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Ivo Šeremet (1900-1991), "Still life (vegetables)", 1935, oil on canvas, 600x810 mm. Courtesy of Art Gallery of Bosnia and Herzegovina, Sarajevo.

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# The role of small journals and their editors in academic communities or: a message to young researchers on why publishing in small journals is important

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When a medical professional becomes a journal editor, he or she is usually not aware of how special and specific their position will be. Being an expert in a certain field and having an academic reputation is usually considered a qualification for an editorial position. Editors are thus often surprised when they learn how complex, technologically advanced and regulated the scientific publishing enterprise in medicine is today (1, 2). For editors of small journals in small scholarly communities, following the developments in medical publishing is probably more important than for those in large, prestigious journals (3). This is because small journals from the so-called scientific periphery not only play a role in communicating new knowledge, but also provide education and service to the local community (4-7). In my life as an editor, I have learned the power of small journals to strengthen the local academic community and promote its visibility in the global academic environment.

Small journals thus provide a door for the local community to mainstream science and open a window to global science to see good local research with global relevance (8, 9). Journals and their editors do this by introducing and following scientific principles and quality criteria in publishing, which is the best way to educate researchers at local institutions (1, 2).

With a background of almost twenty years as editor of a small medical journal, I can give some advice to young academics who are readers of *Acta Medica Academica* or who are thinking of publishing their first research article – *Acta Medica Academica* is a good place to test your writing skills and publish reports of your research. Work-

ing with a journal that embraces the highest international standards in scientific publishing is an important learning experience and preparation for publishing in so-called 'mainstream' scientific journals with greater influence and impact.

Working with journal editors, you will learn about publishing guidelines such as the Uniform Requirements for Manuscripts Submitted to Biomedical Journals of the International Committee of Medical Journal Editors (ICMJE) (10, 11). Editors are responsible for the integrity of the published research recorded in their journals and have the obligation to monitor and ensure that the publication process is fair, timely and thorough. They will provide you with their professional and research expertise, gained from the collective knowledge of larger international editorial organizations, such as the ICMJE, the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), as well as the European Association of Science Editors (EASE). They will also be able to provide guidance for all types of publication disputes or ethical problems, because the editorial community is very aware of the detrimental effect such problems may finally have on health care. COPE's ethical flow charts from the Committee on Publication Ethics (COPE) are particularly useful as algorithms for editors to follow when they have an ethical problem in their journal (12). They are available on the COPE web-site (http://publicationethics.org/flowcharts) and are perhaps even more useful for the authors and their institutions because they can learn about processes in research publishing and the rights and responsibilities of all stakeholders in research, from authors and their institutions to journal editors (1, 2).

They will also learn about the importance of declaring possible conflicts of interest related to the published work or their general research work (13-15). Declaration of conflicts of interest in special forms is routine practice in most international journals, and using a common declaration form both in small and large journals familiarizes researchers with the complexity of conflicts and related terminology. Having experienced misunderstandings about conflicts of interest, the ICMJE has recently developed a special glossary, which has been translated into several languages, so that local research communities and their members can use the forms adequately to provide a transparent account of their work (16).

Finally, you will learn the latest developments in the transparency of clinical research, which are at the heart of clinical research in all scientific and geographical communities. This means that when you send a report on a clinical trial, you have to have already registered the essential information about the trial in a public register. The ICMJE requirement for trial registration (17-19) has been accepted by the World Health Organization, which has developed a special portal for trial registries – the International Clinical Trials Registry Platform (http://www.who.int/ictrp/en/). The latest revision of the World Medical Association Helsinki Declaration on Ethical Principles for Medical Research Involving Human Subjects from 2008 (http://www.wma.net/en/30publications/10policies/b3/index.html), also introduced the requirement for trial registration: "Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject." (20). With the most recent opening of the EudraCT database, European journals and their editors, including those in Bosnia and Herzegovina, can make a significant contribution to the process of increasing the transparency of clinical research for the benefit of the public.

When you publish good articles in a small journal it is not only an exceptional opportunity to learn about the medical pub-

lishing process that is so closely related to academic promotion and research recognition, but you will also contribute to the visibility of the journal itself, so that you will both have great benefits and promote local research (21).

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## References

1. Marušić A, Katavić V, Marušić M. Role of editors and journals in detecting and preventing scientific misconduct: strengths, weaknesses, opportunities, and threats. Med Law. 2007;26:545-66.
2. Marušić A. Approaches to the detection of research misconduct – The role of the peer review process. In: Wells F, Farthing M, eds. Fraud and Misconduct in Biomedical Research. London: The Royal Society of Medicine Press; 2008.
3. Nylena M, Hagve T-A, Marušić A. Small journals and non-English journals. In Godlee F, Jefferson T (ed). Peer review in health sciences. London: BMJ Books; 2003. p. 140-50.
4. Marušić A, Marušić M. Small scientific journals from small countries: breaking from a vicious circle of inadequacy. Croat Med J. 1999;40:508-14.
5. Marušić M, Mišak A, Kljaković-Gašpić M, Fišter K, Hren D, Marušić A. Producing a scientific journal in a small scientific community: an author-helpful policy. Int Microbiol. 2004;7:143-7.
6. Sambunjak D, Huić M, Hren D, Katić M, Marušić A, Marušić M. What do medical professionals know and think about national and international journals: a cross-sectional study. Learned Publishers. 2009;22:57-70.
7. Marušić M, Marušić A. Good editorial practice: editors as educators. Croat Med J. 2001;42:113-20.
8. Marušić M, Markulin H, Lukić IK, Marušić A. Academic advancement of authors receiving tutoring from a medical journal. Teach Learn Med. 2006;18:126-9.
9. Marušić A, Marušić M. Teaching students how to read and write science: a mandatory course on scientific research and communication in medicine. Acad Med. 2003;78:1235-9.
10. Laine C, Horton R, DeAngelis CD, Drazen JM, Frizelle FA, Godlee F et al. Clinical trial registration: looking back and moving ahead. Croat Med J. 2007;48:289-91.
11. Drazen JM, Van Der Weyden MB, Sahni P, Rosenberg J, Marušić A, Laine C, et al. Uniform format for disclosure of competing interests in ICMJE journals. Croat Med J. 2009;50:427-8.
12. Wager E, Barbour V, Yentis S, Kleinert S, on behalf of COPE Council. Retractions: Guidance from the Committee on Publication Ethics (COPE). Croat Med J. 2009;50:532-5.
13. Drazen JM, Van Der Weyden MB, Sahni P, Rosenberg J, Marušić A, Laine C, et al. Uniform format for disclosure of competing interests in ICMJE journals. Croat Med J. 2009;50:427-8.
14. Drazen JM, De Leeuw PW, Laine C, Mulrow C, DeAngelis CD, Frizelle FA, et al. Toward more uniform conflict disclosures: the updated ICMJE conflict of interest reporting form. Croat Med J. 2010;51:287-8.
15. Marcovitch H, Barbour V, Borrell C, Bosch F, Fernández E, Macdonald H, et al. Esteve Foundation Discussion Group. Conflict of interest in science communication: more than a financial issue. Report from Esteve Foundation Discussion Group, April 2009. Croat Med J. 2010;51:7-15.
16. Kušec V, Gelo M. Uniform ICMJE conflict of interest declaration form: report of test use in Croatian Medical Journal in 2009-2010. Croat Med J. 2010;51:285-6.
17. De Angelis CD, Drazen JM, Frizelle FA , Haug C, Hoey J, Horton R, et al. Is this clinical trial fully registered? A statement from the International Committee of Medical Journal Editors. Croat Med J. 2005;46:499- 501.
18. Laine C, Horton R, DeAngelis CD, Drazen JM, Frizelle FA, Godlee F, et al. Clinical trial registration: looking back and moving ahead. Croat Med J. 2007;48:289-91.
19. Marušić A, Huić M. Registration of clinical trials still moving ahead – September 2008 update to Uniform Requirements for Manuscripts Submitted to Biomedical Journals. Croat Med J. 2008;49:582-5.
20. Krleza-Jeric K, Lemmens T. 7th Revision of the Declaration of Helsinki: Good News for the Transparency of Clinical Trials. Croat Med J. 2009;50:105-10.
21. Marušić M, Sambunjak D, Marušić A. Life of small medical journal – how bibliographical indexing and international visibility affected editorial work in Croatian Medical Journal. Croat Med J. 2006;47:372-5.

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# Laser resurfacing of skin flaps: an experimental comparison of three different lasers

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**Objective**. The influence of Coherent Ultrapulse, TruPulse and Erbium: YAG laser skin resurfacing on survival of the skin flaps when performed simultaneously was evaluated. **Material and methods**. We used twelve female Yucatan minipigs in the study. Skin flaps including paniculus carnosus were raised on the animals' back. The flaps were sutured into the defect under tension. We designed 4 experimental groups: Control-Flaps only, Group 2-Flaps + 4 immediate TruPulse laser passes, Group 3-Flaps + 2 immediate Coherent UltraPulse laser passes, Group 4-Flaps – immediate 50J/cm$^2$ total fluence with Erbium: YAG laser. **Results**. Flap survival in Control group was 98.8%. There was no flap in Group 2 with complete survival. Survival of the flaps in Group 2 (Tru-Pulse) ranged from 75-90%, with average flap survival area of 85.2%. In Group 3 (UltraPulse) all 24 flaps had some area of necrosis. Flap survival in Group 3 ranged from 75-95%, with an average of 85.6%. In Group 4 (Erbium: YAG) flap survival area ranged from 70-95%, with all 24 flaps with some area of necrosis, with average flap survival area of 87.3%. There is a significant statistical difference in flap survival area between groups 2, 3 and 4 versus Control ($p<0.001$). **Conclusion.** The results of our study suggest that laser resurfacing of skin flaps sutured under tension in the same operative session is detrimental for skin flap survival. We also found no significant difference in flap survival area between TruPulse, Coherent UltraPulse and Erbium: YAG laser treated flaps.

**Key words:** Laser resurfacing, Flaps, Ischemia injury.

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## Introduction

Rejuvenation of the face is an ever-changing area of aesthetic surgery. New technologies, new concepts and approaches are being introduced constantly. The pathophysiology of the changes induced in tissues is still being explored. The combination of different techniques in one procedure raises the additional question about the effects they have on tissues when applied concomitantly.

Simultaneous use of chemical peel and deep plane face-lift has been studied and no complications related to

their combination have been reported (1). Reports about concomitant use of lasers and different esthetic procedures are available (2, 3, 4, 5, 6), which show no additional complications arising from the combination of procedures. Our experimental data suggest that simultaneous use of pulsed $CO_2$ laser resurfacing on skin flaps sutured under tension is detrimental for flap survival (7). Although the perfect method for treating photoaged skin remains elusive, the search for the best possible result, while minimizing complications, is a constant goal.

This study was designed to evaluate in a systematic and prospective manner the effects of Coherent UltraPulse, TruPulse and Erbium: YAG laser resurfacing on skin flaps sutured under tension when performed simultaneously.

## Material and methods

*Experimental animals*

Female Yucatan minipigs were used in this study. This experimental animal is chosen because of histologic similarities with human skin (1, 8). We used twelve animals 3 months of age and 10-15 kg in weight.

*Experimental flaps*

Skin flaps, including paniculus carnosus (the flaps simulate deep plane facelift skin flaps), were raised on each animal's back. The flaps were oriented with the base toward the animal's spine. Flap size was 2x6 cm (1: 3). Eight flaps were elevated per animal and the treatment modality was randomly assigned for each flap.

The flaps were sutured under tension in the experimental protocol in order to mimic clinical practice. During face lift operative procedures the skin flaps are always sutured under tension and, in our model, flap tension was applied in a systematic fashion by suturing a smaller flap of 2 x 4.5 cm in dimension into a defect measuring 2 x 6 cm. We designed four experimental groups: Control group, Group 2 (Tru Pulse Laser resurfaced), Group 3 (Coherent UltraPulse Laser resurfaced), and Group 4 (Erbium: Yag Laser resurfaced) (Table 1).

*Experimental protocol*

Skin flaps with paniculus carnosus were elevated on the animal's back as described above. The usual aseptic technique was followed - animal skin preparation with Betadine solution and the use of sterile instruments. One preoperative dose of Ancef 50 mg/kg IM was administered to the animals. No flaps were elevated over the pelvic or scapular regions. Hemostasis was obtained with electrocautery and the flaps sutured in place with a two-layered 3/0 Vicryl closure. The distal 1.5 cm of each flap was resected and then sutured into the original flap defect under tension to simulate the tension placed on rhytidectomy flaps. Flap elevation and laser resurfacing was performed with the animals under general anesthesia.

The laser groups had flaps elevated and sutured in defects in same manner as the

Table 1 Experimental groups

| Experimental groups | Flaps | Number of flaps |
|---|---|---|
| Control | Flaps 2x4.5 cm in 2x6 cm defect | 24 |
| Tru Pulse Laser resurfaced | Flaps 2x4.5 cm + immediate 4 laser passes | 24 |
| Coherent UltraPulse Laser resurfaced | Flaps 2x4.5 cm + immediate 2 laser passes | 24 |
| Erbium: Yag Laser resurfaced | Flaps 2x4.5 cm + immediate 50J/cm$^2$ fluences laser pass | 24 |
| Total | | 96 |

control group. Immediately after insetting, the flaps were treated with the different lasers according to a randomization scheme of group assignment (Table 2).

Table 2 Randomization scheme

| Pig | Side | Position front to back | | | |
|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 |
| 1 | Left | 3 | 2 | 4 | 1 |
| 1 | Right | 1 | 4 | 2 | 3 |
| 2 | Left | 4 | 1 | 3 | 2 |
| 2 | Right | 2 | 3 | 1 | 4 |
| 3 | Left | 1 | 3 | 2 | 4 |
| 3 | Right | 4 | 2 | 3 | 1 |
| 4 | Left | 2 | 4 | 1 | 3 |
| 4 | Right | 3 | 1 | 4 | 2 |
| 5 | Left | 3 | 1 | 4 | 2 |
| 5 | Right | 2 | 4 | 1 | 3 |
| 6 | Left | 1 | 4 | 2 | 3 |
| 6 | Right | 3 | 2 | 4 | 1 |
| 7 | Left | 2 | 3 | 1 | 4 |
| 7 | Right | 4 | 1 | 3 | 2 |
| 8 | Left | 4 | 2 | 3 | 1 |
| 8 | Right | 1 | 3 | 2 | 4 |
| 9 | Left | 2 | 3 | 4 | 1 |
| 9 | Right | 1 | 4 | 3 | 2 |
| 10 | Left | 4 | 1 | 2 | 3 |
| 10 | Right | 3 | 2 | 1 | 4 |
| 11 | Left | 3 | 4 | 1 | 2 |
| 11 | Right | 2 | 1 | 4 | 3 |
| 12 | Left | 1 | 2 | 3 | 4 |
| 12 | Right | 4 | 3 | 2 | 1 |

1=control group, 2=TruPulse Laser group,
3=Coherent UltraPulse group, 4=Erbium: YAG Laser group.

