Gram-positive and Gram-negative bacteria. The most active essential oils, eucalyptus and rosemary oils were tested for the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). The activity was more pronounced against Gram-positive bacteria than against Gram-negative bacteria with MIC ranging from 0.097 mg/ml-0.390 mg/ml, and MBC ranging from 0.390 mg/ml-12.5 mg/ml.

Key words: Plants, Medicinal; Anti-Infective Agents; Anti-Bacterial Agents; Oils, Volatile; Essential Oils.

Introduction

The antimicrobial properties of essential oils have been recognized for many years. Several compounds of essential oils are considered to possess biological activities. The antimicrobial activity of essential oils has been the subject of numerous investigations (1). The mechanism of reaction of essential oils

and their components is unclear. A number of factors hamper the evaluation of the antimicrobial activity of essential oils, their volatility at room temperature, their water insolubility and their complexity (2). There are a number of different testing methods with different testing set-ups (2, 3, and 4). The aim of this study was to carry out a comparative analysis of the antimicrobial activity

ties of the essential oils - sage, rosemary, eucalyptus, melissa, lavender, thyme and their active components (1.8-cineole, citral, linalyl acetate, and thymol).

Materials and methods

Essential oils were purchased from commercial samples in local stores.

Essential oils

1. *Salviae aetheroleum*, Sage essential oil, "Aromatica"
 2. *Rosmarini aetheroleum*, Rosemary essential oil, "Aromatica"
 3. *Eucalypti aetheroleum*, Eucalyptus essential oil, "Aromatica"
 4. *Melissae aetheroleum*, Melissa essential oil, "Atea"
 5. *Lavandulae aetheroleum*, Lavender essential oil, "Aromatica"
 6. *Thymi aetheroleum*, Thyme essential oil, "Aromatica"
- Aromatica, Atea, Croatia, producers of essential oils

Active compounds

Pure 1.8-cineole (Sigma-Aldrich), citral (Aldrich), linalyl acetate (Fluka), thymol (Fluka) and a 50% solution in dimethyl sulfoxide DMSO (Merck).

Organisms and media

Test organisms used in this study: *Staphylococcus aureus* (ATCC 6538P), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027). The strains were maintained and tested on medium E (*Bacillus subtilis*), medium A (*Staphylococcus aureus*) and Mueller-Hinton agar (*Escherichia coli*, *Pseudomonas aeruginosa*). Media were made up according to the European pharmacopeia directions (5).

Agar diffusion hole assay

Microorganisms were suspended in a sterile broth with turbidity corresponding to 0.5 McF units (approximately 10^8 CFU mL⁻¹). Suspensions of microorganisms were incorporated in the appropriate medium (1 ml/100 ml media). Holes (0.5 mm diameter) were punched in the agar plate. Pure DMSO was used as a negative control while erythromycin discs (15 µg), gentamicin discs (30 µg) and penicillin discs (6 µg) were used as positive controls. The plates were observed after 18^h at 37°C. The antibacterial activity was expressed as the mean of inhibition diameters (mm). Tests were performed in triplicate. The doze diameters were measured with the Readbiotic apparatus.

Broth dilution assay

(Minimal inhibitory and minimal bactericidal concentration)

Essential oils were serially diluted twofold in Tryptone Soya broth. The final concentration of oils in the medium ranged from 50 %-0,012 % (v/v). A 2 ml of essential oils in the medium was seeded with the broth culture overnight (0.5 McF units). The samples were incubated 18^h at 37°C. After incubation the last tube without any visible growth of the bacteria was taken to represent the minimum inhibitory concentration (MIC). All samples showing no turbidity were sub-cultured but the lowest concentration, from which the microorganisms did not recover, was the minimal bactericidal concentration (MBC).

The minimal inhibitory concentration (MIC) was defined as the lowest concentration of oil or active compound inhibiting the visible growth of bacteria. The minimal bactericidal concentration (MBC) was defined as the lowest concentration of oil or active compound in the test tube showing no growth in sub-culture. Control samples (positive and negative) were incubated under the same conditions.

Results

The results of the diffusion test are listed in Table 1.

Table 1. Antimicrobial activity of essential oils

Essential oils	Diameter of inhibition zone (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
Sage	13.2	14.2	0.7	-
Rosemary	15.9	20.0	13.0	9.0
Eucalyptus	12.5	18.0	13.0	10.5
Melissa	15.0	18.0	-	-
Lavender	11.5	14.5	9.0	-
Thyme	20.1	19.0	12.0	11.5
Positive control	31.8 erythromycin	25.0 erythromycin	16.0 gentamicin	- penicillin
Negative control (DMSO)	-	-	-	-

Table 2. Antimicrobial activity of compounds of essential oils

Compounds	Diameter of inhibition zone (mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
1.8-Cineole	10.4	14.5	-	-
1.8-Cineole 50 %	-	9.0	-	-
Citral	20.0	23.0	9.0	-
Citral 50%	17.1	23.0	-	-
Linalyl acetate	7.0	8.5	-	-
Linalyl acetate 50 %	7.0	8.3	-	-
Thymol	22.0	23.0	14.5	11.5
Thymol 50 %	21.9	23.0	14.0	9.0
Positive control	31.8 erythromycin	25.0 erythromycin	16.0 gentamicin	penicillin
Negative control (DMSO)	-	-	-	-

Table 3. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of eucalyptus and rosemary essential oils

Essential oils	Gram-positive bacteria				Gram-negative bacteria			
	<i>S. aureus</i>		<i>B. subtilis</i>		<i>E. coli</i>		<i>P. aeruginosa</i>	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Eucalyptus	0.390	3.215	0.097	12.5	0.390	0.390	0.390	0.390
Rosemary	0.195	0.781	0.097	6.25	0.390	0.390	0.390	0.781

The results of the antimicrobial activity assays indicated that essential oil of thyme exhibited higher activity against the *S. aureus* (20.1 mm), essential oil of rosemary against the *B. subtilis* (20.0 mm), essential oils of rosemary and eucalyptus against *E. coli* (13.0 mm) and essential oil of thyme against *P. aeruginosa* (11.5 mm).

Table 2. shows antimicrobial activity of active compounds. Thymol, a phenolic constituent of thyme oil, showed the highest activity.

Table 3. summarizes the MIC and MBC of tested essential oils. Rosemary and eucalyptus oils exhibited higher activity against *B. subtilis*.

Discussion

Among the six essential oils tested, thyme, eucalyptus and rosemary oils showed the highest activity. Gram-positive bacteria are known to be more susceptible to essential oils than Gram-negative bacteria (6). *P. aeruginosa* was least susceptible to the essential oils. The weak antibacterial activity against Gram-negative bacteria was ascribed to the presence of their cell wall, lip polysaccharide (7). *B. subtilis* was the most susceptible micro-organism to the rosemary essential oil. Concerning the activity of pure active compounds, the most susceptible bacteria to thymol was *B. subtilis* (23.0 mm) and the most resistant was *P. aeruginosa* (11.5 mm). Eucalyptus essential oil contained about 80 v/v % 1.8-cineole, but the antimicrobial activity of eucalyptus oil was greater than the antimicrobial activity of 1.8-cineole. Other components contributed significantly to the antibacterial activity of eucalyptus essential oil. A similar situation occurred for lavender essential oil. Linalyl acetate (16-30 v/v %)

was a major component to the lavender essential oil, but it was not found to be a major contributor to the antimicrobial activity.

Conclusion

P. aeruginosa appeared to be the most resistant to the essential oils and active compounds. The active compound with the widest spectrum of activity was thymol. Gram-positive bacteria *S. aureus* and *B. subtilis* were more sensitive to essential oils than the Gram-negative bacteria. The antimicrobial activity of essential oils results from the combined effect of compounds.

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Dental Anomalies among Students of Faculty of Dentistry, University of Sarajevo

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The aim of our study was to establish the prevalence of dental anomalies among the student population at the Faculty of Dentistry of Sarajevo University. The aim of the research was to find out the most common dental anomalies in this particular group. The participants were 268 students at the Faculty of Dentistry. The examined students came from different regions of Bosnia and Herzegovina. The students were examined by the dentists employed at the Faculty while the findings were recorded on charts created specifically for this purpose and in accordance with the recommendations of WHO (World Health Organization). The students' age ranged from 18 to 23. The results were statistically analyzed, and compared with the relevant facts from the literature. Our conclusion is that the most prevalent dental anomalies pertain to the teeth position (54%) followed by the teeth crowding at ICS (intercanine segment) (50%). The most negligible changes were found in the tooth shape, and this could be accounted for by the anthropological changes of the human teeth.

Key words: Tooth Abnormalities; Anodontia; Students; Bosnia-Herzegovina.

Introduction

Within congenital anomalies of the human body, dental anomalies can also be studied. The tooth anomalies refer to disturbances during the tooth development. These disturbances are manifested with regard to the number, size, shape, and/or position of the

teeth in jaws. Dental anomalies could appear as isolated, or related to various other syndromes.

Dental anomalies can be congenital or acquired (1, 2, 3). The causes of these anomalies are shown in table 1. As many as 7% of children are born with some disturbances in the orofacial system (1).

Table 1. Causes of development of congenital and acquired dental anomalies

Congenital anomalies	Acquired anomalies
Heritage (approximately one quarter)	Malnutrition (energetic and protein deficit)
External factors (only 1%)	Influence of chemical substances medicines, vitamins, etc. (3-4%)
Multi causal etiology (rest)	Disturbances of metabolism (2%) Infections, especially of viral etiology (2-3%)

The tooth development starts in the sixth week of intrauterine life (4, 2, 3) and it can be divided into five physiological stages:

- Initiation
- Proliferation
- Morphodifferentiation
- Histodifferentiation
- Apposition

Dependent on the tooth development stage, different kinds of dental anomalies can develop. As a result, dental anomalies can be classified as follows:

- Anomalies of number (initiation stage)
- Anomalies of size (proliferation stage)
- Anomalies of shape (morphodifferentiation stage)
- Anomalies of structure (histodifferentiation and apposition stages)
- Anomalies of color (apposition stage)
- Anomalies of position

Anomalies of the number of teeth occur as a result of disturbances in the initiation stage during the tooth bud development. They can be as follows:

- Hyperdontia, supernumerary teeth (enlarged number of teeth)
- Hypodontia or partial anodontia (lack of one tooth, or a group of teeth)
- Anodontia (lack of all teeth – a very rare phenomenon, occurring concurrently with other syndromes).

Anomalies of the size of teeth are a consequence of disturbances in the proliferation

stage of the tooth bud development. They can be as follows:

- Macrodonia (enlarged tooth size in comparison with average measures)
- Microdonia (reduced tooth size in comparison with average measures)

Anomalies of the shape of teeth are caused by disturbances in the morphodifferentiation stage in the tooth bud development. They represent the biggest group of dental anomalies. They are as follows:

- Fusion or gemination (twinning)
- Invagination of teeth
- Evagination of teeth
- Premolarisation or molarisation of teeth
- Abnormal or accessory cusps on occlusal surface
- Taurodontism
- Group of different morphological anomalies (incisors of shovel shape, Hutchinson's incisors, Pfluger's molar, Turner's teeth, enamel pearl, etc.)
- Morphological anomalies of the root (supernumerary roots, accessory roots, dilaceration, angulation, concrescence of the roots).

Anomalies of the structure of teeth are a consequence of disturbances occurring either in the histodifferentiation stage or the apposition stage (layering of mineral components in the organic matrix of the hard tooth tissues). They are as follows:

- Enamel hypoplasia
- Amelogenesis imperfecta
- Dentinogenesis imperfecta
- Odontogenesis imperfecta

Aim of the study

Aim of our study was: 1. to establish the prevalence of dental anomalies among students of the Faculty of Dentistry at Sarajevo University, and 2. to establish the most common dental anomalies.

Subjects and methods

The group of 268 students of the Faculty of Dentistry at Sarajevo University was examined. They came from various parts of Bosnia and Herzegovina. The students' age ranged from 18 to 23. They were examined by dentists employed at the Faculty of Dentistry, with dental probe and mirror, and their findings were recorded in charts. The charts

were specifically created for this purpose and in compliance with the recommendations of World Health Organization (WHO).

Results

The research findings indicate the dental anomalies found among dental students (Table 2, Figure 1- 9).

Table 2. Prevalence of dental anomalies among students (%) of The Faculty of Dentistry, University of Sarajevo

	Anomaly of number	Anomaly of size	ICS crowding	ICS spacing	Anomaly of position	Anomaly of shape	T.anomale Carabelli	Sign of Carabelli	Anomaly of structure	Anomaly of color
Without anomaly	93	89	50	86	46	98	55	52	91	100
Some of the anomalies present	7	11	50	14	54	2	45	48	9	0
Total	100	100	100	100	100	100	100	100	100	100

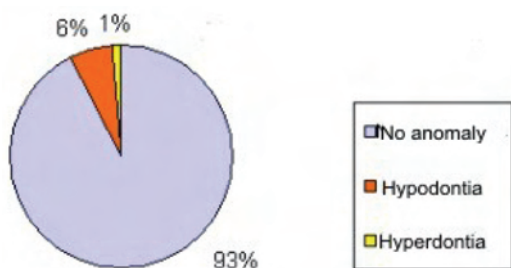


Figure 1. Prevalence of anomalies of number of teeth

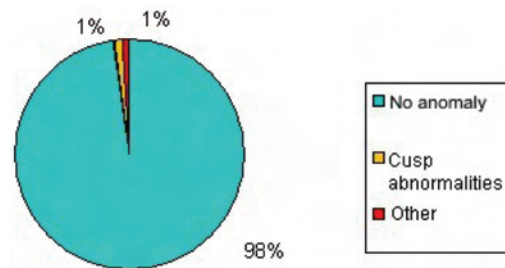


Figure 3. Prevalence of anomalies of shape

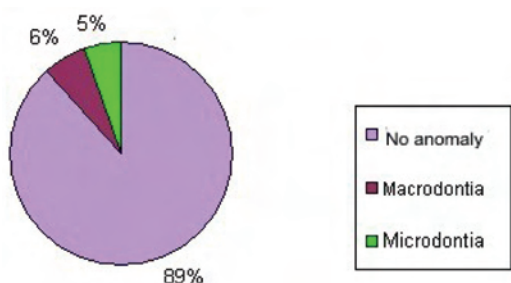


Figure 2. Prevalence of anomalies of size

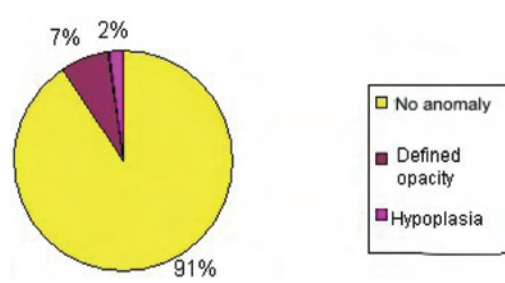


Figure 4. Prevalence of anomalies of structure

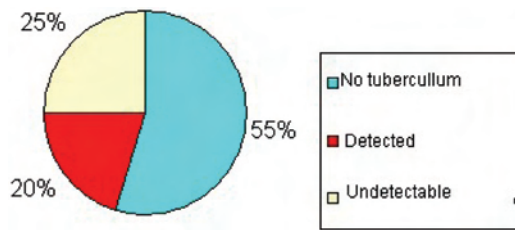


Figure 5. Prevalence of T. anomale Carabelli (Carabelli trait) appearance

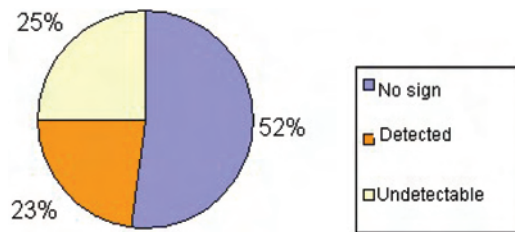


Figure 6. Prevalence of Carabelli's sign appearance

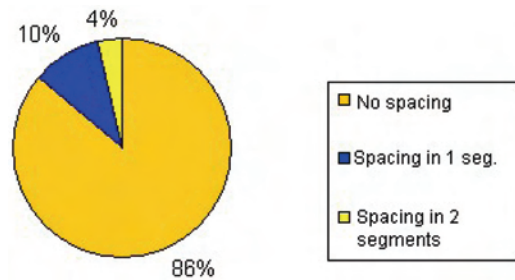


Figure 7. Prevalence of inter canine segment spacing

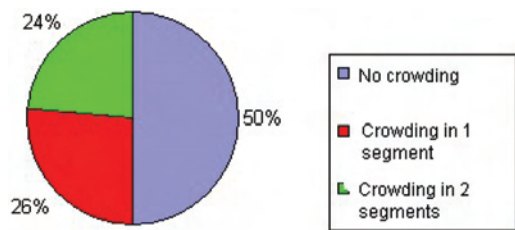


Figure 8. Prevalence of inter canine segment crowding

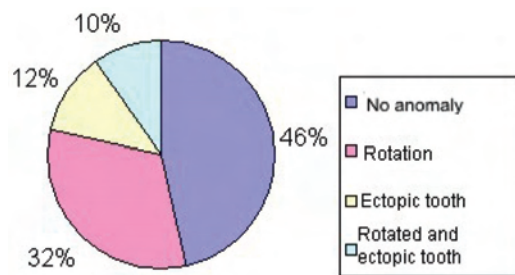


Figure 9. Prevalence of anomalies of position

Correlation between the compression in inter canine sector (ICS) and anomalies of position is 0.55. There is a significant connection between the ICS compression and anomalies of the position of teeth. Figures 11 and 12 show examples of ICS crowding and anomalies of tooth position.



Figure 10. Inter canine sector crowding in lower jaw



Figure 11. Rotation and ectopic upper canine

Discussion

The relevant literature dealing with the prevalence of various dental anomalies is hard to obtain since it appears that few researches have tackled this issue as illustrated in Table 3.

Our study included the group of 268 students of the Faculty of Dentistry aged from 18 to 23. They all come from different parts of Bosnia and Herzegovina.

Table 3. shows the findings of different authors who have researched the dental anomalies problems (5-19).

The most common dental anomalies found in the examined group of dental stu-

Table 3. Prevalence of various dental anomalies according to different authors

AUTHOR	COUNTRY	YEAR	ANOMALY	RESULTS (%)
Hauswright (5)	USA	2002	Hypodontia	2.9-10
Vasconsellos (6)	Brazil	2003	Hypodontia	5.4
			Hyperdontia	2.4
			Macrodontia	0.6
			Microdontia	2.0
Rolling et al. (7,8)	Denmark	2001	Hypodontia	0.16
Thompson et al. (9)	Canada	1974	Hypodontia	7.4
Šutalo (10)	World	1994	Hyperdontia	3.6
			T. anomale Carabelli	17.4
			Carabelli's sign	44
Backman et al. (11)	Sweden	2001	Hyperdontia	1.9
			Fusion / gemination	0.3
			A. imperfecta	<1/800
Knežević et al. (12)	Croatia	2002	Hyperdontia	0.2
			Fusion / gemination	0.2
Schuurs (13)	World	2000	Fusion / gemination	0.1-1
Hovland (14)	World	1977	Dens in dente	0.04-10
Cochran et al. (15)	Ireland	2004	Enamel opacity	61
	Greece	2004	Enamel opacity	28
Keros (1)	World	2000	T. anomale Carabelli	14.7
Konjhodžić (16)	Ex-Yugoslavia	1980	T. anomale Carabelli	56
Maneva et al. (17)	Macedonia	2004	ICS spacing	9.3
			ICS crowding	63.7
			ICS crowding	50
Buchang et al. (18)	USA	2004	ICS crowding	50
Legovic et al. (19)	Croatia	2003	Crowding	45.2

dents from the Faculty of Dentistry, University of Sarajevo, were as follows: anomalies of the number of teeth - 7%, anomalies of the size of teeth - 11%, anomalies of the structure of teeth - 9%, anomalies of the color of teeth - 0%, anomalies of the shape of teeth - 2%, ICS teeth spacing - 14%, ICS crowding - 50 %, anomalies of the position of teeth - 54% .

The most common anomalies were ICS (inter canine segment) crowding - 50%, and anomalies of the position of teeth - 54%.

The above research findings are closely connected with the fact that dental anomalies are commonly caused by various factors such as those related to heritage, disease, endocrine dysfunction, and more specifically, to local factors (trauma, pressure, early loss

of deciduous teeth, bad habits, etc). The hereditary quality has a significant influence on the formation, calcification, eruption, shape, structure, size and number of teeth, but also on the tooth arch width and depth.

Furthermore, we would also like to emphasize the anthropological aspect in respect of evolution of the human jaws and teeth. The last phylogenic changes of the teeth refer both to their shape and size (teeth are atavism). On the other hand, the relations between the size of the teeth, jaws and cranium had changed during the phylogenic transition of human race. The visceral cranium (upper and lower jaw) does not prevail any more, and it is getting smaller. The neurocranium prevails, which is a result of the brain enlargement.



Figure 12. Skull of contemporary man



Figure 13. Skull of Australopithecus

In conclusion, we can state that the research findings of the present study, and of those conducted by other researchers, show that the most prevalent dental anomalies pertain to the position and crowding of teeth in both intercanine segments. These anomalies result from the discrepancy between the size of jaws and the teeth. The jaws have become too small for all the teeth. Looking at this problem in a broader anthropological sense, it is very likely that these anomalies will become more prevalent in the future. As a result, orthodontics will become an increasingly important service in dental medicine.

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Dermatophytoses in Sarajevo Area between 1998-2005

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The progressive increase of zoophilic dermatophytes, especially *Microsporum (M.) canis*, in the etiology of human dermatophytoses has been observed in many regions in Europe. The aim of our study was to assess the frequency of dermatophytes in Sarajevo area during the period 1998-2005.

A total of 3302 samples (skin scrapings, hair, scalp and nail fragments) were collected from patients suspected to have tinea infection and cultured on Sabouraud agar. After three weeks of incubation 633 (19.2%) dermatophytes species were identified based on macroscopic and microscopic morphology. Zoophilic species were found in 554 (87.5%) patients.

The most frequent isolated dermatophyte was *M. canis* (80.3%), followed by *Trichophyton (T.) mentagrophytes* var. *mentagrophytes* (6.7%), *T. mentagrophytes* var. *interdigitale* (4.7%), *Epidermophyton (E.) floccosum* (3.0%), *T. violaceum* (1.4%), *T. schoenleinii* (1.1%), *M. gypseum* (0.9%), *T. rubrum* (0.8%), *T. verrucosum* (0.6%), *T. tonsurans* (0.3%) and *M. ferrugineum* (0.2%). The most common types of *M. canis* infection were tinea capitis (31.7%) and tinea corporis (26.4%).

Our findings indicate increase in the frequency of *M. canis* infection between 1998 and 2002 and the decline over the last years of the observation period, while rate of other zoophilic species *T. mentagrophytes* var. *interdigitale* and *T. verrucosum* did not change significantly.

Key words: Dermatomycoses; Sarajevo; Bosnia-Herzegovina

Introduction

Mycotic infections are among the most common skin diseases. The spectrum of agents of dermatophytosis varies throughout the

world and is constantly changing under the influence of various factors, such as life style, regional ecology, human migration and climatic conditions (1). This change is remarkable especially in the case of zoophilic der-

matophytes (2-4). The increasing frequency of *M. canis* was first observed in southern Europe, especially in Mediterranean countries, and spread from there to northern Europe (5). In some countries, such as Italy, Spain and Greece this species was the most isolated dermatophyte (6-8). In the last decade, an increasing incidence of *M. canis* has been observed in Bosnia and Herzegovina. This study investigated the prevalence of zoophilic dermatophytes and in Sarajevo area during the period 1998-2005.