*Laser treatment*

Laser skin treatment was delivered using two different operative $CO_2$ and Erbium: YAG lasers. In Group 2 the Tru-Pulse Laser System with a 4-mm hand probe was used. Four laser passes were performed with 500 mJ/cm$^2$ of energy to the skin flaps in every pass. In Group 3 we used a Coherent UltraPulse with a CPG scanner. We delivered a fluence of 300 mJ/cm$^2$ with density of 5 in two passes to create a similar depth of injury. In Group 4 we used an Erbium: YAG laser at 2940 nm wavelength. We delivered total fluences of 50 J/cm$^2$ in 3 passes of 17 J/cm$^2$ with 30-50% scanner pattern overlap.

The skin was treated with wet and dry sponges between laser passes. Care was taken to treat the entire surface of the flap uniformly. The settings for the different laser systems were chosen to correspond to the settings used in clinical facial skin laser resurfacing. We decided to use the most frequently used settings published in current laser literature.

Close daily monitoring was secured in the postoperative period and any sign of animal distress was properly treated. Wound cleaning with 0.9% Normal Saline and application of Bacitracin antibiotic ointment was provided daily in the first postoperative week.

*Observations*

Flaps were examined and photographed weekly and flap viability evaluated. Photographs were taken from the same distance every time. Skin flap necrosis area was defined postoperatively by development of full thickness necrotic eschar. An area of flap necrosis or survival was measured from the photos and presented as a percentage of the total flap surface area. Total observation time was 4 weeks after the procedure. Punch knife biopsies were taken from 2 flaps in each group immediately after resurfacing, one week post-resurfacing and at 4 week intervals and H&E stains prepared for histology and evaluation of changes in flap skin thickness, capillary and collagen pattern. Histology evaluations were performed in a blind fashion by the same dermatopathologist (AGB).

*Statistical analysis*

The experiment was carried out on twelve experimental animals in order to examine the difference between four different treatments. As each pig received multiple treatments, we assigned the four treatments in such a way that we systematically checked for variations in necrosis due to the characteristics of the pig, characteristics of the side (right or left) of the pig, and characteristics of the position of the flap on the pig. We used three separate pairs of Latin squares. Each pair of Latin squares consisted of four pigs. One of the squares corresponded to the right hand side of the pig, and the other to the left hand side. Each of the three pairs of Latin squares had a different order of treatment assignments.

## Results

Flap survival in flaps in the Control Group was 98.8%. Only one flap developed distal 5% necrosis. There was no flap in Group 2 with complete survival. Survival of the flaps in Group 2 (Tru-Pulse) ranged from 75-90%, with average flap survival area of 85.2%. In Group 3 (UltraPulse) all 24 flaps had some area of necrosis. Flap survival in Group 3 ranged from 75-95%, with an average of 85.6%. In Group 4 (Erbium: YAG) flap survival area ranged from 70-95%, with all 24 flaps with some area of necrosis. Mean survival area was 87.3%. There is a significant statistical difference in flap survival area between Groups 2, 3 and 4 versus the Control ($p<0.001$). There is no statistically significant difference between laser groups: TruPulse vs. UltraPulse $p=0.84$, TruPulse vs. Erbium: YAG $p=0.31$ and UltraPulse vs. Erbium: YAG $p=0.42$.

### Histology

*Immediately after flap elevation and laser treatment*

Control: Stratum corneum and epidermis are unremarkable. There is a mild superficial and deep perivascular and interstitial lymphohistiocytic infiltrate. The adnexal structures are unremarkable. Full thickness epidermal necrosis was found in histological specimens in all three laser groups. There is a mild superficial and deep perivascular and interstitial lymphohistiocytic infiltrate. The adnexal structures are unremarkable. There was no visible histologic difference between Coherent Ultrapulse, Trupulse and Erbium: YAG immediate specimens.

*Seven days post flap elevation and laser resurfacing*

Control Group specimens obtained at this time point showed the same characteristics as originally described. Erbium: YAG group showed serum crust, reepithelialization of the epidermis. There is a mild superficial and deep perivascular and interstitial lymphohistiocytic infiltrate. The adnexal structures are unremarkable. Coherent Ultrapulse and Trupulse specimens presented same as Erbium: YAG group at 7 days.

*One month after flaps and laser resurfacing*

Control Group specimens obtained at this time point showed the same characteristics as originally described. TruPulse specimens had mild basketweave orthohyperkeratosis, mild irregular acanthosis, mild hyperpigmentation of the basal layer, and melanophages in the papillary dermis, fibrosis and proliferation of blood vessels in the papillary dermis. There is a mild superficial and deep perivascular and interstitial lymphohistiocytic infiltrate. The adnexal structures are unremarkable. CoherentUltrapulse and Erbium: YAG presented the same as at 4 weeks. TruPulse specimens.

## Discussion

Introduction of laser technology in laser rejuvenation has opened new horizons, set

new goals, and defined new perspectives. Also, there is so much to learn about the effects that laser energy induces in tissues. Continuous research efforts constantly improve our knowledge of laser effects on tissue.

This study was designed on a well established experimental model used in prior skin resurfacing experiments (1, 2, 7, 8). The vascularity of the axial pattern flaps in the Yucatan minipig is well established. Flaps of 6 cm length have been shown to survive even when a random pattern was used (9).

The depth of the dermal injury appears to be similar with a 35% TCA peel and the Ultra-Pulse laser (8). The pathophysiology of laser resurfacing producing thermal damage is likely to be responsible for such a difference in flap perfusion and the development of skin necrosis. Histologic data from our study proved the depth of the thermal ablation to the level of the papillary dermis, consistent with published data on the same experimental model (3, 8). Reepithelialization time and histologic sections one month post laser resurfacing in our study were also concordant with previously published data (1, 3, 7, 8).

Despite the results from several studies indicating no additional complications when simultaneous face-lift and laser resurfacing have been used, multiple experimental reports suggest a different approach (2, 3, 7, 10). Our previous experiment suggested that laser thermal injury is detrimental for flap survival when the flap is sutured under tension. Our current data suggest that the specifics of different laser energy do not significantly change that influence.

Different laser pass settings and different numbers of passes have different effects on skin. However, the cumulative effect is not a simple sum of the energy delivered. More passes at a lower setting appear to have deeper penetration in the skin (11). It is yet to be determined what minimal threshold of energy is needed to induce irreversible perfusion changes in skin flaps (12).

Histologic changes in the skin after laser resurfacing continue to occur beyond the initial changes. Most likely, neocollagen formation and shrinkage can be significant factors in obtaining long lasting results of $CO_2$ laser resurfacing (11, 13, 14). Also, skin contraction after laser resurfacing correlates directly to the zone of dermal coagulation induced. That correlation remained in both pulsed $CO_2$ and Erbium: YAG laser resurfacing (9). Similar observations were made in our study regarding the resurfaced flap perfusion, the corresponding energies of three different lasers induced comparable amounts of skin flap necrosis. There is a possibility that pretreatment of skin flaps with retinoic acid could improve flap survival. Four to six weeks pretreatment of skin with retinoic acid before laser resurfacing decreased the depth of injury induced by laser resurfacing, and the wounds seemed to heal faster (14).

There are multiple factors influencing postoperative outcome in laser skin resurfacing (16, 17). Promising results have been obtained with Erbium: YAG laser resurfacing, as well as with a combination of erbium and carbon dioxide lasers (18, 19). In our experiment (erbium resurfacing of skin flaps sutured under tension induced the least amount of necrosis, but there was no statistically significant difference when compared with two other experimental groups. One cannot help asking: Would retinoic acid pretreatment and/or less skin flap tension be beneficial for skin flap survival? The ever-changing field of laser skin resurfacing amazingly brings new technology with decreased morbidity (18, 20).

## Conclusions

The results of our study suggest that laser resurfacing of skin flaps sutured under tension in the same operative session is detrimental for skin flap survival. There was no signifi-

cant difference in flap survival area between TruPulse, Coherent UltraPulse and Erbium: YAG laser treated flaps. Laser treatment induced histologic changes in skin flaps that were similar, corresponding to the similar energy delivered, regardless of laser type used.

**Authors' contributions:** Conception and design: SB and UB; Acquisition, analysis and interpretation of data: SB, AB and UB; Drafting the article: SB; Revising it critically for important intellectual content: SB and RPC.

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## References

1. Dingman DL, Hartog J, Siemionow M. Simultaneous Deep plane face lift and trichloracetic acid peel. Plast Reconstr Surg.1994;93:86-93.
2. Mayl N, Felder DS. $C0_2$ laser resurfacing over facial flaps. Aesthet Surg J. 2004;17(5):285-92.
3. Guyuron B, Michelow B, Schmelzer R, Thomas T, Ellison MA. Delayed healing of rhytidectomy flap resurfaced with $C0_2$ laser. Plast Reconstr Surg. 1998;101:816-9.
4. Alster TS, Doshi SN, Hopping SB. Combination surgical Lifting with ablative laser skin resurfacing of facial skin: a retrospective analysis. Dermatol Surg. 2004;30(9):1191-5.
5. Jackson IT, Yavuzer R, Beal B. Simultaneous face lift and carbon dioxide laser resurfacing: a proven technique. Plast Reconstr Surg. 2001;108(3):802-3.
6. Achauer B.M., Adair S.R., VanderKam M. Combined rhytidectomy and full-face laser resurfacing. Plast Reconstr Surg. 2000;106(7):1608-11.
7. Babovic S, Clay RP, Battey MJ, Trevino MT, Bite U: The effect of laser resurfacing on flap survival. (Non published data).
8. Minoli JJ, Barton FE. A comparison of the histologic effects of chemabrasion, dermabrasion and laserabrasion in the minipig. Aesthetic Surgery J. 1998;18(1):11-8.
9. Stell, P. The pig as an experimental model for skin flap behavior: A reappraisal of previous studies. British J Plast Surg. 1977;30:1-8.
10. Adckok D, Paulsen S, Katzen T, Harper L, Nanney LB, Shack RB. Simultaneous cutaneous flap elevation and skin resurfacing in the rabbit model. Aesthetic Surg J. 1999;19(5):375-80.
11. Grover S, Apferberg DB, Smoller B. Effects of varying density patterns and passes on depth of penetration in facial skin utilizing thew carbon dioxide laser with automated scanner. Plast Reconstr Surg. 1999;104:2247-52.
12. Carniol PJ. The importance of blood supply in combination rhytidectomy and full-face carbon dioxide laser resurfacing. Arch Facial Plast Surg. 2002;4(4):227-33.
13. Kirn DS, Vasckonez HC, Cibull ML, Fink BF. Skin contraction with pulsed $CO_2$ and Erbium: YAG laser. Plast Reconstr Surg. 1999;104:2255-60.
14. Rosenberg GJ, Brito MA, Aportella R, Kapoor S. Long-term histologic effects of the $CO_2$ laser. Plast Reconstr Surg. 1999;104:2239-44.
15. McDonald WS, Beasley D, Jones C. Retinoic acid and $CO_2$ laser resurfacing. Plast Reconstr Surg. 1999;104:2229-35.
16. Weinstein C, Ramirez OM, Pozner JN. Postoperative Care following $C0_2$ laser resurfacing: avoiding pitfalls. Plast Reconstr Surg. 1997;100(7):1855-66.
17. Bernstein LJ, Kauvar AN, Grossman MC, Geronemus RG. The short and long term side effects of carbon dioxide laser resurfacing. Dermatologic Surg. 1997;23(7):519-25.
18. Weinstein C. Erbium laser resurfacing: Current concepts. Plast Reconstr Surg. 1999;103:602-16.
19. Alister TC. Cutaneous resurfacing with $CO_2$ and Erbium: YAG lasers: Preoperative, intraoperative and postoperative considerations. Plast Reconstr Surg. 1999;103:619-32.
20. Schwartz RJ, Burns AJ, Rohrich RJ, Barton FE, Byrd HS. Long-term assessment of $CO_2$ facial laser resurfacing: Aesthetic results and complications. Plast Reconstr Surg. 1999;103(2):592-601.

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# Justifiability of amniocentesis on the basis of positive findings of triple test, ultrasound scan and advanced maternal age

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**Objective.** To assess the effectiveness of antenatal screening for chromosomal abnormalities based on maternal age (≥35 years), positive ultrasound findings or a positive triple test. **Materials and methods.** Retrospective six-year study. The pregnant women routinely underwent established clinical and laboratory practice at the Department of Medical Genetics between 1997 and 2003. The women's case notes were examined to identify indications for karyotyping, gestation period and the outcome of karyotyping and pregnancy. **Results.** Invasive antenatal tests were performed on 1440 cases, 1168 (81.11%) age 35(a), 72 (5.00%) positive triple test (b), 24 (1.67%) positive ultrasound scanning (c) and 176 (12.2%) other (psychological, personal reasons, etc) (d). The overall positive predictive value was 1.67% (1.6%(a), 1.4% (b), 12.5% (c), 0.0% (d). The constructed model of logistic regression gave an odds-ratio of 8.647 for the "positive ultrasound result vs. maternal age ≥35" indication, while the odds-ratio for the triple test vs. maternal age ≥35 was 0.854. **Conclusions.** Amniocentesis and cytogenetic analysis of foetal karyotype should be presented as a diagnostic possibility to all women over 35 years. The application of biochemical markers was far from the expected results. If we compare results for indication positive ultrasound scanning vs. maternal age, an odds-ratio of ~9 was obtained. These results demonstrate that the likelihood of obtaining positive results (i.e. the presence of chromosome alterations) from an amniocentesis having this indication is almost 9 times higher than from having an amniocentesis performed solely for advanced maternal age.

**Key words:** Chromosomopathy, Triple test, Ultrasound, Maternal age, Prenatal screening.

## Introduction

Detection of abnormalities in an unborn child is the subject of interest of prenatal diagnosis, which includes all instrumental and laboratory procedures or techniques used for pregnancy monitoring from the moment of

conception up until the period immediately before delivery. So, this is a matter of preventing and identifying hereditary diseases and congenital anomalies of an unborn foetus/child, which is simultaneously altering the attitudes of medical practice, changing established physician-patient relations in modern perinatology.

Potentially detectable abnormalities can be divided into chromosome, gene and genomic mutation, and those changes (mutations) could cause different consequences i.e. mental retardation and/or the appearance of different somatic malformations. Chromosome abnormalities are present in approximately 50% of all spontaneous miscarriages (1). The most commonly used invasive procedures to obtain material for subsequent genetic diagnostic are villocentesis (Chorionic Villus Sampling-CVS) and amniocentesis (Amniotic Fluid Sampling-AFS) with the rate of spontaneous foetal loss related to amniocentesis, on average, about one in every 200 procedures (2).

There is a direct relationship between foetal trisomies and maternal age, so, as a consequence this can be viewed as the first "screening test" for foetal chromosome abnormalities (3). However, the use of maternal age alone does not appear to be an effective screening method and the traditional estimate is that 30% of Down's syndrome cases can be detected using maternal age alone (3). Furthermore, observations that women younger than 35 years old give birth to about 70 percent of infants with Down's syndrome (4)indicates that it could be necessary to provide younger (<35) pregnant women with non-invasive screening tests. In the last decade there has been a strong development of non-invasive techniques, biochemical serum markers, such as Free Estriol, βHCG, AFP etc., applicable in the first and second trimesters of pregnancy, which has become an established part of non-invasive first-step obstetric practice in many countries.

Four advantages were suggested compared with solely using maternal age (5): It can detect twice as many affected pregnancies for the same rate of amniocentesis; It can identify affected pregnancies in women below the age cut-off; it can reassure older women whose risk was lower than that predicted by age alone, so that they might avoid the need for amniocentesis. Detection rates of these tests are approximately 60%, but in combination with ultrasound examination, the detection rate rises to 85-90% (6).

In the 1990s, Nicolaides et al. realized that the excess skin of individuals with Down's syndrome can be visualized by ultrasonography as increased nuchal translucency in the first 3 months of intrauterine life (6). Foetal nuchal translucency thickness at the 11–14-week scan has been combined with maternal age to provide an effective method of screening for trisomy 21; for an invasive testing rate of 5%, about 75% of trisomic pregnancies can be identified. Other benefits of the 11–14-week scan include confirmation that the foetus is alive, accurate dating of the pregnancy, early diagnosis of major foetal defects, and the detection of multiple pregnancies (6).

The aim of this study is to establish, based on our experience, the most justified diagnostic approach with a special interest in contributing to drawing up guide lines and directions for the most effective clinical practice, especially important in societies with limited economic resources.

## Materials and methods

*Patients*

The pregnant women were selected and enrolled in the study group at the "DIMIMP -Medical Genetic Section, University of Bari, between 1997 and 2003. All pregnant women who had been undergoing routinely established clinical and laboratory practices, consisting of genetic counselling (pre-test

counselling-before AFS), amniocentesis, cytogenetics and if necessary (positive result on cytogenetic analysis) genetic counselling once again (post-test counselling). Where necessary, medical or psychological support/consultation was given.

Pregnant women with indications for amniocentesis such as positive triple test, positive ultrasound scan and advanced maternal age were included in this study (95% of all examined cases). Pregnant women with other indications (molecular prenatal diagnosis) or those with motivations such as personal decision (not psychological nature) were excluded from this study. For ultrasound and biochemical tests, the maternal age was taken into consideration, and in addition, for the triple test a cut-off level (1:250) was used as recommended (7, 8). Furthermore, results of cytogenetic analysis were evaluated in relationwith above mentioned indications.

*Statistical analysis*

A descriptive statistical analysis was undertaken, which took into consideration the age of the pregnant women as well as indications for amniocentesis. The indications for amniocentesis were divided into: maternal age ≥35, a positive triple test, a positive ultrasound test, others (a child with previous genetic disorder, families with positive anamnesis and reasons of a psychological nature). The evaluation of specificity and sensitivity of biochemical analysis could not be carried out because of the unavailability of information regarding the total number of patients who undertook the examination (i.e. those that took the test but had a negative result). On the other hand, it was possible to carry out the evaluation of the positive predictive values for every single indication, with a particular emphasis on the result of the positive triple tests. Furthermore, a model of logistic regression was constructed to ascertain the probability of getting a positive (pathological) result with the foetal karyotype as a function of the different indications. Statistical analysis was performed using the statistical package SPSS 12.0 for Windows and SAS.

## Results

During a period of 6 years (1997-2002) a total of 1440 amniocentesis tests were performed; 82.9% of the examined pregnant women (n=1194) belong to the group of those over 35 years old, while the group of less than 35 years accounts for only 17.1% (n=246) of the patients. 30% of patients were actively working (n=432), while 5% of patients (n=72) smoked during the pregnancy (despite their doctor's recommendation). Figure 1 presents the distribution of pregnant women according to each indication for amniocentesis, in terms of the age of the patient.

Table 1 shows the data regarding the distribution of pregnant women in relation to the indications and obtained results of cytogenetic analyses with the predictive positive value of each indication particularly.

Of the total number (n=1440) of amniocentesis, 81.1% were those done for the maternal age as the only indication (≥35 years old), 5% (n=72) were with the positive triple test as an indication, 1.7% (n=24) for a positive ultrasound result and 12.2% (n=176) other indications. Cytogenetic analysis of the pregnant women with the indication "maternal age ≥35" gave positive results in 19 cases (1.6% of 1.168 amniocentesis tests or 1.3% of the total performed amniocentesis); 15 (78.9%) cases were Down's syndrome, 2 cases (10.5%) were Klinefelter's Syndrome (47,XXY); 1 case was Edwards' Syndrome (5.3%); 1 case was 47,XXX (5.3%) as represented in Table 2.

The positive triple test was the indication in 72 cases (5%) and the largest number of tests were done on pregnant women in the age group from 31 to 34 years (37 cases or 51.4%). Only one case (1.4% of 72 amnio-

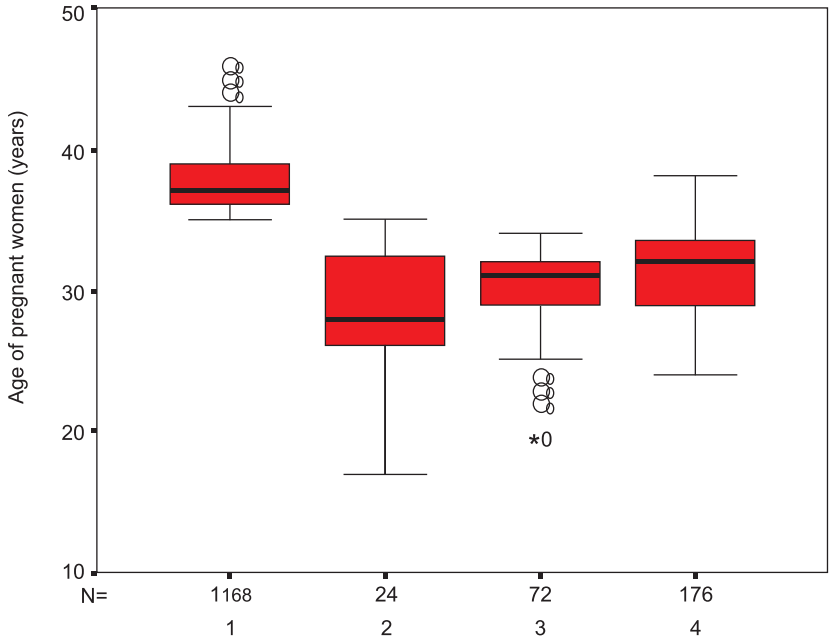


Figure 1 Distribution of the pregnant women according to the age and indications for amniocentesis (N = number of pregnant women; 1 = indication for age group of pregnant women ≥35; 2 = indication for positive ultrasound scanning 3 = positive triple test; 4 = other indications.)

Table 1 Distribution of the pregnant women frequency in relation to the indication and result of the cytogenetic analysis (positive/negative) with the positive predictive value (italic) regarding each indication

| Indications | Cytogenetic analysis (n=1440) | | Total |
|---|---|---|---|
| | Negative (n=1417; 98.4%) | Positive (n=23;1.6%) | |
| | n (%) | n (%) | n (%) |
| Age ≥35 | 1149 (98.4) | 19 (*1.6*) | 1168 (81.0) |
| Ultrasound | 21 (87.5) | 3 (*12.5*) | 24 (1.7) |
| Triple test | 71 (98.6) | 1 (*1.4*) | 72 (5.0) |
| Other | 176 (100.0) | - | 176 (12.2) |

Table 2 Distribution of "positive" cytogenetic analysis for indication "maternal age ≥ 35"

| Kariotype (n) | Age (Years) | | | | | | | | Σ | Positive results (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | | |
| +21* | 2 | 2 | 1 | 2 | 3 | 1 | 2 | 2 | 15 | 78.9 |
| +18* | - | - | - | - | - | - | 1 | - | 1 | 5.3 |
| XXY | - | 1 | - | 1 | - | - | - | - | 2 | 10.5 |
| XXX | - | - | - | - | - | 1 | - | - | 1 | 5.3 |
| Total | 2 | 3 | 1 | 3 | 3 | 2 | 3 | 2 | 19 | 100 |
| Positive results (%) | 10.5 | 15.8 | 5.3 | 15.8 | 15.8 | 10.5 | 15.8 | 10.5 | | 100 |

*Trisomy for the chromosome 18 and 21

centesis tests or 0.07% of the total performed amniocentesis) resulted in the abnormal foetal karyotype (Down's syndrome).

A positive ultrasound marker was found in 24 cases (1.7%) of the total number of amniocenteses performed and for all patients subsequent amniocentesis and cytogenetic analysis of foetal karyotype was performed. A positive result of karyotype analysis was confirmed in 3 cases (12.5% of 24 amniocentesis tests) with the above-mentioned indication (0.3% of total performed amniocenteses). One case of Patau Syndrome (Ultrasound result of multiple malformations) was found; 2 cases of Down's syndrome (the positive NT result); 1 case of Robert's Syndrome (tetraphocomelia).

The constructed model of logistic regression gives an odds-ratio of 8.647 for the positive ultrasound result indication with respect to maternal age (≥35). It actually means that the risk of getting a pathological foetal karyotype result after amniocentesis is almost 9 times higher than the risk in a pregnant woman with only the indication of advanced maternal age ("maternal age ≥35"). On the other hand, the odds-ratio for the triple test vs. maternal age (≥35) is only 0.854.

## Discussion

The aim of this retrospective study is to justify the performing of amniocentesis as an invasive diagnostic technique by analyzing a group of 1440 patients with the following indications: maternal age, positive triple test and positive ultrasound result. The total positive predictive values of all amniocenteses performed in this study was 1.67%, which is completely in accordance with the observations of Howe et al. who reported positive predictive values of 1.8% (3).

The percentage of analyzed patients with the "maternal age ≥35" indication is 81.1% (n=1168), which is very similar to the observation of Chaabouni et al., quoting that precisely maternal age appeared in 63% cases as the main indication for foetal karyogram analysis. Ferguson–Smith and Yater reported similar observations (60%) (9, 10). In contrast, Howe et al. presented data with only 10% of cases with the mentioned indication for amniocentesis (3).

Within this group, 19 abnormal foetal karyotypes were observed which represents 1.6% of all analyses performed in our study, while Chaabouni et al. reported 3.9% and Dupont and Carles 3.2% (7, 9). The most frequent pathological result was Down's syndrome with 15 cases (78.9% of all abnormal karyograms). Our data is in accordance with those given by Howe et al., reporting 66% of Down's syndrome diagnosed (3).

The triple test was an indication in 72 cases or 5% of all performed analyses which completely concurs with the observations of Spenser et al. (6.1%), Wald et al. (5%) while Webley and Halliday reported higher results (9.8%), and Ayme et al. (9.1%) and finally Chaabouni et al. quoted in their study only 1.97% (8, 9, 11, 12, 13). One case of Down's syndrome (or 1.4%) was confirmed by foetal karyotype analysis. This result is in total accordance with those publicized by Dupont and Carles of 2.1%, Spenser of 2.8%, Chaabouni et al. of 3.3%, Ben et al. of 5% and Nyberg et al. of 5.3% (7, 9, 14, 15, 16). A higher percentage was observed by Baenna et al. of 22.8% and Howe et al. of 16.6% of studied cases (3, 17). During the period of six years, a positive ultrasound result as an indication was a means of foetal karyotype analysis in 24 cases, which is 1.7% of all analyses; in contrast, Webley and Halliday observed 20% and Chaabouni et al. gave data of 8.2% of foetal karyotype analysis (9, 12). After cytogenetic analysis, there were 3 pathological cases of foetal karyotype, which was 12.5% of the discovered cases. Baenna et al. quoted data of 45.6%, while Howe et al. presented data of 20.4% of positive cases (17, 3). Nicolaides et al. in their

work reported 64% of discovered trisomy 21 after doing NT, followed by 4.1% false positive results (18). It is necessary to underline the fact that in this study there was total of 4 pregnant women with a positive NT ultrasound result, of which 2 cases (50%) were with the positive abnormal foetal karyotype (Down's syndrome), which is very close to the observations of Nicolaides et al. (18). In contrast, Chaabouni et al. quoted only 9.0% of cases with abnormal karyotypes and Dupont and Carles had similar reports (9.5%) and Benacerraf et al. presented only 1.4% of cases detected (7 , 9, 19).

A limitation of the study was certainly the absence of an evaluation of the specificity and sensitivity of the biochemical analysis, but, as explained in the statistical methods, the information was missing regarding the patients that underwent the examination but had a negative result. In fact these patients generally do not go through genetic counselling and do not have an amniocentesis

## Conclusions

The constructed model of logistic regression gave an odds-ratio of 8.647 for the positive ultrasound result vs. maternal age (≥35) as the only indication. When we add the low price of US, and practically no risk to the foetus and mother, the application of this technique imposes itself as the "gold standard" in prenatal diagnosis. Amniocentesis and cytogenetic analysis of foetal karyotype should be presented as a diagnostic possibility to all women over 35 years of age. Our results completely confirm the indicated observation, taking into consideration the fact that all 19 cases of chromosomopathy discovered belong to that age group. The issue of biochemical markers and application of this non-invasive diagnostic method, at least for the period we studied (1997-2002), is, in our opinion (74/75 cases were found to be false-positive), far from the expected results, as was also confirmed by Howe et al. (3). De Vore and Romero, furthermore, considered that it would be desirable to offer serum marker tests to all younger women (≥ 35 years old) as a routine analysis in pregnancy (20). We could express our agreement especially considering its low price, but with the remark that those tests require setting the laboratory criteria for their conduct, as well as establishing so-called corrected factors (algorithms) which would ensure the adequate increase in quality and sensitivity of these tests (only accredited institutions should perform it).