Patients and methods

Between 1998 and 2005, a total of 3302 samples (skin scrapings, hair, scalp and nail fragments) from patients with suspected tinea infections were collected at the Department of Dermatovenerology and examined in the Mycological Laboratory of the Institute of Microbiology, Parasitology and Immunology, Sarajevo University Clinical Center. All samples were treated with lactophenol to detect the possible presence of fungal elements and inoculated on Sabouraud agar with chloramphenicol and cycloheximide. The plates were incubated at 27°C for up to three weeks. Grown isolates were identified using conventional methods based on macroscopic and microscopic morphology (9).

Results

A total of 3302 samples with suspected tinea infections were collected. Dermatophytes were isolated from 633 (19.2%) patients. The most frequent isolated dermatophyte was *M. canis*, which accounted for 80.3% of all dermatophytes recovered. There followed *T. mentagrophytes* var. *mentagrophytes*, *T. mentagrophytes* var. *interdigitale* and *E. floccosum*, while other species: *T. violaceum*, *T. schoenleinii*, *M. gypseum*, *T. rubrum*, *T. verrucosum*, *T. tonsurans* and *M. ferrugineum* were less frequently isolated (Table 1).

Table 1. Dermatophytes isolated from 633 patients with tinea infection

Species	Number of isolates (n: %)
<i>Microsporum canis</i>	508 (80.3)
<i>Trichophyton mentagrophytes</i> var. <i>mentagrophytes</i>	42 (6.7)
<i>Trichophyton mentagrophytes</i> var. <i>interdigitale</i>	30 (4.7)
<i>Epidermophyton floccosum</i>	19 (3.0)
<i>Trichophyton violaceum</i>	9 (1.4)
<i>Trichophyton schoenleinii</i>	7 (1.1)
<i>Microsporum gypseum</i>	6 (0.9)
<i>Trichophyton rubrum</i>	5 (0.8)
<i>Trichophyton verrucosum</i>	4 (0.6)
<i>Trichophyton tonsurans</i>	2 (0.3)
<i>Microsporum ferrugineum</i>	1 (0.2)
TOTAL	633 (100)

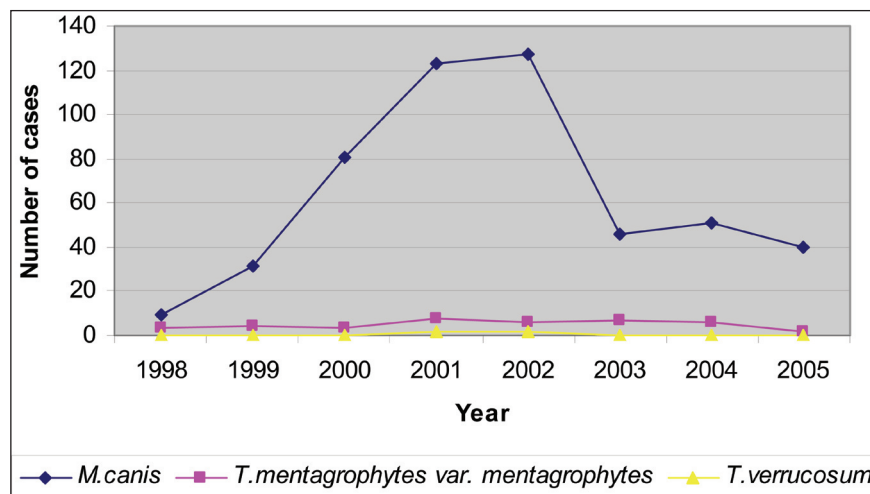


Figure 1. Frequency of zoophilic dermatophytes during the period 1998-2005

Table 2. Distribution of dermatophytes by location of infection

Location of infection	<i>M. canis</i>	<i>M. gypseum</i>	<i>M. ferrugineum</i>	<i>T. mentagrophytes</i> <i>var. mentagrophytes</i>	<i>T. mentagrophytes</i> <i>var. interdigitale</i>	<i>T. violaceum</i>
Tinea capitis	161 (31.7%)	2 (33.3)	1 (100)	3 (7.1)	/	4 (44.4)
Tinea corporis	134 (26.4%)	2 (33.3)	/	22 (52.4)	/	2 (22.2)
Tinea faciei	67 (13.2)	1 (16.7)	/	14 (33.3)	/	3 (33.3)
Tinea pedum	55 (10.8)	1 (16.7)	/	/	24 (80.0)	/
Tinea manuum	35 (6.9)	/	/	3 (7.1)	/	/
Tinea unguium	39 (7.7)	/	/	/	3 (10.0)	/
Tinea cruris	17 (3.3)	/	/	/	3 (10.0)	/
TOTAL (%)	508 (80.3)	6 (0.9)	1 (0.2)	42 (6.7)	30 (4.7)	9 (1.4)

Location of infection	<i>T. schoenleini</i>	<i>T. rubrum</i>	<i>T. verrucosum</i>	<i>T. tonsurans</i>	<i>E. floccosum</i>	TOTAL (%)
Tinea capitis	5 (71.4)	2 (40.0)	/	1 (50.0)	/	179 (28.3)
Tinea corporis	1 (14.3)	3 (60.0)	1 (25.0)	/	8 (42.1)	173 (27.3)
Tinea faciei	/	/	1 (25.0)	/	/	86 (13.6)
Tinea pedum	/	/	/	/	1 (5.3)	81 (12.8)
Tinea manuum	1 (14.)	/	1 (25.0)	/	2 (10.5)	42 (6.6)
Tinea unguium	/	/	1 (25.0)	/	1 (5.3)	44 (7.0)
Tinea cruris	/	/	/	1 (50.0)	7 (36.8)	28 (4.4)
TOTAL (%)	7 (1.1)	5 (0.8)	4 (0.6)	2 (0.3)	19 (3.0)	633 (100.0)

M=Microsporium; T=Trichophyton, E=Epidermophyton

Lesions of tinea capitis were the most prevalent type of *M. canis* infection (31.7%), followed by tinea corporis (26.4%), tinea faciei (13.2%), tinea pedis, tinea unguium, tinea manuum and tinea cruris (10.8%, 7.7%, 6.9% and 3.3%, respectively) (Table 2).

Figure 1 shows the frequency of zoophilic dermatophytes (*M. canis*, *T. mentagrophytes* var. *mentagrophytes* and *T. verrucosum*). In the period 1998-2002 a constant increase in frequency of *M. canis* was observed, while during the last three years we recorded a decline in the rate of this dermatophyte species. The frequency of other zoophilic dermatophytes remained unchanged (Figure 1).

Discussion

Zoophilic dermatophytes were the most common pathogens recovered from our patients during the period 1998-2005. They were isolated from 87.5% of positive cul-

tures, clearly outnumbering anthropophilic species.

Dermatophytes flora in Bosnia and Herzegovina in period 1964-1978 was characterized by *T. violaceum* and *T. tonsurans* as the agents of superficial trichophytosis and *T. schoenleinii* as the agent of the favus. Zoophilic dermatophytes were represented by *T. mentagrophytes* var. *mentagrophytes* and *T. verrucosum*, but no isolate of *M. canis* was found in clinical patients. Microsporiasis was detected only from affected animals and no one case of human infection was noted until 1998 (10, 11).

Since then, the number of infected persons has been constantly growing to up 508 positive isolates in 2005. The prevalence of *M. canis* in our patients is one of the highest in Europe and is comparable only with rates reported from Italy (accounting for 90.5% of all dermatophytes), Brazil (70.5%) and Spain (62.6%) (12-14). In other countries on

the territory of ex-Yugoslavia, such as Slovenia and Croatia, the isolation rate of this fungus is also very high (46.8% and 36.5%, respectively) (15, 16). On the contrary, some other European laboratories revealed a step increase in infection caused by *T. rubrum*, whereas the frequency of *M. canis* remained unchanged (17-19). A similar pattern has been observed in Brazil, Malaysia and Mexico (20-22). In the United States, *M. canis* has been superseded by *T. tonsurans* as well (23).

After the dramatic increase in the rate of *M. canis* infection, recorded in the first years, a significant decline was noted over the last three years of the observed period. Similar to our results, a decrease of this fungus is noted in Greece (8). The rate of two other zoophilic species, *T. mentagrophytes* var. *mentagrophytes* and *T. verrucosum* did not change significantly.

This dramatic change in dermatophytes flora of our patients could be explained as the results of antimycotic campaign in Bosnia and Herzegovina carried out by griseofulvin. Those few cases of *T. violaceum* and *T. schoenleinii* as well as *T. tonsurans* reflect migration of rural population from occupied territories in urban regions. The prevalence of *M. canis* is probably related to the increase in the number of domestic animals particularly cats living outside of homes and consequently an increase in the phenomenon of animals stray and semistray (24). Presumably stray cats are the most important carriers and transmitters of *M. canis*. The elimination of obvious vectors, such as stray animals, could improve conditions of life and hygiene, may be able to reduce dermatophytes, particularly *M. canis*.

The distribution of dermatophytes in this study is similar to the epidemiological pattern reported in some European countries (2-5). *M. canis* remains one of the most important dermatophytes in southern Europe. Further studies are needed to find out

whether the present trend in decreasing frequency of *M. canis* infection will continue.

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Molecular Imaging

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Molecular imaging is a new medical discipline that integrates cell biology, molecular biology and diagnostic imaging. It is generally defined as the *in vivo* characterization and measurement of biological processes at the cellular or molecular level. Compared to conventional diagnostic imaging, it examines the specific molecular abnormalities that are the origin of disease, rather than providing images of the resulting condition. Molecular imaging has two basic applications. The first is diagnostic imaging, which is used to determine the location and extent of targeted molecules specific to the disease being assessed. The second is therapy, which is used to treat specific disease-targeted molecules. The basic data about molecular imaging are reviewed in this paper.

Key Words: Diagnostic Imaging; Positron-Emission Tomography, Magnetic Resonance Imaging; Molecular Biology; Review.

Introduction

Molecular imaging is a new medical discipline that integrates cell biology, molecular biology and diagnostic imaging. It is generally defined as the *in vivo* characterization and measurement of biological processes at the cellular or molecular level. The earlier diagnosis of disease will be possible, since changes at the molecular level always precede anatomical structural changes. Com-

pared to conventional diagnostic imaging, it examines the specific molecular abnormalities that are the origin of disease, rather than providing images of the resulting condition (1). Clinical applications of molecular imaging include the use of nuclear medicine, computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound (US). The nuclear medicine applications utilize devices such as single photon emission computerized tomography (SPECT) and

positron emission tomography (PET). Molecular imaging has two basic applications. The first is diagnostic imaging, which is used to determine the location and extent to targeted molecules specific to the disease being assessed. The second is therapy, which is used to treat specific disease-targeted molecules. The basic principle of the diagnostic imaging application is derived from the ability of cell and molecular biologist to identify specific receptor sites associated with target molecules that characterize the disease process to be studied (2, 3).

Application

Significant scientific effort has gone into the understanding of complex biological systems. These efforts have yielded much information about the molecular changes that are causative or arise as a result of disease. Molecular imaging is a relatively newer field that is attempting to use these molecular data to generate images that report on changes in gene expression. It has been demonstrated that generating images based on molecular differences rather than anatomical differences between tissues has resulted in more sensitive detection of diseased tissues and has allowed imaging of drug efficacy against particular drug targets (2). Positron emission tomography images the biology of diseases at the molecular level, often before anatomic changes are visible or, in some cases, before symptoms appear. Diseases are biological processes and it is these processes that PET examines.