It is evident that the important goal for future parents, and society in general, is giving birth to healthy offspring, or if the diagnoses is positive and the parents decide to continue with the pregnancy, this permits preparation for the delivery in the best way, with the best hospital/personal organization to obtain optimal results.

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**Authors' contributions**: Conception and design: DB and NB; Acquisition, analysis and interpretation of data: DB, NB and MF; Drafting the article: DB and NB; Revising it critically for important intellectual content: GG and NB.

### References

1. Mueller R, Young I. Chromosome disorders. In: Emery's elements of medical genetics. 10th ed. London: Churchill Livingstone;1998. p. 245-64.
2. Lončar J, Barnabei VM, Larsen JW. Advent of maternal serum markers for Down syndrome screening. Obstet Gynecol Surv. 1995;50:316-20.
3. Howe TD, Gornall R, Wellesley D, Boyle T, Barber J. Six year survey of screening for Down's syn-

drome by maternal age and mid-trimester ulltrasound scans. BMJ. 2000;320:606-10.

4. Saller DN, Canik JA. Maternal serum screening for Down szndrome: clinical aspects. Clin Obstet Gynecol.1996;39:783-92.
5. Royal College of Obstetricians and Gynaecologists. Report of the working party on biochenical markers and the detection of Down's syndrome. London: RCOG Press; 1993.
6. Nicolaides KH. Nuchal translucency and other first-trimester sonographic markers of chromosomal abnormalities. Am J Obstet Gynecol. 2004;191:45-67.
7. Dupont JM, Carles E. Three-year national survey of prenatal cytogenetic activity in France 1998-2000. European Cytogeneticists Association Newsletter No. 13, January 2004. p. 3-8.
8. Spenser K, Spenser CE, Power M, Dawson C, Nicolaidis KH. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in one stop clinic:a review of three years prospective experience. Br J Obstet Gynecol. 2003;110:281-6.
9. Chaabouni H, Chaabouni M, Maazoul F, Rad MR, Jemaa BL, Smaoui N,Terras K, Belghith N, Ridene H, Oueslati B, Zouari F. Prenatal diagnosis of chromosomal disorders in Tunisian population. Ann Genet. 2001;44:99-104.
10. Fergusson-Smith MA, Yater JR. Maternal age specific rates for chromosomes aberrations and factors influencing them, report of a collaborative European study on 52965 amniocentesis. Prenat Diagn. 1984;4:5-44.
11. Wald NJ, Cukle HS, Densem JW, et al. Maternal serum screening for Down syndrome in early pregnancy. BMJ. 1988;297:883-7.
12. Webley C, Halliday J. Report on prenatal diagnostic testing in Victoria 2001. http://www.health.vic.gov.au/perinatal/downloads/report_diagnostictest2001.pdf
13. Ayme S, Morichon N, Goujard J, Nisand. Prenatal diagnosis in France. Eur J Hum Genet. 1997;5:26-31.
14. Spencer K. Screening for trisomy 21 in tvin pregnancies in the first treimester using free β – HCG and PAPP-A, combined with fetal nuchal translucency thickness. Prenat Diagnosis. 2000;20:91-5.
15. Benn PA, Horne D, Briganti S, Greenstein RM. Fetus-Placenta-Newborn: Prenatal diagnosis of diverse chromosomal abnormalities in population of patients identified by triple-marker testing as screen positive for Down's syndrome. Am J Obstet Gynecol. 1995;173(2):496-501.
16. Nyberg DA, Luthy DA, Cheng EY, Sheley RC, Resta RG, Williams MA. Role of prenatal ultrasonography in women with positive screen for Down's syndrome on the basis of maternal serum markers. Am J Obstet Gynecol. 1995;173(4):1030-35.
17. Baenna N, De Vigan C, Cariati E, Clementi M, Stoll C, Caballin MR, Guitart M and Euroscan Working Group. Prenatal detection of rare chromosomal autosomal abnormalities in Europe. Am J Med Genet. 2003;118A:319-27.
18. Nicolaides KH, Azar G, Snijders RJM, Gosden CM. Fetal nuchal edema: associated malformations and chromosomal defects. Fetal Diag Ther. 1992;6:46-57.
19. Benacerraf BR, Frigoletto FD, Laboda L A. Sonographic diagnosis of Down syndrome in the second trimester. Am J Obstet Gynecol. 1985;160:319-21.
20. DeVore RG, Romero R. Genetic sonography. An option for women of advanced maternal age with negative triple-marker maternal serum screening results. J Ultrasound Med. 2003;22:1191-99.

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# CGH microarray studies in idiopathic developmental/ cognitive impairment: association of historical and clinical features and the De Vries Score

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**Objective.** Studies have confirmed that copy number variations (CNV) in the human genome contribute to the etiology of mental retardation/ development delay/ congenital anomalies. We sought to evaluate the use of a microarray in the context of a clinical genetics practice, to determine if there were any specific clinical findings that predict the discovery of a CNV. **Patients and methods.** 334 cases with idiopathic mental retardation/impairment/development delay/ disability or a combination of these findings were studied using array comparative genomic hybridization (Signature Chip Version 4). The subjects had previously had a non diagnostic medical genetics evaluation. Clinical findings were collated by a chart review. Each patient was scored according to a previously published clinical checklist by de Vries and colleagues. **Results.** Of 334 patients, 8 were excluded due to a syndromic diagnosis being established by clinical and/or microarray testing. Out of the remaining 326 patients, 33 (10%) showed CNVs, of which 5 were maternally inherited, 4 paternally inherited, 11 were *de novo*, and the origin of 13 remained unknown. The mean de Vries score was greater in the CNV group than in the non CNV group (4.17 and 3.95, respectively). No patient in the CNV group had a score of less than 3, while in the non CNV group, 12% of patients had scores less than 3. **Conclusions.** The De Vries clinical score was higher in CNV cases compared to those with no CNV ($p=0.04$) but this difference is unlikely to be clinically meaningful. Several features reached statistical significance of $p<0.05$ but we were unable to delineate patterns of features that might increase the yield of positive CNV results.

**Key words:** Copy number variation, Developmental delay, Dysmorphism, Birth defects, Prenatal.

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## Introduction

Mental retardation/subnormality/impairment/disability/ or developmental delay, with or without other clinical findings, comprises a substantial portion of patients referred to medical geneticists for diagnosis. Around 2% of the human population has mental retardation, defined by

the World Health Organization as a "global and noticeable deficiency in the development of motor, cognitive, social, and language functions". The most recent version of the Diagnostic and Statistical Manual of Mental Disorders cites the criteria for diagnosing mental retardation (MR; more commonly now called mental deficiency or mental subnormality) as:

1) Significantly sub-average intellectual functioning: An IQ of approximately 70 or below on an individually administered IQ test. For infants, a clinical judgment for significantly sub-average intellectual functioning, and

2) Concurrent deficits or impairments of onset prior to age 18 years in adaptive functioning in at least two of the following areas: communication, self-care and home living, social skills, use of community resources, self-direction, functional academic skills, work, leisure, health issues, and safety. If onset is after age 18 years, then the diagnosis is dementia (1).

The cause of mental subnormality is identified in only 40-60% of affected individuals, and a very specific syndrome or genetic diagnosis is found in a few as 20% (2, 3). Recent advances in molecular cytogenetics have increased the diagnostic yield. Historically, traditional karyotyping identified chromosomal aneuploidy in around 14 percent of all cases of severe mental subnormality, with Down syndrome accounting for the majority (4). The ability to detect smaller and smaller copy number variations by use of molecular karyotyping, also called array comparative genomic hybridization (aCGH), has reduced the number of individuals who lack a diagnosis. A review of 13 studies that used bacterial artificial chromosome (BAC) arrays showed abnormalities of copy number in 4-17% of affected individuals who had apparently normal karyotypes (5). A meta-analysis of 19 studies, most using densely spaced oligonucleotide arrays, had a diagnostic yield of 10% for convincing abnormalities, but also a 7% chance of a "false positive", defined as chromosomal variants that were judged to be non causal or were of unknown significance. These facts lead to two important conclusions. First, molecular karyotyping can meaningfully increase the diagnostic yield in individuals with mental subnormality. Second, there is a need for methods to better select candidates for testing as 90% of those tested have non diagnostic results.

De Vries et al. studied 29 subjects with documented subtelomeric deletions and then developed criteria in the form of a simple check list to describe the pre- and postnatal clinical features that might correlate with abnormal karyotyping (6). The checklist included data on birth history, craniofacial and non facial dysmorphism, congenital malformations and family history. The few studies that have looked at frequencies of CNVs have reported this level of clinical detail about their series, likely because of the lack of availability of this detail.

The aim of the current study was to determine if there were any specific features or combination of clinical findings in our clinical medical genetics practice that was significantly associated with the discovery of CNV using a clinically available microarray study.

## Patients and methods

This cross-sectional study included the first 334 individuals on whom aCGH was performed in the context of the medical genetics practice at Mayo Clinic, Rochester, Minnesota between 2004 and 2007. All of the individuals had different combinations of cognitive impairment, mental subnormality, development delays, seizures, autism, congenital abnormalities, dysmorphic features, in whom no diagnosis had been achieved prior to their referral to our department. Proper consent had been attained for chart review for research purposes. All patients

were evaluated by one of the five clinical geneticists in the Department of Medical Genetics and all had a normal G-banded karyotypes of peripheral blood lymphocytes. Each patient was scored according to the proposed criteria of de Vries et al., shown in Table 1 (6). Only patients for whom there was no permission to review their medical record were excluded from this study. No other patients were excluded, although some had some data elements missing, hence the changing denominator for some data elements shown in on-line supplements.

Array CGH was performed on blood from each patient using Signature Chip Version 4 (Signature Genomics Laboratory in Spokane, Washington), which included 622 loci using 1887 BAC clones, to look for subtelomeric or pericentromeric interstitial rearrangements, or alterations in known deletion areas. Once the results for the patients were obtained, the parents of the patients with CNVs were also invited to be tested to determine if the alteration seen was *de novo* or familial. Out of 33 patients with CNVs, both parents were tested in 20 cases, and in 13, either 1 or neither parents was tested.

A detailed chart review was conducted and more than 200 features were abstracted from each chart regarding maternal health, prenatal, perinatal, and postnatal events, growth, developmental achievements (including motor, language, speech, psychosocial, cognitive), functional abnormalities, behavioral disorders, autism, attention deficit hyperactivity disorder, congenital anomalies, dysmorphic features, and a detailed family history. All findings were considered of potential importance and were recorded. In addition, each patient was scored strictly according to de Vries Criteria (Table 1) in which each category was given a maximum of 2 points and maximum final score was 10 points.

A detailed family history included known major or minor congenital anomalies, a syndrome diagnosis in a relative, including those caused by chromosomal disorder, history of recurrent miscarriages (two or more in any relative including the mother), diagnosis of chromosomal anomaly in a fetus or child, mental retardation or cognitive impairment in relative, and Sudden Infant Death Syndrome.

Prenatal events included low maternal weight gain during pregnancy (less than 5 pounds), a diagnosis of intrauterine growth restriction (birth weight less than third percentile for gestational age), decreased fetal movements compared to previous pregnancies, and abnormal prenatal ultrasound results. Perinatal data included birth weight, length and head circumference, placental or cord anomalies, immediate newborn problems including respiratory problems, hypo-

Table 1 Checklist for patients, adapted from de Vries et al. (6) A maximum score of 10 points can be achieved. No more than 2 points can be achieved in categories A-E

| Finding | Points Scored |
|---|---|
| A. Family history of mental retardation | |
| Compatible with Mendelian inheritance | 1 |
| Incompatible with Mendelian inheritance, including discordant phenotypes within one family | 2 |
| B. Growth retardation of prenatal onset | 2 |
| C. Growth abnormalities of postnatal onset: 1 point each for microcephaly or macrocephaly or short stature or tall stature | 2 |
| D. Two or more facial dysmorphic features, notably hypertelorism, anomalies of the nose or ears | 2 |
| E. Non-facial dysmorphism and congenital abnormalities: maximum of 2 points for hand anomaly (1 point), heart anomaly (1 point), hypospadias+/- undescended testis (1 point) | 2 |

glycemia, seizures, hypotonia, and hyperbilirubinemia.

Post natal growth and development parameters included most recent height, weight and head circumference, and attainment of developmental milestones with regard to motor, speech, language, communication, and cognitive development. Short stature was defined as less than third percentile for age and tall stature as greater than 97$^{th}$ percentile for age. The same percentiles were used to define micro- and macrocephaly. "Later onset" (defined as onset older than 5 months) seizures, hypotonia, or autism spectrum disorders diagnoses were noted. Functional abnormalities of vision and hearing were noted.

Chart abstraction for dysmorphism included abnormal head size, shape, fontanelles, dysmorphic aspects of the forehead (sloping, prominent brow, high or low hairline), unusual formation of the eyebrows (sparse, busy, synophrys), facial asymmetry and midfacial abnormalities; abnormal shape, size or orientation of palpebral fissures; strabismus; epicanthic folds, hyper- or hypotelorism, abnormalities of the eyes (aniridia, enophthalmos, abnormalities of the nasolacrimal duct, cornea, retina, optic nerve), abnormal morphology of the ears including pits, tags, or creases; dysmorphism of the nose, lips, philtrum, and abnormalities the mandible, tongue, palate, and dentition.

Non craniofacial abnormalities recorded included defects of the palate, heart, central nervous system (e.g., abnormal MRI), chest wall, nipples, abdominal wall or viscera, internal and external genitalia, spine, long bones and joints, hands, feet, skin and hair.

For the purposes of analysis, all studied individuals were assigned either to the group with no CNV or to the group with CNV. Individual features and bundled features were compared between these two groups and p-values were calculated. A threshold of $p<0.01$ calculated using Chi square analysis was considered significant, though we also noted those $<0.05$ with interest.

## Results

Out of the total 334 individuals evaluated, 10% (n=33) had a copy number variation (CNV; details in Supplement 1; note that one individual had two CNVs) that was not known to cause a defined syndrome. Eight of the original 334 patients had a syndromic diagnosis made, sometimes clinically, sometimes with CGH array assistance (e.g., velocardiofacial 22q deletion). These were excluded from our analysis, so the final dataset included a total of 326 cases of which 293 showed no CNV. Table 2 compares general characteristics between these groups. Of the 33 cases with CNVs, 5 were shown to be maternally inherited, 4 were paternally inher-

Table 2 Description of the individuals included in this study

| Characteristics | CNV found (n=33) | No CNV detected (n=293) |
|---|---|---|
| Average age of males (range) | 10.7 years (0.8-47) | 6.9 years (0.2-37) |
| Average age of females | 8.1 (0.5-33) | 7.1 (0.1-43) |
| Average paternal age in years | 30.5 (available on 24) | 31.4 (available on 192) |
| Average maternal age in years | 27.4 (available on 30) | 28.4 (available on 244) |
| Average parity of mother at time of pregnancy with proband | 1.14 (38% were primigravid) | 1.2 (36% were primigravid) |
| Mean number of miscarriages | 0.93 (available information on 29) | 0.62 (available information on 199) |

CNV=chromosome copy number variation

ited, 11 were *de novo*, and the origin could not be determined in 13 as biparental testing could not be accomplished. All CNVs were included in our analyses, regardless of origin, as inherited CNVs may be pathogenic (7, 8) (See Supplement 1 for details).

For all cases, we attempted to generate a score using the criteria of de Vries (Table 1). Due to missing data, especially regarding pregnancy, postnatal growth or family history, not all cases could be scored. We were able to provide a de Vries score for 29 patients with CNVs, and 231 patients without CNVs. A comparison of the two groups is shown in Figure 1.

In our study no patients in the CNV group had scores lower than 3, while in the CNV negative group, 28 out of 231 patients

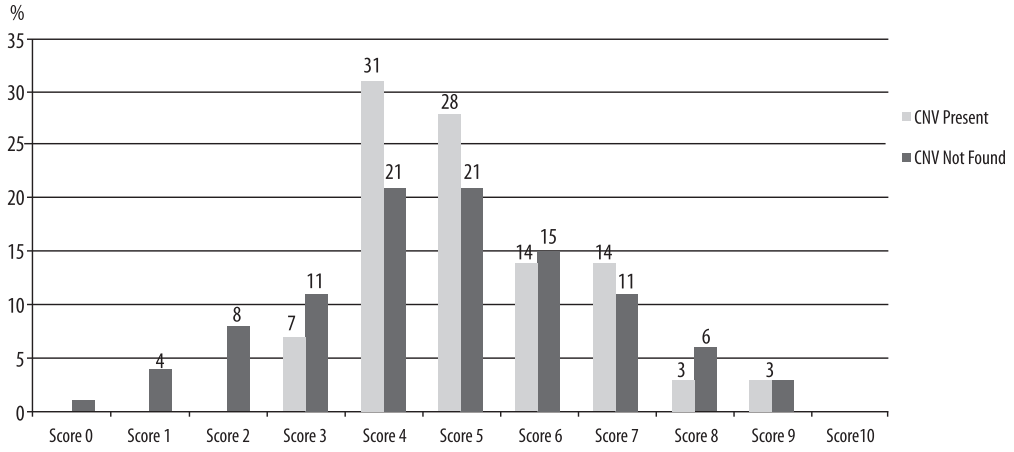



Figure 1 Overall comparison of individuals with CNV detected and those with no detectable CNV using the De Vries scoring system shown in Table 1

Table 3 Analysis of different combinations of scoring in the de Vries table (see Table 1) for cases with sufficient information available to use the de Vries system. P values were all greater than 0.05

| Combinations of scoring in de Vries system | CNV Detected (n=29) | | No CNV Detected (n=231) | |
|---|---|---|---|---|
| | n | % | n | % |
| Prenatal growth retardation | | | | |
| Prenatal growth retardation with 1 or more features | 4 | 14 | 16 | 7 |
| Prenatal growth retardation with 2 or more features | 3 | 10 | 15 | 6 |
| Postnatal growth abnormality | | | | |
| Postnatal growth abnormalities with 1 or more features | 16 | 55 | 101 | 44 |
| Postnatal growth abnormalities with 2 or more features | 4 | 14 | 45 | 20 |
| Family history positive | | | | |
| Family history with 1 or more features | 19 | 66 | 138 | 60 |
| Family history with 2 or more features | 7 | 24 | 37 | 16 |
| Facial dysmorphism | | | | |
| Facial dysmorphism with 1 or more features | 24 | 83 | 205 | 89 |
| Facial dysmorphism with 2 or more features | 20 | 69 | 186 | 81 |
| Nonfacial dysmorphism | | | | |
| Nonfacial dysmorphism with 1 or more features | 28 | 97 | 198 | 86 |
| Nonfacial dysmorphism with 2 or more features | 23 | 79 | 165 | 71 |

CNV = copy number variation

(12%) had scores of 2 or lower. The average de Vries score in the CNV group was 4.17 and in the CNV negative group was 3.95 (p=0.04). In Table 3, different combinations of findings were compared between the CNV and non CNV groups but none seemed to select for CNV cases efficiently.

The presence or absence of over 200 specific individual findings beyond the deVries criteria were also evaluated and compared between individuals with CNVs and those with no CNV. A statistically significant difference was seen at the $p<0.05$ level (but no p values were less than 0.01) in the frequency of specific features was noted between the CNV-positive and the CNV-negative groups in 3 categories (full details in Supplements 2-6):

1) Developmental delay within first 5 months in the CNV group was reported in 21% and in the non CNV group in 6% (p=0.020) and

2) Congenital hypotonia was observed in 28% of CNV cases while only in 12% of non CNV cases (p= 0.0496).

3) Macrocephaly, excluding relative macrocephaly, was present in 17% of CNV cases versus 5% in non-CNV cases (p=0.026).

Trends favoring a higher frequency in those with CNVs were observed for intrauterine growth retardation (p=0.051), two or more miscarriages in the mother (p=0.076), and a history of decreased fetal movements as felt by the mother in this pregnancy as compared to previous ones (p=0.067).

## Discussion

In this study we sought to determine if there were any specific clinical features that individually or in combination would provide increased yield of CNVs when studying individuals with aCGH. Three hundred and thirty four chromosomally normal individuals having diagnostic evaluations in the Department of Medical Genetics for developmental delay, with or without other anomalies, were included in this retrospective chart review. The major strength and novelty of this study was access to very detailed and quite complete clinical information, allowing comparison between those with CNVs to those with no findings. We also applied the checklist proposed by de Vries to this cohort to determine its utility (6). Our results showed trends toward higher scores using the de Vries check list, and several individual features were identified that may merit further clinical studies of this type. Overall, however, no finding was found at a frequency that was sufficiently different between the CNV group and the group without CNVs to inform changes in current clinical practice.

This finding is not surprising and several explanations likely contribute to this finding. The first generation clinical microarray was targeted toward areas of known clinical significance and the coverage in other parts of the genome was poor. The newer oligoarray studies yield modestly increased numbers of CNVs in similar populations. Undoubtedly there could be individuals in our study that had CNVs not detected on this first generation microarray (5, 9-12). Secondly, there may not be a phenotype resulting from CNVs that is so characteristic as to distinguish it from non genetic causes of mental subnormality, with or without birth defects. Also, single gene disorders can cause mental subnormality, dysmorphism and birth defects, and none of these will be detected by any type of CGH array.

In our analyses, we chose to include all CNVs regardless of whether they were familial or *de novo*, rejecting the assertion that a familial CNV will be non causal. We have observed a number of families in which the carrier parent was functioning adequately but appeared to share phenotypic features with the proband. In addition, further genetic change in the size of a familial CNV

across meiosis has also been reported (7, 8). As some familial CNVs may truly be without consequences, we may have diluted our "affected" group by making this choice.

## Conclusion

De Vries clinical score was higher in CNV cases compared to those with no CNV ($p=0.04$) but this difference is unlikely to be clinically meaningful. Several individual features reached statistical significance at the $p<0.05$ threshold but we were unable to delineate patterns of features that might increase the yield of positive CNV results. What we can do as clinicians to increase our ability to increase the diagnostic yield of tests we order? Can we make a more informed selection of candidates for microarray testing? It will be of interest to see this type of clinical correlative analysis repeated on the newer platforms used for molecular karyotyping. Only by detailed reflection on our outcomes will we know if we could be practicing in a more efficient manner.

**Authors' contributions**: Conception and design: PP and NML; Acquisition, analysis and interpretation of data: PP and NML; Drafting the article PP and NML; Revising it critically for important intellectual content: PP and NML.

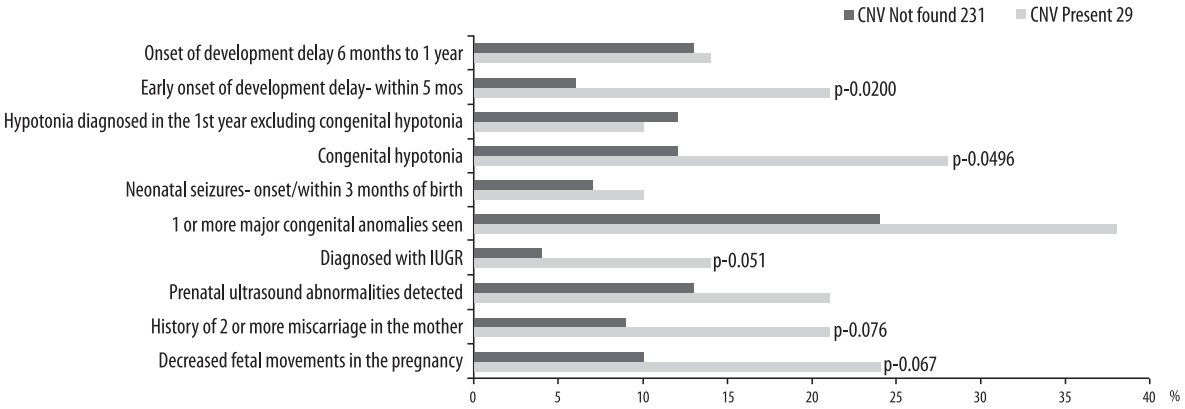
**Conflict of interest**: The authors declare that they have no conflict of interest. This study was not sponsored by any external organization.

### References

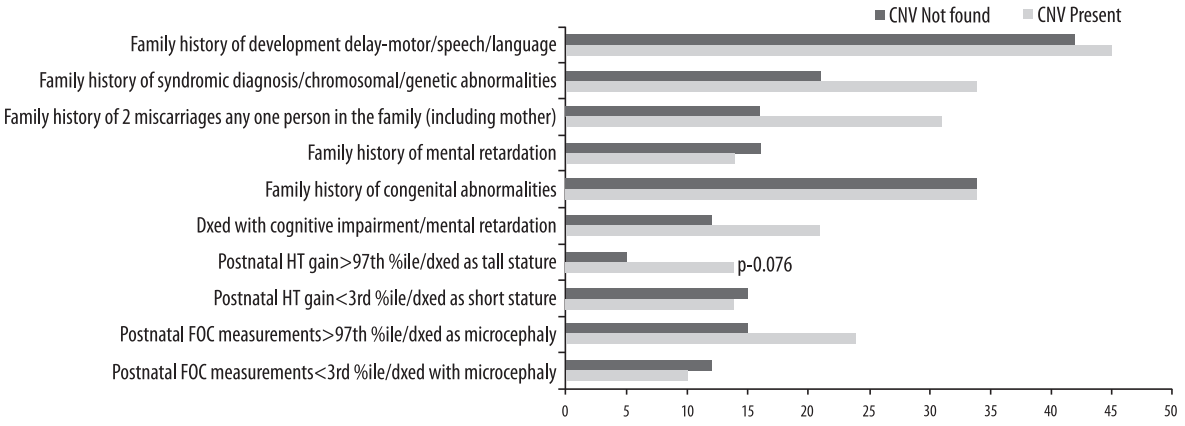
1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Washington, DC: American Psychiatric Publishing, Inc.; 2000.
2. Curry CJ, Stevenson RE, Aughton D, Byrne J, Carey JC, Cassidy S, et al. Evaluation of mental retardation: recommendations of a Consensus Conference: American College of Medical Genetics. Am J Med Genet. 1997;72(4):468-77.
3. Hunter AG. Outcome of the routine assessment of patients with mental retardation in a genetics clinic. Am J Med Genet. 2000;90(1):60-8.
4. Opitz JM, Kaveggia EG, Durkin-Stamm MV and Pendleton E. Diagnostic/genetic studies in severe mental retardation. Birth Defects Orig Artic Ser. 1978;14(6B):1-38.
5. Shao L, Shaw CA, Lu XY, Sahoo T, Bacino CA, Lalani SR, et al. Identification of chromosome abnormalities in subtelomeric regions by microarray analysis: a study of 5,380 cases. Am J Med Genet A. 2008;146A(17):2242-51.
6. de Vries BB, White SM, Knight SJ, Regan R, Homfray T, Young ID, et al. Clinical studies on submicroscopic subtelomeric rearrangements: a checklist. J Med Genet. 2001;38(3):145-50.
7. South ST, Rope AF, Lamb AN, Aston E, Glaus N, Whitby H, et al. Expansion in size of a terminal deletion: a paradigm shift for parental follow-up studies. J Med Genet. 2008;45(6):391-5.
8. Merritt JL and Lindor NM. Further clinical description of duplication of Williams-Beuren region presenting with congenital glaucoma and brachycephaly. Am J Med Genet A. 2008;146A(8):1055-8.
9. Shevell MI, Bejjani BA, Srour M, Rorem EA, Hall N and Shaffer LG. Array comparative genomic hybridization in global developmental delay. Am J Med Genet B Neuropsychiatr Genet. 2008;147B(7):1101-8.
10. Hoyer J, Dreweke A, Becker C, Göhring I, Thiel CT, Peippo MM, et al. Molecular karyotyping in patients with mental retardation using 100K single-nucleotide polymorphism arrays. J Med Genet. 2007;44(10):629-36.
11. Sagoo GS, Butterworth AS, Sanderson S, Shaw-Smith C, Higgins JP and Burton H. Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects. Genet Med. 2009;11(3):139-46.
12. Bernardini L, Alesi V, Loddo S, Novelli A, Bottillo I, Battaglia A, et al. High-resolution SNP arrays in mental retardation diagnostics: how much do we gain? Eur J Hum Genet. 2010;18(2):178-85.