PET/CT is an imaging technology that combines the biological examination of patients by PET with the CT images of the body's structural detail. This technology improves the diagnostic accuracy and treatment management of patients. It provides surgeons, radiation oncologists and other physicians with precise anatomical landmarks associated with the disease condition

as determined by PET. PET whole-body imaging capability helps physicians improve their ability to detect and determine the location, extent and stage of cancer, neurological disorders and cardiac disease. By improving diagnosis, PET scans aid physicians in selecting better courses of treatment, as well as assessing whether treatment is effective or should be changed. Recent published clinical trials have shown that in a wide array of cancers, the use of PET has caused the treatment to be changed for 15 to 50% of patients, depending on the specific clinical question. In addition, PET and PET/CT provide both the patient and their physician with a degree of certainty that is often unavailable through other imaging methods (4). PET holds an edge over SPECT for imaging colon and head and neck cancers. SPECT offers opportunities to pair long-lived radioisotopes with low-molecular-weight agents that target cell receptors. Because of the relatively long half-life of gamma-emitting radioisotopes, SPECT tends to be easier to work with than PET agents, but PET produces much higher resolution. Radiation scattering reduces spatial resolution, as it is not possible to precisely locate the origin of the scattered photons from the body. SPECT is generally not in the same league as PET for cancer imaging. The sensitivity of PET for staging lung cancer in the mediastinum is 76% to 100%, according to various studies. In comparison, the sensitivity of SPECT for this role is 43% to 75% (4). But the value of SPECT should not be underestimated. It is diagnostically powerful and versatile, despite its alleged second-class status. Its molecular imaging capabilities include: uncovering, staging, and monitoring numerous cancers; examining deep venous thrombosis; measuring multi drug resistance to chemotherapy; imaging angiogenesis and apoptosis for early diagnoses and measures of therapeutic response; and diagnosing and evaluating Parkinson's disease and other neurodegenerative conditions (1,

4). SPECT lends insight to the Darwinian world of cellular multi drug resistance to therapy. Chemotherapy triggers natural selection, killing off susceptible cells while allowing resistant cells to replicate. Although cancer cells can employ various pathways to combat therapy, research was concentrated on efforts on the so-called multi drug resistance gene and its product, P-glycoprotein. It is expressed in many tissue types, including the liver, where it pumps substrates into the bile, and the kidneys, where it pumps xenobiotics into the urine. In the absence of P-glycoprotein, the lipophilicity of Tc-99m MIBI enables it to translocate across the cell membrane, and its cationic charge allows it to concentrate inside the cell and be sequestered in the mitochondria. Agent uptake is consequently high. With the presence of P-glycoprotein, Tc-99m MIBI acts like a therapeutic agent and is pumped out of the cell, so uptake is low. Because uptake is quantifiable, the radiopharmaceutical can measure the effectiveness of drugs designed to treat multi drug resistance (4, 5).

Gene expression imaging is one form of molecular imaging used to visualize, characterize, and quantify, spatially and temporally, normal as well as pathologic processes at cellular and sub cellular levels within intact living organisms. This rapidly developing field can be expected to provide useful new tools with which to study gene expression in transgenic animals and in humans during gene therapy.

The application of magnetic resonance imaging (MRI) to molecular imaging begins with a review of the basis for magnetic resonance image generation and how manipulation of different parameters of the system can be applied to molecular imaging. Several examples demonstrate the utility of MRI to generate high-resolution, noninvasive images of molecular events occurring *in vivo* (6). A century after the discovery of X-rays, the low-energy range of the electromagnetic

spectrum also attained broad application in radiology. Radiofrequency waves allow excitation in a magnetic field of the magnetic resonance of spin-bearing nuclei in tissue. Using the intense signal of the water protons, morphological images of the human body can be obtained, while at a higher frequency resolution also endogenous metabolites as well as pharmaceuticals, which contain MR-visible nuclei, can be detected non-invasively and *in vivo*. Accordingly, *in vivo* MR spectroscopy is a technique which is sensitive to molecules and molecular properties and which can be applied to repeated examinations. Its major limitation is the low signal intensity vs. noise, which implies long measurement times and poor spatial resolution. Using spectroscopic imaging, the distribution of metabolites within an organ can be monitored selectively and displayed as a molecular image.

Sarcomas are often characterized by significant histopathology heterogeneity, both between and within tumors. This heterogeneity reflects physiologic, biochemical and genetic processes that are amenable to characterization by functional imaging. Although anatomically based imaging modalities such as plain radiography, X-ray computed tomography (CT) and magnetic resonance imaging (MRI) remain the primary diagnostic modalities for staging sarcomas, nuclear medicine approaches including gamma camera scintigraphy and positron emission tomography (PET) are being used increasingly to provide complementary information in specific clinical situations. These include biopsy guidance within anatomically complex masses, staging, therapeutic response assessment and evaluation of residual mass lesions after treatment (7). Nuclear cardiology has historically played an important role in detection of cardiovascular disease as well as risk stratification. With the growth of molecular biology, new therapeutic interventions and the requirement

for new diagnostic imaging approaches have come. This progress has been made possible with the availability of transgenic animal models along with many technological advances. Future adaptations of the developing experimental procedures and instrumentation will allow for the smooth translation and application to clinical practice (6). In the management of prostate cancer, combined anatomic and metabolic imaging is already in clinical use. In daily clinical practice, fusion of magnetic resonance imaging and magnetic resonance spectroscopic imaging is improving the evaluation of cancer location, size, and extent and is simultaneously providing assessment of tumor aggressiveness (8).

Discussion

Long-term program goals include enabling preclinical disease detection for a wide range of medical disorders and creating personalized, targeted therapies for them. This will be based on molecular profiling of cell and tissue function and delineating data on cell physiology and function to guide development of personalized treatment and computational modeling. The biology teams develop molecular imaging agents, which will bind specifically to the targeting therapy which is based on an extension of the diagnostic imaging principle. Basically, it is assumed that if the molecular probe does target the specific disease molecules of interest, the same molecular agent can be loaded with an agent that will deliver therapy to the targeted cells (1, 2).

The eventual clinical owners of molecular imaging may be a specialty group that is a hybrid by conventional measures. For example, the clinical owner should have fundamental knowledge in basic cellular and molecular biology but must also be certified as well as competent in the specific diagnostic imaging specialty applied (i.e. nuclear,

MRI or US). Another issue relates specifically to the therapy applications in oncology. Clearly, radiology and its associated diagnostic imaging subspecialties is the most logical owner of molecular imaging (1).

It is very important to develop a database of all ongoing molecular imaging efforts, including imaging programs and development of molecular imaging probes. Also it is necessary to provide funding which will provide much-needed support for promising molecular imaging programs and also encourage more investigation in the field. By linking programs in molecular imaging, molecular probes, and molecular libraries, it will bring together the critical elements for development of new, more specific therapies for a wide range of medical maladies. Decoding of the human genome has yielded a catalog of three billion genetic sequences, which constitute the building blocks for discoveries related to the genetic pathways and networks responsible for health and disease. Effort contributed to the identification of tens of thousands of potential genetic pathways and molecular targets that provide opportunities for development of new therapies. As a result, development of a molecular library to catalog the existing and emerging information is a priority (4).

Conclusion

Molecular imaging and the benefits it offers for cancer research and clinical care, which include noninvasive, *in vivo* imaging of specific cellular and molecular processes, nearly simultaneous monitoring of multiple molecular events, real-time imaging of the trafficking and targeting of cells, optimal patient-specific adjustment of drug and gene therapy, and assessment of disease progression at a molecular pathologic level, bring the revolution in medical care and patients treatment.

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Oral Mucositis

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Oral mucositis develops in 100% of patients receiving radical radiotherapy for head and neck malignancies, 70%-80% of patients on chemotherapy for bone marrow transplantation, 75% of patients on high-dose chemotherapy, and 40% of patients on standard chemotherapy. The oncologic team managing this casuistics should also include a dental medicine doctor to take active part in the treatment of these patients before, during and after therapy administration because of the associated oral complications. Oral mucositis and xerostomia pose difficulties in the intake of food and drinks, leading to malnutrition and dehydration. An array of agents with a varying therapeutic efficacy is now available for the treatment of oral mucositis. Recently, a novel agent named Gelclair, manufactured by Helsin Birex Pharmaceuticals from Dublin, Ireland, has appeared on the market. Gelclair has proved highly efficacious in the management of oral mucositis, ulcerous lesions of oral mucosa of other etiologies, postoperative wounds in oral cavity, etc. Gelclair has been approved by the US Food and Drug Administration FDA, CE and Drug Commission of the Republic of Croatia as a class 1 medical aid.

Key words: Mucositis; Stomatitis; Antineoplastic Agents; Radiation Injuries; Radiotherapy; Gelclair; Review.

Introduction

Oral mucositis is inflammation of the oral mucosa caused by ionizing radiation and chemotherapeutics (Figure 1).

Stomatitis is inflammation of the oral mucosa caused by infection (Figure 2). Epidemiologic data show cardiovascular diseases to account for 52%, malignancies for

22%, and all other causes of death for 26% of total mortality (1, 2). The prevalence of malignant neoplasms of the head and neck region in men is threefold that in women (3-5). A study conducted in 1993 in the USA found oral carcinoma in 20,300 of 600,000 (3.4%) and 9,500 of 577,000 (1.6%) carcinomas in male and female population, respectively (6). Therapeutic methods for the

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Figure 1. Oral mucositis



Figure 2. Ulceronecrotic stomatitis in AIDS

management of malignancies include surgical therapy, radiotherapy, chemotherapy, hormone therapy, immunotherapy, and their combinations (7).

Radiotherapy

In medicine, radioactive irradiation is used for experimental, diagnostic and therapeutic purposes. Orofacial region is an area where nuclear medicine finds application in the

radiation casuistics of the head and neck, involving specialist disciplines of neurosurgery, ophthalmology, otorhinolaryngology, cervicofacial surgery, maxillofacial surgery, oral surgery, oral medicine, endocrinology (pituitary, thyroid and parathyroid glands), and dermatology (8, 9).

The potential oral complications of radiotherapy may be acute and chronic. Acute complications include mucositis, xerostomia, infection, dysgeusia, dysphagia and malnutrition, whilst chronic complications are xerostomia, cervical caries, telangiectasias, myofibrosis, trismus, impaired vascularization, soft tissue necrosis, osteoradionecrosis, dentofacial malformations (if exposed to radiation before adolescence), and malnutrition.

Chemotherapy

Along with surgical therapy and radiotherapy, chemotherapy is frequently used as an adjuvant therapeutic method or as a method of choice in bone marrow transplantation. Chemotherapy administered for malignant lesions beyond the head and neck region may also induce oral complications. Local oral secondary infection consequential to myelosuppression is the most common oral complication of chemotherapy; it may lead to sepsis and occasionally to lethal outcome (10). Other complications associated with chemotherapy are electrolyte imbalance, hemorrhage, acute drug toxicity (including nausea and vomiting), photosensitivity, central nervous system dysfunction, alopecia, and inadequate nutrition. Sonis *et al.* describe oral complications (mucositis, ulceration and xerostomia) in 40% of patients treated with standard chemotherapy and free from malignant lesions in the head and neck region (11). Oral complications are reported in 75% of patients on high-dose chemotherapy, 70%-80% of patients with bone marrow transplantation, and 100% of

patients receiving radiotherapy for head and neck malignancies (12-14).

Chemotherapeutics exert an effect on bone marrow and lead to reduced myeloproliferation which results, among other sequels, in thrombocytopenia, leukopenia and neutropenia. These agents also elicit an effect on oral mucosa, manifesting as a decreased mitotic activity of the oral epithelial cells, which in turn results in epithelial atrophy, reduced epithelial resistance to mechanical irritation, mucositis and oral ulcerations. Ulcerations provide free access to secondary infection from massive and virulent oral flora, while the presence of neutropenia may lead to sepsis and its serious sequels, occasionally with lethal outcome.

Dental doctor in oncology team

Malignancies are managed by an oncology team that consists of an oncologist, pathologist, radiologist, hematologist, radiation physicist, dosimetrist, radiology technician, radiology nurse, physiatry technician, psychologist, dietitian, social worker, and specialists in various health care fields, including dental doctor, depending on the given casuistics. Dental doctor as member of the oncology team can upgrade the quality of life in these patients by reducing the severity of acute irradiation complications and preventing the development of chronic irradiation complications (15-17). Patients scheduled for radiotherapy of the head and neck region or for chemotherapy undergo dental examination and dental treatments, which are divided into those administered before, during and after radiotherapy or chemotherapy.

Procedures performed before radiotherapy or chemotherapy:

- oral clinical examination with x-ray of the teeth and jaws (orthopantomography or panoramix and retroalveolar images);

- patient education, instructions and motivation for a higher level of oral hygiene before, during and after therapy administration;
- an aggressive protocol of oral hygiene;
- complete and thorough dentition and jaw treatment;
- radical approach to dental treatment;
- extraction of all teeth that lack the prognosis of being retained in the oral cavity for >5 years;
- indications for extraction are pulpless teeth, apical periodontitis, teeth requiring endodontic treatment, teeth with true periodontal pockets of >6 mm in depth and furcation involvement, teeth with destroyed crowns, retained root, impacted tooth, no dilemma between extraction and apicoectomy, and teeth adjacent to a tumor (8);
- in patients scheduled for radiotherapy and chemotherapy, tooth extraction should be performed 14-20 days (minimum 10 days) and 7 days (minimum 5 days) before the respective therapeutic modality;
- extraction wound should not be left with sharp margins or alveolar prominence, therefore alveoloplasty should be performed;
- upon tooth extraction, the extraction wound should be sutured to allow for healing at primary intention; a fresh coagulum is sensitive to radiation;
- the regimen of antibiotic administration after tooth extraction is the same as in patients with infective endocarditis, with possible continuation (18);
- cystectomy should be performed when jaw cysts are present;
- removable prostheses should not be used during radiotherapy and for a prolonged time after this therapy; removable prostheses can only be worn at meal and social contacts, as approved and regularly controlled by dental doctor; and
- individual splint for fluorine application in 1% gel should be designed.

Procedures performed during radiotherapy or chemotherapy:

- control of mucositis (ultra soft toothbrush, dental floss for interdental space hygiene without provoking bleeding, chlorhexidine, topical application of 1% sodium fluoride gel by use of the specially designed individual splint);
 - pain control (local anesthetics in the form of gel); and
 - prevention of secondary infection (mouth wash several times a day with a mixture of sodium bicarbonate and table salt, chlorhexidine, nystatin, miconazole).

Procedures performed after radiotherapy or chemotherapy:

- all efforts should be invested for thorough health care of the teeth, gingiva, oral mucosa and pharynx (19, 20);
 - teeth should be washed with a soft toothbrush after each meal and before bedtime;
 - fluorinated toothpastes should be used;
 - tooth necks (cervical caries) and interdental spaces (tooth floss and interdental stimulators) require special hygienic measures that do not induce gingival bleeding;
 - mouth should be washed several times a day with physiologic saline with the addition of sodium bicarbonate;
 - mouth wash shower with moderate pressure dosage should be used, for interdental spaces in particular;
 - post-irradiation xerostomia is quite common; these patients should be instructed as follows: discomforts are alleviated by sipping some fresh drink; a vacuum bottle with water and ice cubes should be brought along in the morning and occasionally sip ice-cold water; sugar-free chewing gum and candies are helpful; if there is no syndrome of burning mouth, it is recommended to spread a mixture of virgin olive oil and lemon juice over oral mucosa (21, 22);

- artificial saline (Glandosan spray, Oral Balance gel, Xero-Lube) should be prescribed;
 - sialogogues (pilocarpine hydrochloride solution, Salagen tablets, mallow-root demulcent) should be prescribed;
 - vitamin creams (d-panthenol) should be spread over lips; and
 - control visits to dental doctor office at appointment, at least once in three months.

Maxillomandibular complications of radiotherapy

Maxillomandibular complications pose a specific problem as post-irradiation sequels with a clinical picture of osteomyelitis, post-irradiation osteonecrosis (PRON) and sequestration (23-26). The risk of these complications is minimized by taking appropriate pretherapeutic dental measures. However, irradiation reduces the bone regenerative ability, impairs interosseous vascular flow, and reduces osteocyte-osteoclast count. The mandible is more vulnerable to these effects than the maxilla (Figure 3).



Figure 3. Post-irradiation osteonecrosis of the mandible.

When these complications have set in, the following measures are suggested:

- high doses of antibiotics according to antibiotic sensitivity report;

- oxygenation in hyperbaric chamber to increase tissue oxygenation which stimulates angiogenesis, osteoblast function and fibroblast function (27); and
- critical consideration of surgical therapy if there is no sequestration.

Dietary regimen

Dietary management is extremely demanding in patients receiving radiotherapy and chemotherapy. Oral difficulties (pain, mucositis, ulceration, xerostomia, thick and sticky saliva, absence of tooth and mouth self-cleaning, dysgeusia, depressive mood, stress situations, difficult communication, etc.) require nutritionist's assistance to avoid tube feeding or parenteral nutrition. Low bacteria diet should be introduced. All these measures require education and great patience on patient management. Dietary regimen guidelines are listed below (28):

- caloric protein rich diet containing adequate amounts of vitamins, minerals and water;
- raw foods, uncooked vegetables, fresh unwashed fruits, dried fruit, salad, nuts, hazelnut, uncooked or semi-cooked meat, fish, eggs should be avoided; vegetarian foods should be thoroughly cooked; unboiled milk, old cheese, all kinds of yoghurt (containing bacterial flora), shells, non-pasteurized fruit drinks, uncooked honey, zwieback, and sweets between meals should be avoided;
- only vacuum packed meat with clearly stated expiry date should be used;
- food prepared by street vendors should not be used;
- irritant drinks and sour food should not be used;
- cigarette smoking and alcohol drinks are forbidden;
- alcohol-free mouth wash should be used because alcohol induces mucosal dryness;
- food is taken when the patient is hungry rather than waiting for meal time;

- at least three meals should be taken daily, in the form most appropriate for a particular patient;
- if the amount of food taken by the patient is inadequate, it should be calorie enriched (butter, creams, dressings, cheese, thoroughly washed or peeled sweet fruit);
- perishable food (milk, meat, sandwich) should not be left at room temperature for more than 2 hours;
- food should be tasteful, soft, chopped, nicely served in a cozy atmosphere with nice music and consumed in pleasant company;
- the patient should kindly order food with certain modifications that suit him/her best;
- a popular saying states that "the strength enters the body by mouth"; and
- attention should be paid to the organization of the patient's free time.

Mucositis

Oral mucositis is inflammation of the mucosa caused by ionizing radiation or chemotherapy. Oral mucositis interferes with the patient's quality of life. The intake of food and drinks is difficult or even impossible, leading to malnutrition and dehydration. Communication is hampered due to severe pain (29-31). Clinical evaluation of mucositis is done according to the World Health Organization criteria modified by Scully et al. (32) (Table 1).

Table 1. Clinical evaluation of mucositis

Grade	Clinical feature
0	No mucosal lesions
1	Mucosal sensitivity – erythema
2	Erythema – ulcerations, solid food intake possible
3	Ulcerations, liquid diet required
4	Oral feeding impossible

The casuistics of oral clinical complications following ionizing radiation is presented in Figures 4, 5, 6 and 7.



Figure 4. Oral mucositis.

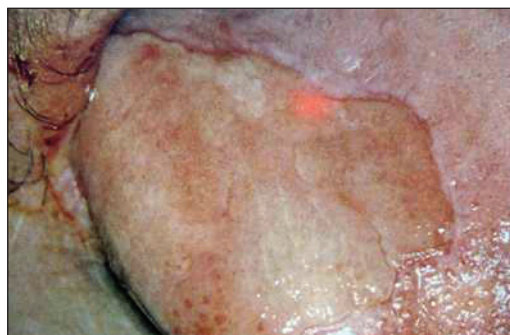


Figure 6. Ulcus linguae.

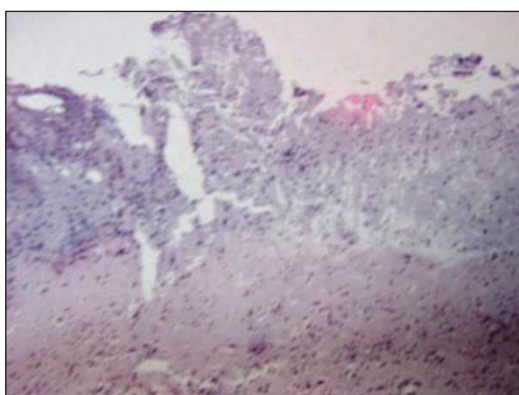


Figure 5. Histopathology finding in oral mucositis.



Figure 7. Cervical caries.

Treatment of oral mucositis

The management of oral mucositis includes a wide array of drugs and procedures: local anesthetics, corticosteroids, systemic analgesics, systemic or topical anti-inflammatory agents, antiseptics, antibiotics, mucosal dressing (Orabase), keratinocyte growth factor (stimulating proliferation and differentiation of epithelial cells), interferon, Lysobact, mixture of physiologic saline and sodium bicarbonate, artificial saliva, and various teas.

Patients with poor oral hygiene and untreated teeth have a higher incidence of mucositis, a more severe clinical picture, and longer time to treatment than those with properly treated teeth and good oral hygiene based on an aggressive protocol (Figure 8).



Figure 8. Neglected mouth – severe post-irradiation sequelae.

Gelclair, a novel agent for the management of acute symptoms of oral mucositis manufactured by Helsin Birex Pharmaceuticals, Ltd., from Dublin, Ireland, and distributed by Pharmaswiss, Ltd., Zagreb – Sarajevo, has appeared on the market. Gelclair is a viscous oral gel for the treatment of oral

mucositis lesions and oral ulcerative lesions of other etiologies. Gelclair alleviates painful sensitivity by creating mechanical protection in the form of a bioadherent coating that covers and spans mucosal surface discontinuities (ulcers), fills uneven areas while moisturizing damaged tissue, alleviates irritation of denuded nerves in the ulcer area, thus helping the patient to take of food and drinks *per os* as well as in the speech function (Figures 9 and 10).

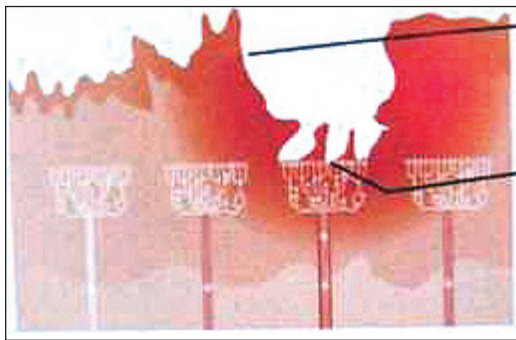


Figure 9. Schematic presentation of oral ulcer.

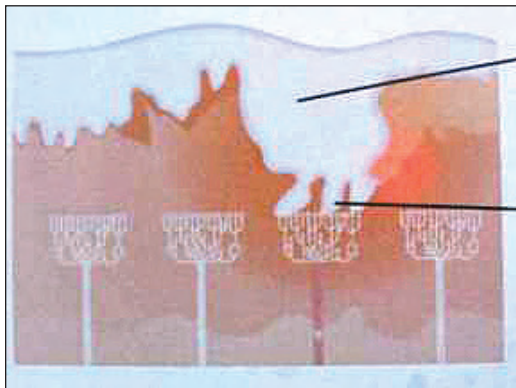


Figure 10. Gelclair dressing adheres to the mucosa, covers the ulcer and shields nerve endings.

Gelclair contains 16 different components, of which the following should be noted:

- polyvinylpyrrolidone (PVP), sodium hyaluronate and glycyrrhetic acid;
- PVP is a hydrophilic polymer which creates a muco adherent coating, increases

tissue moisturizing and accelerates wound healing;

- sodium hyaluronate protects the coating thus formed; its molecules possess the properties of hydration, lubrication and wound repair; and

- the glycyrrhetic acid molecules exert topical antiallergic and anti-inflammatory effects.