| Origin of CNV | Deletion | BAC clones | Duplication | BAC clones |
|---|---|---|---|---|
| De novo | 19p13.3 | 3 | – | – |
| De novo | 22q13.33 | 1 | – | – |
| De novo | 1q44 | 23 | – | – |
| De novo | 20q11.23 | 3 | – | – |
| De novo | 5q35.2 | ~3 MB | – | – |
| De novo | 1q41q42.12 | 9 | – | – |
| De novo | 13q34 | 26 | 18q23 | 2 |
| Mat | 6p21.1 | 2 | – | – |
| Mat | 11p15.4 | 3 | – | – |
| pat | 11p12 | 1 | – | – |
| pat | 6p25.1 | 3 | – | – |
| Unknown | 2q13 | 1 | – | – |
| Unknown | 17p13.2 | 2 | – | – |
| Unknown | 6q16.3 | 9 | – | – |
| Unknown | 6q27 | 3 | – | – |
| Unknown | 2q13 | 1 | – | – |
| Unknown | 6q27 | 14 | – | – |
| Unknown | 2q13 | 1 | – | – |
| De novo | – | – | 1q44 | 24 |
| De novo | – | – | 2p25.3 | RP11-1N7 → RP11-97B21 |
| De novo | – | – | 11p12p11.12 | 25 |
| De novo | – | – | 18q23 | 2 |
| Mat | – | – | 17p13.3 | 2 |
| Mat | – | – | Xp22.31 | 3 |
| Mat | – | – | 7q11.23 | CTD--2069P19 → RP11-97B21 |
| pat | – | – | Yp11.32 | 3 |
| pat | – | – | 8q24.3 | 2 |
| Unknown | – | – | 10p15.3 | 3 |
| Unknown | – | – | 15q26.3 | 3 |
| Unknown | – | – | 1p36.3 | Duplication of 3 BAC and triplication of 3 BAC |
| Unknown | – | – | 20p12.2 | 3 |
| Unknown | – | – | 11p13 | RP11-293B1, RP11-26B16 RP11-133E13 |
| Unknown | – | – | 7q11.23 | 6 |
| Unknown | – | – | 8p23.3 | RP11-388B22 |

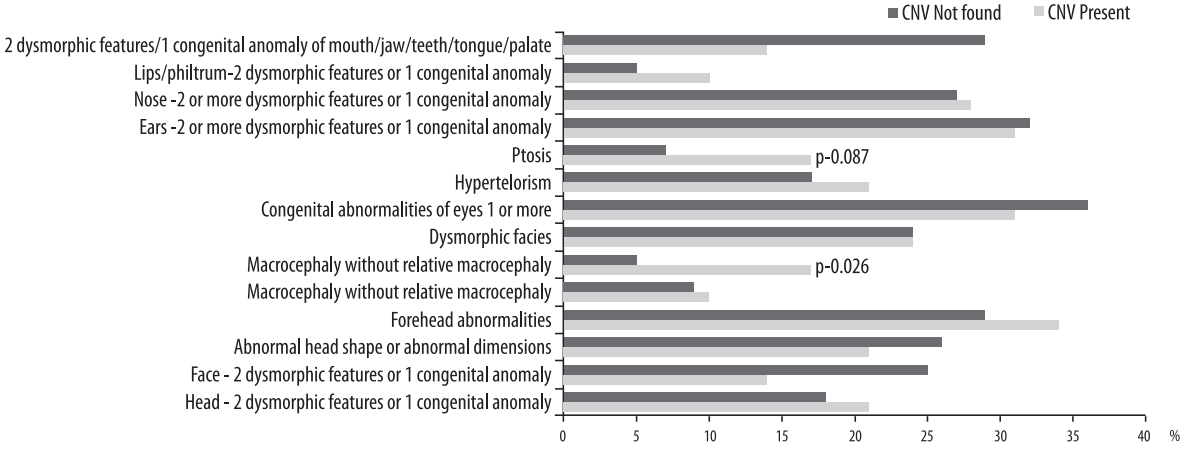
Supplement 1 Summary of the CVNs included in this study



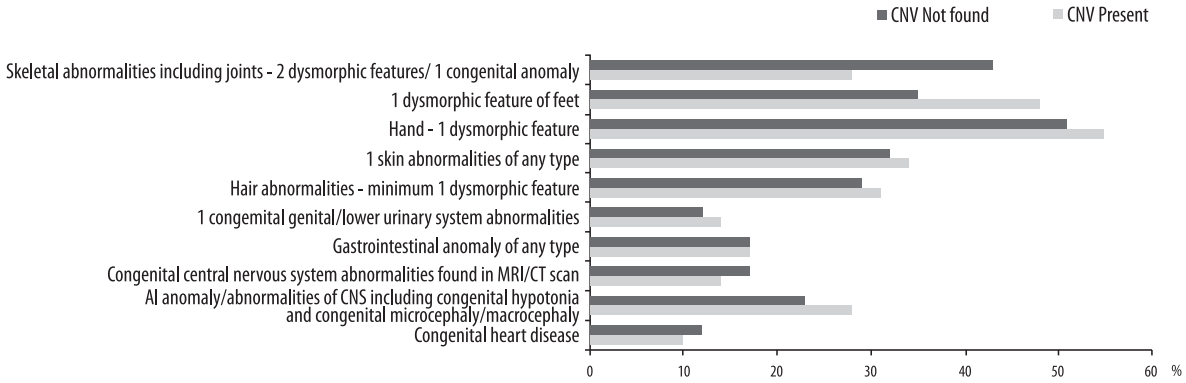

Supplement 2 Comparisons of specific features for the history between 33 individuals with CNV detected and 293 with no CNV detected. CNV=copy number variation



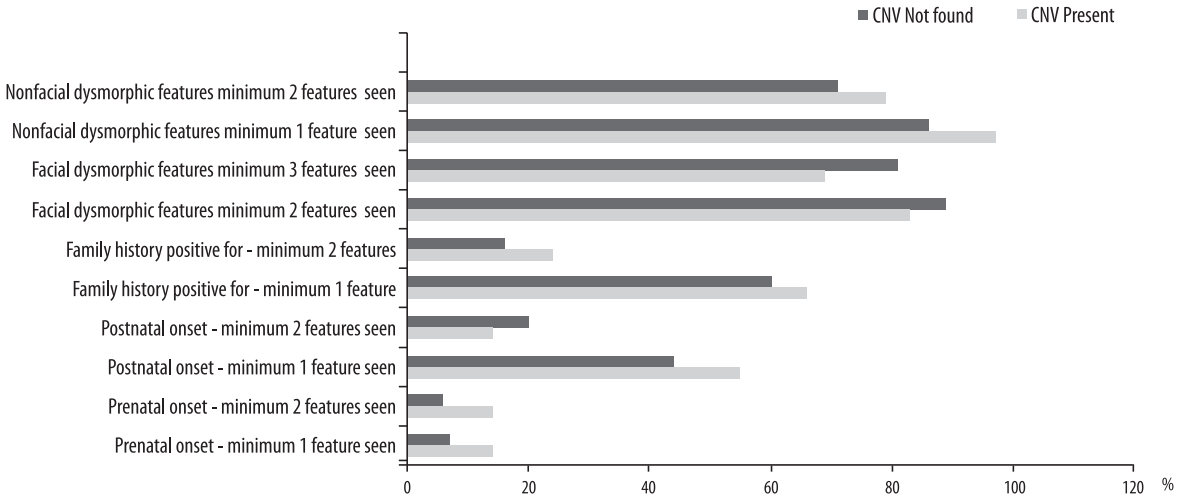

Supplement 3 Comparisons of additional historical features between 33 individuals with CNV detected and 293 with no CNV detected. Using a p value of less than 0.01, none were statistically significantly different. CNV=copy number variation




Supplement 4 Comparison of craniofacial features between 33 individuals with CNV detected and 293 with no CNV detected. Using a p value of less than 0.01, none were statistically significantly different. CNV=copy number variation




Supplement 5 Comparison of noncraniofacial features between 33 individuals with CNV detected and 293 with no CNV detected. CNV=copy number variation. Using a p value of less than 0.01, none were statistically significantly different.




Supplement 6 Comparison of different combinations of findings between the CNV and non CNV groups. Using a p value of less than 0.01, none were statistically significantly different.

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# The importance of combining of ultrasound and mammography in breast cancer diagnosis

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**Objective.** The aim of this study was to analyse individual and combined sensitivity and specificity of ultrasound and mammography in breast cancer diagnosis and emphasize the importance of combining breast imaging modalities. **Patients and methods.** By means of a cross-sectional study, ultrasound and mammographic examinations of 148 women (mean age 51.6 ± 10.8 years) with breast symptoms were analysed. All women underwent surgery and all lesions were examined by histopathology analysis which revealed the presence of 63 breast cancers, and 85 benign lesions. In relation to age, the women were separated in to a group under 50 years and a group 50 years and older. Ultrasound and mammographic findings were classified on the BI-RADS categorical scale of 1-5. Categories 1, 2 and 3 were considered negative, while categories 4 and 5 were positive for cancer. For statistical data processing the McNemar chi-square test for paired proportions was used. The differences on the level of $p<0.05$ were considered statistically significant. **Results.** In the group under 50 years, the ultrasound sensitivity was significantly higher than the mammographic sensitivity ($p=0.045$, $\chi 2=4$), without a statistically significant difference in specificity ($p=0.24$, $\chi 2=1.39$). In the women over 50, a significant difference between sensitivity of ultrasound and mammography was not proved ($p=0.68$, $\chi^2=0.17$), nor any difference in the specificities ($p=0.15$, $\chi^2=2.08$). In the group consisting of all patients, the sensitivity of ultrasound was statistically significantly higher in comparison with the sensitivity of mammography ($p=0.04$, $\chi^2=4.27$) with higher specificity ($p=0.04$, $\chi^2=4$). By combining the two methods in all patients sensitivity of 96.8% was achieved, in patients up to 50 sensitivity was 90.47% and in patients over 50, sensitivity was 100%. When the two methods were combined in all patients, a decrease in specificity was noted. **Conclusion.** The combination of ultrasound and mammography in breast cancer diagnosis achieves high sensitivity and the number of undetected breast cancers is reduced to minimum.

**Key words**: Breast cancer, Ultrasound, Mammography, Sensitivity, Specificity.

## Introduction

Breast cancer is the most common malignancy in women (1). After lung cancer, it is the most common cause of death from malignancies that affect the female gender (2) and in many countries is in the first place, so it is known as the leading female cancer (3). Thanks to mammography, breast ultrasound and the appropriate surgical and postoperative treatment, in recent years there has been a trend reducing the breast cancer mortality rate. Mammography is used as a screening method for early detection of breast cancer in women after 40, in some countries after 50 years of life, while breast ultrasound is the imaging of choice in women under 40 years (1). With an appropriate combination of ultrasound and mammography, the number of undetected breast cancers can be reduced to a minimum.

The aim of this study was to analyse the individual and combined sensitivity and specificity of ultrasound and mammography in breast cancer diagnosis and emphasize the importance of combining of breast imaging modalities.

## Patients and methods

By means of a cross-sectional study, ultrasound and mammographic examinations of 148 women with breast symptoms or positive family history for breast cancer in the period from January 2009 to November 2010 were analysed. All the women underwent surgery and all 148 breast lesions were examined by histopathology analysis. Histopathology results revealed the presence of 63 breast cancers, and 85 benign lesions. Diagnostic imaginings were performed at the Department of Radiology and Nuclear Medicine, surgical treatment at the Department of Surgery, and pathohistological analysis at the Department of Pathology of the Polyclinic for Laboratory Diagnostics of the University Clinical Centre, Tuzla. The group pattern was made consecutively. In relation to age, the women were separated into two groups, group A: women up to 50 years and group B: women 50 years and older. The breast ultrasound was performed on a "Sonoline G60 S"- Siemens ultrasound machine with 12 MHz-linear array transducer and if needed with 7.5 MHz-linear array transducer. Mammography was performed by a "Mammomat Nova 3000"-Siemens. Standard mediolateral oblique and craniocaudal views of each breast were taken.

To obtain and read mammography images, cassettes with phosphorus imaging plates (18x24 and 24x30), mamma-laser drystar DT-2 films and digitizer type CR 85-X with an NX workstation were used.

The findings were interpreted by two radiologists. Additionally, according to the radiological features of the described pathological changes, ultrasound and mammographic findings were classified on the BI-RADS categorical scale of 1-5 (4) as follows:

1. No significant abnormality,
2. Benign finding,
3. Probably benign finding,
4. Suspicious lesions- suspicious abnormality and
5. Highly suggestive of malignancy -malignant lesion.

Categories 1, 2 and 3 were considered negative, while categories 4 and 5 were considered as positive for cancer. To test the overall sensitivity and specificity of the two methods, a positive finding to cancer was considered if only one or both tests were positive, and negative to cancer when both tests had negative findings.

The standard methods of descriptive statistics (mean and standard deviation), standard statistical parameters, and parametric McNemar chi-square test for paired proportions were used for statistical data processing. The sensitivity and specificity of the methods were determined by the 2x2 table

diagnostic test. The differences on the level of $p<0.05$ are considered statistically significant.

## Results

The study included 148 patients, 63 patients with breast cancer and 85 patients with benign lesions. The mean age of all the patients was 51.6±10.8 years, ranging from 19 to 79 years. The mean age of patients with breast cancer was 55.4±11.4, ranging from 26 to 79 years, while the mean age of patients with benign lesions was 48.8±9.4, ranging from 19 to 74 years.

In the group A (patients up to 50) there were 69 (46.6%) patients. Of these, 21 (30.4%) patients had breast cancer and 48 (69.6%) of them had benign lesions. The ultrasound sensitivity in the group under 50 years was 33.3% higher than the mammographic sensitivity. In this group the ultrasound sensitivity was significantly higher than the mammographic sensitivity ($p=0.045$, $\chi2=4$). The specificity of ultrasound was 12.5% higher than mammographic but the difference in the specificities of the two imaging tests was not statistically significant ($p=0.24$, $\chi2=1.39$). Combining the two methods, high sensitivity of 90.5% was achieved, which was 4.8% higher compared to the sensitivity of ultrasound alone, and 38.1% higher compared to the sensitivity of mammography alone. The overall specificity of the two methods was 12.5% lower than mammographic specificity alone, and 25% lower relative to ultrasound specificity alone (Table 1).