The National Cancer Institute describes Gelclair as follows: "Gelclair (approved by FDA). This gel soothes oral mucositis pain by forming protective coating that shields exposed and over stimulated nerve endings" (33). The journal *Hospital Medicine* reports: "Gelclair, a new concentrated oral gel, may provide an interesting new way managing the pain associated with oral mucositis, and it may help patients to eat and drink more easily" (34). A large body of clinical data on the efficacious management of oral sequelae of radiotherapy and chemotherapy by use of Gelclair has been reported in the literature. So, Innocenti *et al.* prescribed Gelclair for 10 days, 3 times daily before meal, in 30 patients diagnosed with ulcerative mucositis, while monitoring pain and deglutition (35). Pain intensity was evaluated according to the Visual Analog Scale (VAS). At 5-7 hours of Gelclair application, pain intensity was reduced by 92%, from 8.2 to 0.6 (Figure 11).

In 7-10 days of Gelclair application, pain associated with swallowing various food contents, e.g., saliva, liquid, liquid creams, semi-solid food, chopped food and normal diet, showed a statistically significant reduction ($p < 0.05$) (Figure 12).

Bonassi *et al.* treated 15 grade III and IV mucositis patients with Gelclair applied 3 times daily (36). Five of these patients had to be hospitalized due to their inability to take food *per os*. The symptom of pain was evaluated according to VAS. After three days of Gelclair application, all patients experienced substantial improvement, on day 7 mucositis showed significant reduction, and on day 19

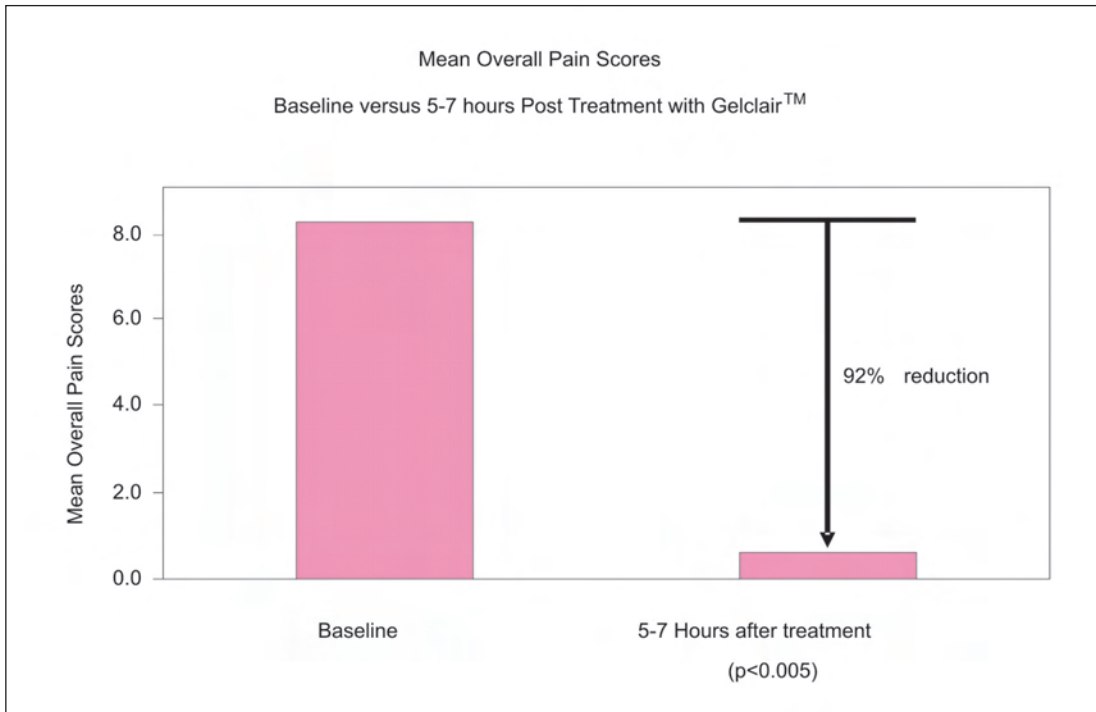


Figure 11. Quantification of pain reduction in mucositis.

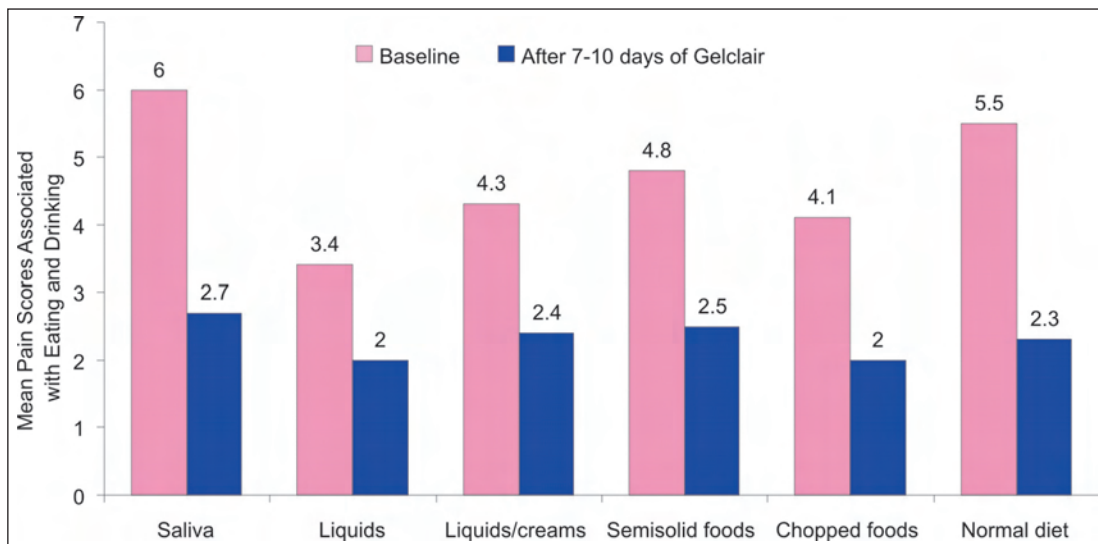


Figure 12. Pain severity on various food intakes.

oral mucosa was completely normal. All patients had the symptom of dysgeusia, which vanished with the regression of mucositis, and the patients showed interest in and need of an increased intake of food and drinks.

In Sweden, ten patients with the diagnosis of oral mucositis were treated with Gelclair during radiotherapy or chemotherapy, and they all continued and completed their therapy without interruption (37).

Gelclair also finds application in oral and maxillofacial surgery after operative procedures in the oral cavity, as exemplified by a Spanish sample of 60 patients (30 in experimental and control group each) with benign tumor lesions and treated with CO₂ laser. Experimental group patients were postoperatively prescribed Gelclair 3 times daily for 7 days. On days 1 and 7, pain reduction and easier food and drink intake yielded statistically significant differences between the experimental and control groups of patients (38). At Department of Oral Medicine, Zagreb University School of Dental Medicine, Gelclair was administered in five patients diagnosed with erythema exudativum (n=2), allergic stomatitis (n=1), ulcus linguae (n=1) and pemphigus vulgaris (n=1). If the area of erosive-ulcerative lesions at zero time point is expressed as 100%, the area involved by the lesion was 47.8% on day 2, 26.4% on day 4, and 9.9% on day 8. Pain severity according to VAS score was 7.6 at zero time point, 6.5 on day 2, 4.5 on day 4, and 2.2 on day 8. All patients reported easier food and drink intake following the application of Gelclair. Gelclair also proved efficacious in soothing pain in patients with ulcer due to dental trauma, after oral surgery procedures, in relapsing aphthae, leukoplakia, oral and gingival lesions associated with AIDS, and oral erosive lichen (33, 39).

Conclusions

1. Oral mucositis is the most common acute oral complication of radiotherapy and chemotherapy.

2. Oral mucositis makes food and drink intake difficult, thus leading to malnutrition and dehydration in these patients. Tube feeding or parenteral nutrition may be needed in some patients.

3. An array of agents to soothe the painful symptoms of oral mucositis is available.

4. A number of clinical studies demonstrated Gelclair, an agent manufactured by Helsin Birex Pharmaceuticals, Ltd. from Ireland, to be efficacious in the management of oral mucositis, oral ulceration of other etiologies, and postoperative treatment of operative wounds in oral cavity.

5. Gelclair has been approved by the Food and Drug Administration, CE and Drug Commission of the Republic of Croatia as a class 1 medical aid.

6. Due to oral sequels of radiotherapy and chemotherapy, an oncologic team should also include a dental medicine doctor for prevention and treatment of the possible oral sequels before, during and after radiotherapy and chemotherapy.

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Guide for Peer Reviewers of Scientific Articles in the *Croatian Medical Journal**

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People to whom we send articles for review sometimes ask us why they should waste time on the free reviewing of other people's articles. A guide for peer review of a scientific article should begin with an answer to that question (Box 1)

Box 1: Why peer review?

- **Obligation** – peer review is a part of scientific publishing; whoever wants to publish, must be ready to peer review
- **Benefit** – increasing of knowledge and awareness, strengthening professional reputation
- **Satisfaction** – scientific debate, exchange of information, fulfilling the responsibility

Despite its shortcomings, peer review is still the best tool of scientific publishing. It brings benefits not only to the journal and its authors, but to the peer reviewers: they are privileged to have an insight into the latest research and still unpublished results in their scientific field. Reviewers also build up their ability to critically assess scientific papers, which may be useful in their own professional work and development. We wrote these brief guidelines to help the reviewers for the *Croatian Medical Journal* learn about the specificities of the journal and editor's expectations from their partnership with peer reviewers. The guidelines were created primarily for new reviewers, but they may be useful as a refresher text for experienced reviewers.

What is the Benefit of Peer Review?

A good review – one that gets to the essence of a reviewed article, keeping its clarity and simplicity at the same time – can considerably increase the scientific merit of the reviewed article (1). The reviewer acts as an educator: his or her suggestions and comments enrich authors' knowledge and ability to perform research and report about it.

It is true that the peer review process has many imperfections and shortcomings. It is subjective and difficult to control and standardize (2,3). Critics claim that the peer review process is slow, expensive, partial,

and subject to abuse (4). However, without peer review it would be almost impossible for editors to publish journals. Peer review is the pillar of scientific publishing, which in turn is a basis of accumulating human knowledge. It follows that anyone who wants to publish his or her own scientific reports must inevitably accept the obligation to be a peer reviewer.

Peer review also brings direct benefits to the reviewer. It is a chance for learning, a valuable source of up-to-date scientific information, and actually an exciting job. It increases the reviewer's knowledge, brings the pleasure and beauty of scientific debate, and creates a feeling of fulfilled responsibility. Reviewers are privileged to have an insight into the latest research and still unpublished results in their scientific field. Reviewers also build up their ability to critically assess scientific papers, which may be useful in their own professional work and development. Writing high quality reviews strengthens a reviewers' scientific reputation. Reviewing can also be a significant part of the *curriculum vitae*. There is an international initiative to provide peer reviewers with continuing medical education (CME) credits for their work (5).

What is Necessary for a Good Peer Review?

Responsibility. A prerequisite for a good reviewer is a strong sense of responsibility towards research and their colleagues. The reviewers assess the manuscript timely, fairly, and to the best of their abilities.

Conversance with the literature. The reviewers must be thoroughly conversant with the pertinent literature and be able to apply general scientific principles to the given problem. Good reviewers can place the article in the context of relevant previous research, recognize the limitations and weaknesses of the hypothesis, and understand the way in which the conclusions of the article

can relate to clinical practice (6). Reviewers should also be acquainted with the guidelines for authors of the journal for which they are refereeing (7).

Time. Depending on the complexity of the reviewed article and relevance to the reviewer's expertise, the time for a fair assessment of an article worth reviewing has been estimated to about three hours (8). Badly written articles increase the time needed for a review.

Knowing the journal. Different journals have different publishing priorities, review policies, and rejection rates. A good peer reviewer should know these aspects of the journal, so that the review process could identify the best articles for the journal. Publishing priorities of the *Croatian Medical Journal* can be found in the Guidelines for Authors (Table 1).

Table 1. Publishing priorities in the Croatian Medical Journal*

Topics of the manuscript	Acceptance priority
<i>Field of study:</i>	
basic sciences	high
clinical sciences	very high
public health	very high
health care organization	very high
medicine in developing and emerging countries	very high
war and post-war related medicine	very high
health and human rights	very high
medical education	very high
<i>Types of articles:</i>	
original research articles	absolute preference
reviews	solicited only
forum	discussion on an important topic
short communications	low
case reports†	low
correspondence	high
poetry and other artwork	very welcome

*Rejection rate of papers submitted to the Croatian Medical Journal is approximately 60%.†Unique case of hitherto unknown symptom or disease; new correlations of two or more diseases; new variant of known disease's course; disease course indicating new therapeutic or side effects.

How to Review a Manuscript

The first principle is to be respectful but resolute. This entails demanding explanations, arguments, and clarity. The seriousness of peer review should not be watered down, inconsistencies should not be concealed, and the editor must be given a clear recommendation (9).

The process of peer review has a common structure (Box 2): reading the abstract, reading the text of the article, final appraisal, and writing comments for authors and the editors. It is important to finish the review in the time limit set by the editor (10). If for some reason the reviewer cannot do so, he or she should immediately inform the editor and agree whether the editor will wait longer or send the manuscript to someone else, in which case the reviewer can recommend some less busy colleagues. It is also important to recognize possible conflicts of interest and, if necessary, decline reviewing the article, with an appropriate explanation to the editor (11).

Box 2: Process of peer review

- Reading the abstract
 - the message of the article
 - the type of study
 - broad questions
- First reading of the article – detecting shortcomings and limitations
 - specific questions
 - logic of “the story”
 - rules for presentation of research data (see Boxes 4 and 5)
- Second reading of the article – value assessment
 - intelligibility
 - scientific power
 - novelty
- Final appraisal
 - accept
 - minor revision
 - major revision
 - reject
- Writing a peer review
 - comments for the editor (up to 200 words)
 - comments for the authors (up to 1,000 words)

First Reading

In the first reading, the reviewer should try to understand the article and question all ambiguities. It is best to write down all the questions in the text of the manuscript, on its margins, or on the back of the paper. The first reading is like a triage (12), where the reviewer decides on the importance and relevance of the study (Box 3).

Box 3: Triaging manuscripts

- Treatment study
 - is it a randomized controlled trial?
 - if not, are there good reasons for not randomizing?
- Diagnosis study
 - is the test compared in a prospective and blind manner with a gold standard?
 - does the test population include patients with the condition, with related conditions that could be confused with the main condition, and people without the condition?
 - is there information on sensitivity, specificity, and other appropriate measures
- Prognosis study
 - is there a cohort of patients followed prospectively from when they were first identified with the disease?
 - are 80% of patients followed up?
- Qualitative study
 - were qualitative methods appropriate for the question?
 - were the methods and the analysis described in detail and justified?
- Questionnaire study
 - does it report what people say they do or what they really do?
 - are there other ways to answer the question?
 - is the response rate over 55%?
- Case report
 - not so common that everybody knows it?
 - not so rare?
 - written in an engaging and amusing way?
- Systematic review
 - the question asked is clear?
 - was search strategy clearly described?
 - were quality criteria set?
 - were studies appraised and discarded?

Reading the Abstract. In the abstract, authors disclose what they consider most

important in their report. Therefore, the reading of the abstract can help the reviewer to look for the crucial elements of the study design, methods, results, and conclusions.

At this point, it is good to note general, broad questions that arise from the abstract, such as “Is this really a double-blinded randomized study?”, “What is new here?”, “Is the sample big enough?” or “This is diagnostic research – is it reported according to STARD statement?”

Reading the body of the article. In the first reading, the reviewer has to focus on the science of the article. The reviewer has to be able to understand all scientific messages that the authors try to convey. Sometimes it is not easy to discern incoherent presentation from the author’s incoherent thinking. If there is anything that reviewer does not fully understand, he or she has to think about it, examine the literature or discuss the problem (not the article!) with a more adept colleague.

Specific questions can arise from any part of the article. Looking for the clear answers on those questions can help reviewer not to overlook some deficiency in the article (13).

– *Title:* does it accurately reflect the content, does it specify the type and the setting of the study?

– *Abstract:* is it structured, is it concise, does it specify outcome measures, are numerical data presented, does the conclusion relate directly to the results of the study?

– *Introduction:* does it justify performing the study, does it end with the hypothesis, and does the hypothesis arise logically from the theoretical framework?

– *Patients or Participants:* is the sample and its formation described in detail, are inclusion and exclusion criteria stated, is there a study flowchart?

– *Methods:* are they supported by references?

– *Statistical analysis:* is the test suitable, presentation appropriate, and interpretation correct?

– *Results:* are they clear and convincing? Each table and figure has to be self-sufficient and carry a single message.

– *Discussion:* does it begin with the most important finding, does it relate exclusively to the results of the study, are the limitations of the study clearly stated?

– *Conclusions:* are they based only on the presented results?

– *References:* are they accurate and up-to-date, are they written according to guidelines for authors, are there any obvious mistakes?

Article as a whole. During the first reading, the reviewer has to pay attention not only to the individual parts, but also to the article in its entirety.

– A properly written article begins with the introduction and continues with the description of materials and methods, presentation of the results, and finally with a discussion. Such a structure is known by the acronym IMRaD and is accepted as a standard in scientific journals (14).

– Some articles are poorly focused, ie “the story” about the idea, methods, and results does not flow well, and the parts of the article do not correspond to one another logically. There are many guidelines and instructions on writing scientific articles (15,16) and authors should do their best to write the article properly.

– Certain types of clinical studies have specific guidelines for data presentation (Box 4). Reporting on prospective randomized trials follows the CONSORT statement (17). There are similar guidelines for diagnostic trials (STARD) (18), meta-analyses of prospective randomized trials (QUOROM) (19), and meta-analyses of observational studies in epidemiology (MOOSE) (20). Furthermore, research results have to be presented with appropriate statistical indicators (Box 5).

Box 4: Obligatory guidelines for research data presentation**CONSORT** – Consolidated Standards of Reporting Trials (17)**STARD** – Standards for Reporting of Diagnostic Accuracy (18)**QUOROM** – The Quality of Reporting of Meta-analyses (19)**MOOSE** – Meta-analysis of Observational Studies in Epidemiology (20)**Box 5: Presenting the results with appropriate statistical indicators (24)****Treatment study**

- relative risk reduction – RRR
- absolute risk reduction – ARR
- number needed to treat – NNT
- control event rate – CER
- experimental event rate – EER

Diagnostic study

- sensitivity
- specificity
- likelihood ratio – LR
- accuracy
- positive predictive value – PPV
- negative predictive value – NPV

Etiology study

- cohort study - relative risk – RR
- case-control study - odds ratio – OR

Prognosis study

- confidence interval (95% CI)

Second Reading

The second reading should be done after a few hours or days, depending on the time available. It begins with checking the questions and remarks previously written on the manuscript. After that, the reviewer should assess the value of the article, keeping in mind several important points.

Firstly, if an expert reviewer does not understand something in the article, an average reader would probably be even more puzzled. Therefore, the reviewer should freely object to anything that disturbs him or her in reading and comprehending the article. In so doing it is not necessary to judge the

general style of the article, because the tastes in that regard can differ. Also, the reviewer is not required to rectify the errors in grammar, spelling, and punctuation – that is the job of a language editor. Still, an overall assessment of language quality can be useful to the editor.

Secondly, the reviewer should assess the scientific value of the article, especially the quality of reasoning, following the scientific principles and knowledge in the particular field of science.

Finally, an assessment is made about the importance of the science in article. The reviewer's judgment should not be biased with current popularity of some research areas, but depend upon the strength of the research methods, data, and conclusions. An important article is one that is scientifically sound and really brings new information into the body of human knowledge. It does not matter whether the study is applied or basic. Applied studies may be relevant for clinical practice, and basic studies may have a broader significance, but in both fields so much great work has been done that the field itself should not influence the judgment about the value of the report (9).

Final Appraisal and Recommendations to the Editor

The fame or reputation of the author should not be taken into account when judging the article. The reviewer who consciously or unconsciously eases the criteria in reviewing the articles of well-known scientists does a disservice both to the authors and the journal. On the other hand, peer review should not be abused as an opportunity for revenge. Any kind of personal remarks are utterly inappropriate and editors usually do not convey them to the authors (21).

Generally, the appraisal of the article can lead to different types of recommendations:

- If the article presents an interesting idea, but is not sufficiently scientifically sound, the reviewer should suggest the au-

thors how to improve it, and put forward the problem to the editor (9).

– If the article has good science in it, but presents only a minor novelty, the reviewer should ask the authors to explain what they consider new in their work.

– If the article is scientifically acceptable, but the text itself is poorly written, the reviewer can be tolerant, but only to a certain point: a carelessly written and messy article should be rejected.

Depending on the shortcomings detected during the first and second reading of the article, the reviewer will suggest the editor to accept or reject the article, or to send it back to the authors for revision.

Reasons for Recommending a Revision of the Article

In principle, if the reviewer sees the opportunity for authors to improve the scientific value and data presentation in their article, they can be given a chance to do so (Box 6).

Problems with science. The first group of problems stems from authors' illogical reasoning: contradictions, ill-founded conclusions, groundless generalizing or attributing causality, inappropriate extrapolations, circular reasoning, and studying irrelevant details. The reviewer may also notice inconsistencies in the classification and inaccuracy of measurements (Box 6).

Problems with presentation. There are many possible problems with presentation. These include redundancies, elaborating unimportant questions, and digressing into irrelevant issues. The reviewer has to point out the imprecise use of the words or phrases, ill-chosen words in translation to another language, use of jargon, and above all – non-standard abbreviations. One should not overlook the errors, such as incorrect sums and tables which do not correspond to the text.

Box 6: Reasons for revision of the article

Scientific problems

- contradictions
- ill-founded conclusions
- groundless generalizing or attributing causality
- inappropriate extrapolations
- circular reasoning
- studying irrelevant details
- inconsistencies in classification and measuring

Poor presentation of results

- redundancies
- elaborating unimportant questions
- imprecise use of words or phrases
- ill-chosen words in translation
- use of jargon and nonstandard abbreviations
- tables and figures not corresponding with text, incorrect sums

Reasons for Recommending Rejection

In spite of being aware that every article submitted for publishing is the result of more or less long and arduous labor of its authors, the reviewer should not hesitate to recommend rejection if the limitations of the article are insurmountable (Box 7).

Fundamentally flawed study. The reviewer can conclude that the study does not bring anything new or that it engages in completely unimportant subject matter, and therefore does not deserve to be published in a scientific journal. The fundamental structure of the study can be flawed, for example when the study does not really test the hypothesis. Unacceptable ethical doubts regarding the study can also be a reason for recommending rejection. The reviewer has to bear in mind that the approval of an institutional ethical committee is not always a guarantee that the study is ethically acceptable. It is the reviewer's duty to independently assess the ethical integrity of the study (9). The reviewer should also help in disclosure of plagiarisms and duplicate publications.

Box 7: Reasons for rejecting the article**Flawed study**

- does not bring anything new
- unimportant subject matter
- fundamentally flawed structure
- major ethical doubts

Unacceptable shortcomings

- no hypothesis
- no control
- weak evidences
- inappropriate statistical analysis

Unacceptable shortcomings of the study.