In group B (patients 50-years and older) there were 79 (53.4%) patients. Of these, 42 (53.2%) patients had breast cancer and 37 (46.8%) of them benign lesions. The ultrasound sensitivity was 4.7% higher than mammographic sensitivity, while the specificity of ultrasound was 16.2% higher than mammography. In this group the difference in the sensitivities of the two imaging tests was not statistically significant $p=0.68$ ($\chi^2=0.17$). Also, the difference in the specificities of the two imaging tests was not statistically significant $p=0.15$ ($\chi^2=2.08$). Combining the two imaging tests, 100% sensitivity was achieved, but the overall specificity was 8.1% lower relative to the specificity of mammography, and 24.3% lower relative to specificity of ultrasound (Table 2).

In the group consisting of all patients, the sensitivity of ultrasound was statistically significantly higher relative to the sensitivity of mammography $p=0.04$ ($\chi^2=4.27$). The higher specificity of ultrasound relative to mammographic was noted, also $p=0.04$ ($\chi^2=4$). Combining the two methods high sensitivity (96.8%) was achieved, but the overall speci-

Table 1 Individual and overall sensitivity and specificity of ultrasound and mammography in women under 50 years

| Reliability | Diagnostic procedures | | |
|---|---|---|---|
| | Mammography | Ultrasound | Mammography+ Ultrasound |
| Sensitivity (%) | 52.4 | 85.7 | 90.5 |
| Specificity (%) | 66.7 | 79.2 | 54.2 |
| Positive predictive value (%) | 40.7 | 64.3 | 46.3 |
| Negative predictive value (%) | 76.2 | 92.7 | 92.9 |
| Positive likelihood ratio | 1.6 | 4.1 | 2 |
| Negative likelihood ratio | 0.71 | 0.18 | 0.17 |

Table 2 Individual and overall sensitivity and specificity of ultrasound and mammography in women 50 years and older

| Reliability | Diagnostic procedures | | |
|---|---|---|---|
| | Mammography | Ultrasound | Mammography+ Ultrasound |
| Sensitivity (%) | 90.5 | 95.2 | 100 |
| Specificity (%) | 64.9 | 81.1 | 56.8 |
| Positive predictive value (%) | 74.5 | 85.1 | 71.9 |
| Negative predictive value (%) | 85.7 | 93.8 | 100 |
| Positive likelihood ratio | 2.6 | 5 | 2.3 |
| Negative likelihood ratio | 0.15 | 0.06 | - |

Table 3 Individual and overall sensitivity and specificity of ultrasound and mammography in all 148 patients

| Reliability | Diagnostic procedures | | |
|---|---|---|---|
| | Mammography | Ultrasound | Mammography+ Ultrasound |
| Sensitivity (%) | 77.8 | 92.1 | 96.8 |
| Specificity (%) | 65.9 | 80 | 55.3 |
| Positive predictive value (%) | 62.8 | 77.3 | 61.6 |
| Negative predictive value (%) | 80 | 93.2 | 95.9 |
| Positive likelihood ratio | 2.3 | 4.6 | 2.2 |
| Negative likelihood ratio | 0.34 | 0.1 | 0.06 |

Table 4 Sensitivity of ultrasound and mammography in 63 patients with breast cancer

| Mammography | Ultrasound | | |
|---|---|---|---|
| | Positive (n; %) | Negative (n; %) | Total (n; %) |
| Positive (n; %) | 46 (73) | 3 (4.7) | 49 (77.8) |
| Negative (n; %) | 12 (19.1) | 2 (3.2) | 14 (22.2) |
| Total (n; %) | 58 (92.1) | 5 (7.9) | 63 (100) |

ficity was lower than the specificity of each method separately. Thus, the overall specificity was 10.6% lower than the specificity of mammography, and even 24.7% lower than the specificity of the ultrasound (Table 3).

Table 4 shows that, of all breast cancers, 19.1% were correctly identified as cancer by ultrasound but not by mammography, while 4.7% of all cancers were correctly identified as cancer by mammography, but not by ultrasound. Combining the two methods, in which case the finding is considered positive if both or either test is positive, in two cases (3.2%) the finding was false negative.

## Discussion

Since there is no factor that prevents breast cancer, like other cancers, the only possible way to reduce mortality is early detection of breast cancer.

Mammography is currently the only breast imaging modality that is widely used for the screening, while ultrasound is a wide-

ly used imaging technique in the diagnosis of mammographic detected or palpable lesions (3, 5). The sensitivity and specificity of mammography in breast cancer diagnosis is variable and depends primarily on the age of the patient and breast density (6).

Considering the age of the patients in most published studies, the higher sensitivity and specificity of mammography at 50 years and over has been shown compared to women aged from 40 to 49 (7, 8, 9).

Sibbering and al. (8) compared the sensitivity but not the specificity of mammography in women aged from 50 to 70 (257 women) and women under 50 years of age (143 women). The sensitivity of the mammography in the first group was 83%, and in the second, 65% (p=0.001).

The higher sensitivity of mammography in women aged over 50 was proven in research conducted by Ciatto and al. (7) and by Dixon and al. (9). In these studies, similar to Sibbering's study, the sensitivity of mammography was analysed, but not the sensitivity of the ultrasound.

In our study, the sensitivity of mammography was 38.1% higher in women 50 years and older in relation to women under 50 years, which is in accordance with published results (7, 8, 9).

Houssami and al. (10) proved that for women younger than 50 years, the sensitivity of ultrasound and mammography in relation to age has little variability, but the sensitivity of mammography increases substantially after age 50. Their study also showed that the difference between the specificities of these two methods is small and age does not have any important influence on the specificity of the methods.

Devolli-Disha and al. (11) showed the higher accuracy of ultrasound than mammography in symptomatic women below 45 years as well the fact that the sensitivity of mammography progressively increases after 60 years.

In our study, in the group of women younger than 50, the sensitivity of ultrasound was significantly higher (33.3%) in relation to mammography, while in women over 50 years, this difference was only 4.7%, which is in accordance with published results.

In 1977, Teixidor and al. (12) published a study about the individual and combined sensitivity of these two methods. The study involved 200 patients and 30 of them had breast cancer. The sensitivity of mammography was 94%, sensitivity of ultrasound 78%, while the combined sensitivity was 97%.

The higher sensitivity of mammography in relation to ultrasound was shown by Negri and al. (13). Combining the two methods, they achieved overall sensitivity equal to the sensitivity of mammography (89%) while overall specificity was only 46%.

In the study conducted by Moss and al. (14) the sensitivity of ultrasound was 88.9% and mammography 78.9%. Combining the two methods, overall sensitivity was 94.2%, but overall specificity was only 67.9% as before.

The significantly higher sensitivity of ultrasound in relation to mammography was shown by Rotten and Levaillant (15). In their study specificity values were not published.

Housami and al. (16) conducted research on a group of patients aged from 25-55 years. Combining ultrasound and mammography, they achieved sensitivity of 76.3% and even 100% specificity, which is a difference from other studies.

Devolli-Disha and al. (11) proved in their study that ultrasound is a 20.5% more sensitive and 14.6% more specific method in relation to mammography.

In our study, combining the two methods high sensitivity of 96.8% was achieved, while specificity decreased to 55.3%, which is in accordance with the results of most of the studies above.

When mammography and ultrasound are combined, the possibility that breast

cancer will be misdiagnosed is 2.4% according to most results (11, 13-18). Devolli et al. (11) in their study correctly identified 25.1% of all breast cancers by ultrasound but not by mammography, while 4.6% of all cancers were correctly diagnosed by mammography but not by ultrasound, which is similar to our results (Table 4). In our study we had 3.2% misdiagnosed cancers, which means that both methods were negative in only two cases. This result is in accordance with the results of other studies (11-18) which indicates the importance of combining ultrasound and mammography in order to reduce the number of undetected breast cancers to a minimum.

## Conclusion

In breast cancer diagnosis, in women under the age of 50, ultrasound is a significantly more sensitive imaging test than mammography, but in women over 50 years the sensitivity of both imaging tests is almost equal. The combination of ultrasound and mammography achieves high sensitivity so the number of undetected breast cancers is reduced to a minimum, but specificity is decreased.

**Authors' contributions:** Conception and design: SM; Acquisition, analysis and interpretation of data: SM and MB; Drafting the article: SM, GS and JMM; Revising it critically for important intellectual content: SM and MB.

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### References

1. McPherson K, Steel M, Dixon J. Breast cancer-epidemiology, risk factors, and genetic. Brit Med J. 2000;321:624-8.
2. Jemal A, Tivvari RC, Murrav T, Ghafoor A, Samuels A, Ward E, et al. Cancer statistics. CA Cancer J Clin. 2004;54(1)8-29.
3. Goldner B, Dodić M, Mijović Z, Stević R. Klinički ultrazvuk u bolestima dojke. Beograd: Medicinski fakultet Beograd; 1998. p. 15-153.
4. American College of Radiology. Illustrated Breast Imaging Reporting and Data System (BI-RADS) 3rd ed. Reston, Va: American College of Radiology; 1998.
5. Boris B, Renata HK. - prevod s engleskog Leksikon ACR BI-RADS, mamografija. Branko Š (ur.), ACR-BIRADS Postupci oslikavanja dojki i sustav tumačenja i kategorizacija nalaza; Oslikavanje dojki - Atlas mamografija Utrazvuk; 2006.
6. Kerlikowske K, Grady D, Barclay J, Sickles EA, Ernster V. Effect of age, breast density, and family history on the sensitivity of first screening mammography. JAMA. 1996;276:33-8.
7. Ciatto S, Rosselli del Turco M, Catarzi S, Morrone D. The contribution of ultrasonography to the differential diagnosis of breast cancer. Neoplasma. 1994;41:341-5.
8. Sibbering DM, Burrell HC, Evans EJ, Yeoman LJ, Wilson ARM, Robertson JF, et al. Mammographic sensitivity in women under 50 years presenting symptomatically with breast cancer. The Breast. 1999;4:127-9.
9. Dixon JM, Anderson TJ, Lamb J, Nixon SJ, Forrest APM. Fine needle aspiration cytology, in relationships to clinical examination and mammography in the diagnosis of a solid breast mass. Br J Surg. 1986;71:593-6.
10. Housami N, Irvvig L, Simpson M, McKessar M, Blome S, Noakes J. Comparative sensitivity and specificity of mammography in young women with simptoms. AJR Am J Roentgenol. 2003;180:935-45.
11. Devolli-Disha E, Manxhuka-Kërliu S, Ymeri H, Kutllovci A. Comparative accuracy of mammography and ultrasound in women with breast symptoms according to age and breast density. Bosn J Basic Med Sci. 2009;9(2):131-6.
12. Teixidor H S, Kazam E. Combined mammographic ± sonographic evaluation of breast masses. Am J Roentgenol. 1977;409-17.
13. Negri S, Bonetti F, Capitanio A, Bonzanini M. Preoperative diagnostic accuracy of fine-needle aspiration in the management of breast lesions: comparison of specificity and sensitivity with clinical examination, mammography, echography, and thermography in 249 patients. Diagn Cytopathol. 1994;11:4-8.
14. Moss HA, Britton PD, Flower CDR, Freeman AH, Lomas DJ, Warren RML. How reliable is modern breast imaging in differentiating benign from malignant breast lesions in the symptomatic population? Clin Radiol. 1999;54:676-82.

15. Rotten D, Levaillant J M. The value of ultrasonic examination to detect and diagnose breast carcinomas. Analysis of the results obtained in 125 tumors using radiographic and ultrasound mammography. Ultrasound Obstet Gynecol. 1992;2:203-14.
16. Houssami N, Irwig L, Loy C. Accuracy of combined breast imaging in young women. Breast. 2002;11:36-40.
17. Zonderland HM, Coerkamp EG, Hermans J, van de Vijver MJ, van Voorthuisen AE. Diagnosis of breast cancer: contribution of US as an adjunct to mammography. Radiology. 1999;213:413-22.
18. Rahbar G, Sie AC, Hansen GC, Prince JS, Melany ML, Reynolds HE et al. Benign versus malignant solid breast masses: US differentiation. Radiology. 1999;213:889-94.

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# Office hysteroscopy, transvaginal ultrasound and endometrial histology: a comparison in infertile patients

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**Objective.** To evaluate accuracy of transvaginal sonography (TVS) and hysteroscopy in detection of intrauterine pathology in infertile women. **Subjects and methods.** This retrospective study was conducted in 56 infertile women with abnormal transvaginal ultrasound findings of the uterine cavity which was performed during the midfollicular phase as a part of routine infertility workup. Hysteroscopy was performed between 6$^{th}$ and 10$^{th}$ day of cycle. **Results.** The mean age of the subjects was 31.9±4.0. The most frequent ultrasound finding was endometrial polyp in 34 (60.7%) patients, septate uterus in 8 (14.3%) patients, submucosal myoma in 7 (12.5%) patients, endometrial hyperplasia in 5 (8.9%) patients and Syndroma Ascherman in 2 (3.6%) patients. Hysteroscopy confirmed 20 (35.7%) polyps, the same number of myomas, septate uterus and Syndroma Ascherman as detected by ultrasound, (7 (12.5%), 8 (14.3%) and 2 (3.6%), respectively) and 19 (33.9%) endometrial hyperplasia. In 46 women with histological excamination, the sensitivity of TVS and hysteroscopy in the diagnosis of endometrial polyps were identical - 100%, while the specificity was higher in hysteroscopy than in TVS (92.3% versus 56.4%, p<0.001). The sensitivity of TVS in the diagnosis of endometrial hyperplasia was higher than that of hysteroscopy (86.4% versus 22.7%, p<0.001), while specificity was identical, of 100%. Accordance between hysteroscopy and histology was good (κ=0.79), between ultrasound and hystology was moderete (κ=0.59). **Conclusion.** Hysteroscopy appeared to be more reliable in diagnosis than TVS. The use of a high frequency ultrasound probe leads us to a lack of diagnostic clarity between endometrial polyps and hyperplasia.