The article should be rejected if the authors did not use basic scientific principles (e.g. setting up the hypothesis, forming the sample and control group), if they did not objectively consider the reliability of methods used in the study, if they did not notice significant bias factors, or if they did not employ appropriate statistical methods. Improper statistical analysis is not necessarily a reason for recommending rejection, because the authors can correct it. However, appropriate analysis often shows that there are no substantial differences needed to prove the hypothesis, which makes the article unacceptable for publishing.

Writing a Peer Review Report

A peer review report consists of two main parts – one for the editor, and the other for the authors. The reviewers commonly receive a printed review form in which they can grade each aspect of the submitted article (22). This review form should be carefully filled out. Additionally, the reviewer is usually asked to write comments for the editor and, separately, for the authors. No part of the report should be written by hand, because important remarks could be overlooked or disregarded due to indistinct handwriting.

Comments for the Editor

The part intended for the editor should be brief, approximately 200 words. It is good, but not necessary, to begin with a brief summary of the main topic, approach, results, and conclusions of the article. In that way, the editor can find out what the reviewer recognized as the essential message.

After that, the main objections and open questions should be stated, beginning with the most important ones. Sometimes it is useful to divide the remarks into general and specific. The reviewer should explain why he or she considers certain objections and questions important, and suggest the way the authors could work them out. At this point one could also express any doubt as to whether authors would be able to satisfactorily resolve the problems. Finally, this is the place for possible praise or recommendation, for example: “This is an original idea, so in spite of the shortcomings of the article, it deserves to be revised instead of rejected” (9).

Comments for the Authors

If the editor decides that the article should be revised before publishing (which is usually the case), he or she will send the reviews to the authors. Although the identity of the reviewer usually remains unknown to the authors, the review should be written as though it would be signed – politely, constructively, and collegially. Some journals have an open peer review, where both reviewers and authors are known to each other (23). The *Croatian Medical Journal* does not have such a system, but leaves an option for the reviewer to sign his or her comments for the author.

The part intended for authors can be as long as 1,000 words or more, but length itself does not always guarantee quality. A few clear, well thought out, and focused questions can be more than enough to help authors to

improve the article. A review has to be written in such a way that all comments can be understandable to authors, and if possible, accompanied by examples. The reviewer should avoid any kind of censure, but also any kind of praise. The purpose of review is to call attention to possible shortcomings of the article and help the authors to correct them, not to feed the authors' ego.

The first paragraph can be identical to the brief summary from the comments to the editor. The authors might find it useful to see what the reviewer understood as the main message of their article. If the reviewer could not evaluate certain aspects of the article, he or she should openly admit it. For example, an immunologist can evaluate the analysis of cytokines and growth factors in an article on immunological disturbances in schizophrenia, but will not go into reliability of division of patients according to subtypes of schizophrenia. By going beyond his or her own area of expertise, a reviewer not only does a disservice to the authors of the reviewed article, but also compromises his or her own reputation and credibility.

The comments for authors should be divided and numbered so that the authors can clearly answer each one of them.

Major comments. The reviewer should first state the comments which were described to editor as the most important. Every comment or question should be well-explained and well-founded. Instead of general remarks like "sampling was bad", it is necessary to clarify why certain aspects of the article are problematic. It is crucial to write precisely and to make clear if the comment is the result of personal reasoning or it is based on available scientific evidences.

If the article is scientifically strong, but poorly written, the reviewer will help authors the most by explaining what he or she did or did not understand, or by indicating where he or she "got lost" while reading (9).

Minor comments. The reviewer finally mentions minor faults like unnecessary repetitions, incorrect symbols, or abbreviations. They should be ordered in the same way they appear in the text, and identified by page, paragraph, and line.

In Conclusion

Reviewing scientific articles is an essential part of a scientist's job, equal with reading literature or conducting research. It is a very important and responsible work. There are certain rules which a peer reviewer should follow, at least in general. Although relatively unrecognized, the benefits of peer review are significant and valuable.

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Instructions to Authors

Acta Medica Academica

(continuation of Radovi Akademije nauka i umjetnosti Bosne i Hercegovine,
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Language. Manuscripts must be written in clear, concise, grammatical English. Authors from non-English speaking countries are requested to have their text translated by a professional, or thoroughly checked by a native speaker with experience in writing scientific manuscripts in English. Revision of the language is the responsibility of the author. All manuscripts should be spellchecked using a Microsoft Word or Dorland's spellchecker before they are submitted. Spelling should be US English or British English, but not a mixture. Manuscripts may be rejected on the grounds of poor English.

Font and spacing. The manuscript should be prepared in Microsoft Word format (for PC, 6.0 or a later version). Paper version should be typewritten on white bond paper of A4 size, with margins 3 cm each. Write on one side of each sheet, using a font not smaller than 12 points, preferably Times New Roman or Ariel. All pages must be numbered. Prepare texts with double spacing (except those of tables). Double spacing of all portions of the manuscript (including the title page, abstract, text, acknowledgments, references, and legends), makes it possible for editors and reviewers to edit the text line by line, and add comments and queries, directly on the paper copy.

Length. The length of a manuscript depends on its type. On the title page, author should specify total word count and/or character count. Microsoft Word can count them for you. With **double spacing** (1800 characters per page), the limits are as follows:

- for reviews - up to 24 pages (maximum count 43200 characters),
- for original research or clinical reports - up to 20 pages (max. 36000 characters),
- for statistical and methodological compilations - up to 16 pages (max. 28800 characters), and
- for case reports and letters - up to 3 pages (max. 5400 characters).

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Organization of the text. The text of observational and experimental articles is usually (but not necessarily) divided into sections with the following headings: Introduction, Methods, Results, and Discussion. This so-called “IMRAD” structure is not simply an arbitrary publication format, but rather a direct reflection of the process of scientific discovery. Long articles may need subheadings within some sections (especially the Results and Discussion sections) to clarify their content. Other types of articles, such as case reports, reviews, and editorials, are likely to need other formats.

Title Page (the first page). The title page should carry the following information:

1. Type of the article.
2. Title of the article. Concise titles are easier to read than long, convoluted ones. Authors should include all information in the title that will make electronic retrieval of the article both sensitive and specific.
3. Authors’ names and institutional affiliations (full first name followed by family name, separated by a comma from the next name; using Arabic numerals in superscript format relate names and institutions).
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5. Corresponding authors. The name, mailing address, telephone and fax numbers, and e-mail address of the author responsible for correspondence about the manuscript. The name and address of the author to whom requests for reprints should be addressed (if different from the corresponding author), or a statement that reprints will not be available from the authors.
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9. The number of figures and tables.

Abstract and Key Words (second page). Because abstracts are the only substantive portion of the article indexed in many electronic databases, and the only portion many readers read, authors need to be careful that abstracts reflect the content of the article accurately.

An abstract in English (up to 250 words each) should follow the title page. The abstracts should have titles (in English and in Bosnian/Serbian/Croatian), **without** authors’ names and institutional affiliations. Its structure should be similar to that of the text. For original articles, the abstract should provide the context or background for the study; it should state the study’s purposes, basic procedures (selection of study subjects or laboratory animals, observational and analytical methods), main findings, and principal conclusions. It should emphasize new and important aspects of the study or observations.

Following the abstract, authors provide, and identify as such, 3 to 5 key words or short phrases that capture the main topics of the article. Terms from the Medical Subject Headings (MeSH) list of Index Medicus should be used; if MeSH terms are not available, natural language terms may be used. MeSH terms are available from: www.nlm.nih.gov/mesh/.

Introduction. Provide a context or background for the study. State the specific purpose or research objective of, or hypothesis tested by, the study or observation. Give only strictly pertinent references and do not include data or conclusions from the work being reported.

Methods. The Methods section should include: *Selection and Description of Participants, Technical information* (describe the methods, apparatus, and procedures in sufficient detail to allow other workers to reproduce the results; give references to established methods, including statistical methods; identify precisely all drugs and chemicals used, including generic names, doses, and routes of administration), and *Statistics*.

Results. Present your results in logical sequence in the text, tables, and illustrations, giving the main or most important findings first. Restrict tables and figures to those needed to explain the argument

of the paper and to assess its support. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. The text must contain a clear designation as to where the tables and illustrations are to be placed relative to the text. Do not duplicate data by presenting it in both a table and a figure.

Discussion. Emphasize the new and important aspects of the study and the conclusions that follow from them. Do not repeat in detail data or other material given in the Introduction or the Results section. For experimental studies it is useful to begin the discussion by summarizing briefly the main findings, then explore possible mechanisms or explanations for these findings, compare and contrast the results with other relevant studies, state the limitations of the study, and explore the implications of the findings for future research and for clinical practice.

Conclusion. Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data. In particular, authors should avoid making statements on economic benefits and costs unless their manuscript includes the appropriate economic data and analyses. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledge anyone who contributed towards the study by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship. List the source(s) of funding for the study, for each author, and for the manuscript preparation in the acknowledgements section.

References (separate page). Small numbers of references to key original papers will often serve as well as more exhaustive lists. Avoid using abstracts as references. References to papers accepted but not yet published should be designated as “in press” or “forthcoming”; authors should obtain written permission to cite such papers as well as verification that they have been accepted for publication. Information from manuscripts submitted but not accepted should be cited in the text as “unpublished observations” with written permission from the source. Avoid citing a “personal communication” unless it provides essential information. For scientific articles, authors should obtain written permission and confirmation of accuracy from the source of a personal communication.

References should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by Arabic numerals in parentheses at the end of a sentence. Use the same number in the reference list. References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure.

The titles of journals should be abbreviated according to the style used in Index Medicus. Consult the list of Journals Indexed for MEDLINE, published annually as a separate publication by the National Library of Medicine (available from: www.nlm.nih.gov/tsd/serials/lij.html).

Sample References

Articles in Journals

Standard journal article (List the first six authors followed by et al.):

Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. *N Engl J Med.* 2002;347(4):284-7.

More than six authors:

Rose ME, Huerbin MB, Melick J, Marion DW, Palmer AM, Schiding JK, et al. Regulation of interstitial excitatory amino acid concentrations after cortical contusion injury. *Brain Res.* 2002;935(1-2):40-6.

Organization as author:

Diabetes Prevention Program Research Group. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension*. 2002;40(5):679-86.

No author given:

21st century heart solution may have a sting in the tail. *BMJ*. 2002;325(7357):184.

Volume with supplement:

Geraud G, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache*. 2002;42(Suppl 2):S93-9.

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Glaser TA. Integrating clinical trial data into clinical practice. *Neurology*. 2002;58(12 Suppl 7):S6-12.

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Banit DM, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop*. 2002;(401):230-8.

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Tor M, Turker H. International approaches to the prescription of long-term oxygen therapy [letter]. *Eur Respir J*. 2002;20(1):242. ; Lofwall MR, Strain EC, Brooner RK, Kindbom KA, Bigelow GE. Characteristics of older methadone maintenance (MM) patients [abstract]. *Drug Alcohol Depend*. 2002;66 Suppl 1:S105.

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Mansharamani M, Chilton BS. The reproductive importance of P-type ATPases. *Mol Cell Endocrinol*. 2002;188(1-2):22-5. Corrected and republished from: *Mol Cell Endocrinol*. 2001;183(1-2):123-6.

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Article published electronically ahead of the print version:

Yu WM, Hawley TS, Hawley RG, Qu CK. Immortalization of yolk sac-derived precursor cells. *Blood*. 2002 Nov 15;100(10):3828-31. Epub 2002 Jul 5.

Books and Other MonographsPersonal author(s):

Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. *Medical microbiology*. 4th ed. St. Louis: Mosby; 2002.

Editor(s), compiler(s) as author:

Gilstrap LC 3rd, Cunningham FG, VanDorsten JP, editors. *Operative obstetrics*. 2nd ed. New York: McGraw-Hill; 2002.

Organization(s) as author:

Royal Adelaide Hospital; University of Adelaide, Department of Clinical Nursing. *Compendium of nursing research and practice development, 1999-2000*. Adelaide (Australia): Adelaide University; 2001.

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Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. *The genetic basis of human cancer*. New York: McGraw-Hill; 2002. p. 93-113.