**Key words:** Endometrium, Infertility, Ultrasound, Hysteroscopy, Hystology.

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## Introduction

Evaluation of the uterine cavity is the important part of examination of infertile women. Findings as a fibroids, polyps and Müllerian anomalies can impair fertility and result in poor outcome of pregnancy. Assessment of the endometrium is an important step in the management of women with diagnosed infertility. Abnormalities in the

process of implantation could be the basis of many cases of unexplained infertility.

The first line diagnostic tool for uterine abnormalities is transvaginal sonography (TVS). It is an easy, fast and cheap technique that has became widely used. TVS has already proved to be a method with high reliability in diagnosing this pathology.

Hysteroscopy is the second step, which serves after an ultrasound examination as a form of screening. The advantage of hysteroscopy is that we diagnose and treat the changes we notice at the same time. Over recent years hysteroscopy is being increasingly used in out-patient facilities which alongside the standard advantages of hysteroscopy also provide greater comfort for the patients, since it excludes the need to stay in hospital and decreases the time of treatment, but also the time needed to prepare the patient for further procedures, e.g. medically assisted conception (1, 2, 3).

The aim of this study was to evaluate accuracy of transvaginal ultrasound and hysteroscopy in detection of intrauterine pathology in infertile women.

## Patients and methods

This retrospective study was conducted from July 2008 to December 2010 in 56 infertile women. At the time of the study all patients had a history of primary or secondary infertility of one-year duration. The inclusion criteria was abnormal transvaginal ultrasound findings of uterine cavity which was performed as a part of routine infertility workup afterwards every women was underwent the office hysteroscopy procedure in the same centre.

## Ultrasound evaluation

TVS was performed during the midfollicular phase of the cycle ($6^{th}$ to $10^{th}$ day) using an Volusion 730 expert (General Electric, New York, USA) equipped with a 9.0 MHz transvaginal probe. The endometrial cavity contours and echo patterns of the endometrium were evaluated for irregularities in both the transverse plane and the long axis. All examinations were performed by the same operator.

## Hysteroscopy technique

Hysteroscopy was performed in the early follicular phase (6 to 10. day) using a 9 mm continuous-flow operative office hysteroscope based on a 4 mm rod lens scope (Karl Storz, Tuttlingen, Germany). With intravenous anaesthesia with Diprivan cervical dilatation was performed up to Hegar 9.5. The uterine cavity was distended by normal saline solution and intra-uterine pressure was controlled by an irrigation–suction device (Varioflow, Ljubljana, Slovenia). Intrauterine pressure was set around 30 mmHg, resulting in a balance of the irrigation flow of around 200 ml/minute and a vacuum of 0.1 bars. Endometrial specimens were obtained by 5Fr crocodile biopsy forceps (Karl Storz). Endometrial polyps, myomas and septa were treated utilising the operative facilities of the hysteroscope with Versapoint eletrodes (Johnson and Johnson, New Yersy, USA).

Whenever a lesion was found by hysteroscopy, except in the case of septate uterus and Syndroma Ascherman, it was hysteroscopically excised during the same procedure, fixed in formalin and sent for histological examination.

## Statistical analysis

Numerical data are presented as mean ± standard deviation, while categorical data are expressed as proportions. The diagnostic accuracy characteristics as sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for both

hysteroscopy and TVS were calculated using histological diagnosis as the gold standard. Differences between proportions were tested by the chi-squared test. The level of agreement between methods was measured by Kappa test. A p value less than 0.05 was considered statistically significant. Data were analyzed using the PASW 18 software (SPSS Inc., Chicago, IL, USA).

## Results

In this retrospective study 56 infertile patients were included with abnormal ultrasound appearance of the uterine cavity. The mean age (± standard deviation) of the patients was 31.9±4.0. The distribution of the various abnormalities detected by ultrasound and hysteroscopy is reported in Table 1. The most frequent ultrasound finding was endometrial polyp in 34 (60.7%) patients, then septate uterus in 8 (14.3%) patients, submucosal myoma in 7 (12.5%) patients, endometrial hyperplasia in 5 (8.9%) patients and Syndroma Ascherman in 2 (3.6%) patients.

Hysteroscopy confirmed 20 (35.7%) endometrial polyps, the same number of myomas, septate uterus and Syndroma Ascherman was detected by ultrasound [7 (12.5%), 8 (14.3%) and 2 (3.6%), respectively, and 19 (33.9%)] endometrial hyperplasia. There were no failed hysteroscopic procedures.

In 46 women, the histological examination of endometrial specimen was performed. In the diagnosis of submucosal myoma, all measured diagnostic accuracy characteristics, according to histological examination, for hysteroscopy and TVS were identical (Table 2). In the diagnosis of en-

Table 1 The incidence of different abnormalities diagnosed by hysteroscopy, ultrasonography and histology in 56 infertile women

| Endouterine abnormalities | Hysteroscopy | | TVS* | | Histology | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Polyp | 20 | 35.7 | 34 | 60.7 | 17 | 37.0 |
| Submucosal myoma | 7 | 12.5 | 7 | 12.5 | 7 | 15.2 |
| Syndroma Ascherman | 2 | 3.6 | 2 | 3.6 | 0 | 0 |
| Septate uterus | 8 | 14.3 | 8 | 14.3 | 0 | 0 |
| Endometrial hyperplasia | 19 | 33.9 | 5 | 8.9 | 22 | 47.8 |
| Total | 56 | 100 | 56 | 100 | 46 | 100 |

*Transvaginal sonography

Table 2 Sensitivity and specificity of ultrasonography and hysteroscopy, based on histology in 46 infertile women

| Diagnostic method | Sensitivity (%) | PPV*(%) | Specificity (%) | NPV§ (%) |
|---|---|---|---|---|
| Ultrasonography (polyp) | 100 | 50 | 56.4 | 100 |
| Ultrasonography (myoma) | 100 | 100 | 100 | 100 |
| Ultrasonography (endometrial hyperplasia) | 22.7 | 100 | 100 | 66.7 |
| Hysteroscopy (polyp) | 100 | 85 | 92.3 | 100 |
| Hysteroscopy (myoma) | 100 | 100 | 100 | 100 |
| Hysteroscopy (endometrial hyperplasia) | 86.4 | 100 | 100 | 91.9 |

*Positive predictive value; §Negative predictive value

dometrial polyps, the sensitivity of hysteroscopy and TVS were of 100%, both. However, the specificity and PPV were higher in hysteroscopy than in TVS (92.3% versus 56.4%, $\chi^2$=11.36, p<0.001, and 85% versus 50%, $\chi^2$=5.20, p=0.023, respectively). In the diagnosis of endometrial hyperplasia, the sensitivity and NPV of hysteroscopy was higher than that of TVS (86.4% versus 22.7%, $\chi^2$=15.49, p<0.001, and 91.9% versus 66.7%, $\chi^2$=6.40, p=0.011, respectively), while specificity was the same (of 100% in both). Accordance between hysteroscopy and histology was good (κ=0.79, 95% CI: 0.67, 0.91) and between ultrasound and hystology was moderete (κ=0.59, 95% CI: 0.44, 0.75).

In the diagnosis of endometrial polyps and hyperplasia, the concordance between hysteroscopy and histology (0.93) was significantly higher than the concordance between TVS and histology (0.63) (χ2=12.52, p<0.001). However, in the diagnosis of submucosal myoma, the concordance of hysteroscopy and TVS with histology was identical (concordance index=1).

## Discussion

Intrauterine pathology such as polyps, submucosal myoma and uterine septa are relatively common and may impair fertility. Traditionally, the assessment of the uterine cavity for the basic infertility work-up has been performed by TVS and hysteroscopy. In the last decade, improvements in ultrasound and hysteroscopy technologies have changed the diagnostic approach. On the one hand the introduction of a high-frequency transvaginal probe increases the possibility of diagnosis, and on the other hand the introduction of "office hysteroscopy" enables direct visualisation of the cervix and the uterine cavity and at the same time the possibility of treating the abnormalities diagnosed. The results of this study whith sensitivity of 100% and specificity of 100% in diagnosis of submucosal myoma and uterine septa confirmed the results of other studies according to hysteroscopy as the gold standard in the diagnosis of endouterine pathologies, such as submucosal myomas and anomalies of the uterine cavity (2, 4).

In this study the endometrial polyps and hyperplasia are the most frequent findings of endouterine pathologies. But we noticed differences between TVS and hysteroscopy in their diagnostic accuracy: lower specificity in the diagnosis of polyps (56% versus 92%) and lower sensitivity in the diagnosis of hyperplasia (23% versus 86%) with TVS in comparison to hysteroscopy. In the analysis of the results we found that in those cases in which hysteroscopy and histology did not confirm the existence of an endometrial polyp, it was actually a case of endometrial hyperplasia, but we had diagnosed it as an endometrial polyp by TVS. Mukhopadhayay et al. (5) compard TVS, hysteroscopy and histology in perimenopausal women with abnormal uterine bleeding and found similar results in sensitivity and specificity of ultrasound in diagnosis of polyps and endometrial hyperplasia as are results of our study. Shalev et al. (6) compared TVS and hysteroscopy findings in infertile patients and found disagreement between methods in diagnosis of polyps end endouterine synechia.

Since the ultrasound examinations were performed by a single operator on the same ultrasound apparatus, we tried to analyse and find the reasons for this discrepancy between ultrasound and hysteroscopy diagnosis. We believe that a possible reason lies precisely in the use of a high frequency probe and images with better resolution, which show changes in the structure of the endometrial hyperplasia clearly. So, for example echogenic zones are shown as small polyps in the surrounding less echogenic tissue. Looking for data in the literature, we came across similar results obtained by

authors following changes caused to the endometrium in women who take Tamoxifen. In those cases too TVS proved to be less reliable than hysteroscopy (7) as in the postmenopausal women when endometrium appears as a normal (8, 9).

The question arose whether this in fact had any clinical significance when both endometrial hyperplasia and polyps are conditions which require treatment in infertile patients, and indicate hysteroscopy for further diagnosis and treatment.

This could lead us to think about the fact that advances in ultrasonography technology by introducing a high frequency probe improve diagnostic possibilities but impose the need to set new diagnostic criteria related to the structure of endometrial echo structures.

If we take in consideration that ultrasound and hysteroscopy was done by the same observer we could think about his subjectivity as a limitation of the study (10). The second limitation is a small number of participants.

## Conclusion

Hysteroscopy appeared to be more reliable in diagnosis than TVS and offers the possibility of simultaneous diagnosis treatment of endouterine pathologies in infertile women. Introduction of a high frequency probe leads us to a lack of diagnostic clarity between endometrial polyps and endometrial hyperplasia.

**Authors' contributions:** Conception and design: DB; Acquisition, analysis and interpretation of data: DB and AB; Drafting the article: DB; Revising it critically for important intellectual content: DB and AB.

**Conflict of interest:** The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

**References**

1. Ozturk E, Ugur MG, Balat O, Kutlar I, Dikensoy E, Cebesoy B. An analysis of hysteroscopy experience over a seven-year period. Clin Exp Obstet Gynecol. 2010;37(2):150-1.
2. Ait Benkaddour Y, Gervaise A, Fernandez H. Which is the method of choice for evaluating uterine cavity in infertility workup? Gynecol Obstet Biol Reprod. 2010;39(8):606-13.
3. Bosteels J, Weyers S, Puttemans P, Panayotidis C, Van Herendael B, Gomel V at al. The effectiveness of hysteroscopy in improving pregnancy rates in subfertile women without other gynaecological symptoms: a systematic review. Hum Reprod Update. 2010;16(1):1-11.
4. Loverro G, Nappi L, Vicino M, Carriero C, Vimercati A, Selvaggi L. Uterine cavity assessment in infertile women: comparison of transvaginal sonography and hysteroscopy. Eur J Obstet Gynecol Reprod Biol. 2001;100(1):67-71.
5. Mukhopadhayay S, Bhattacharyya SK, Ganguly RP, Patra KK, Bhattacharya N, Barman SC. Comparative evaluation of perimenopausal abnormal uterine bleeding by transvaginal sonography, hysteroscopy and endometrial biopsy. J Indian Med Assoc. 2007;105(11):624.
6. Shalev J, Meizner I, Bar-Hava I, Dicker D, Mashiach R, Ben-Rafael Z. Predictive value of transvaginal sonography performed before routine diagnostic hysteroscopy for evaluation of infertility. Fertil Steril. 2000;73(2):412-7.
7. Liedman R, Lindahl B, Andolf E, Willen R, Ingvar C, Ranstam J. Disaccordance between estimation of endometrial thickness as measured by transvaginal ultrasound compared with hysteroscopy and directed biopsy in breast cancer patients treated with tamoxifen. Anticancer Res. 2000;20(6C):4889-91.
8. Kasraeian M, Asadi N, Ghaffarpasand F, Karimi AA.Value of transvaginal ultrasonography in endometrial evaluation of non-bleeding postmenopausal women. Climacteric. 2011;14(1):126-31.
9. Dreisler E, Sorensen SS, Ibsen PH, Lose G. Value of endometrial thickness measurement for diagnosing focal intrauterine pathology in women without abnormal uterine bleeding. Ultrasound Obstet Gynecol. 2009;33(3):344-8.
10. Kasius JC, Broekmans FJ, Veersema S, Eijkemans MJ, van Santbrink EJ, Devroey et al. Observer agreement in the evaluation of the uterine cavity by hysteroscopy prior to in vitro fertilization. Hum Reprod. 2011;26(4):801-7.

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# Acute respiratory distress syndrome in patients with Legionella pneumonia

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**Objective.** The relationship between specific causative organisms and development of ARDS in pneumonia patients has not been explored. Several case reports have described the development of ARDS in patients with *Legionella* pneumonia. The aim is of this study was to determine frequency and outcomes of ARDS in patients with *Legionella* Pneumonia. **Methods.** A retrospective cohort study of patients with *Legionella* pneumonia hospitalized at two Mayo Clinic Rochester hospitals was conducted. To identify the patients with *Legionella* pneumonia we searched the Mayo Clinic Life Sciences System (MCLSS) database from 01/01/2003 to 12/31/2007. Electronic medical records of patients with active Legionella pneumonia based on positive cultures and/or urinary antigen were reviewed. ARDS was diagnosed on the basis of the criteria of the North American/European consensus conference definition. **Results.** We identified 15 patients with microbiologically proven *Legionella* pneumonia of whom 11 were admitted to the intensive care unit (ICU), 6 required mechanical ventilation and 5 met the criteria for ARDS. Age (median 42 vs. 50 years, p=0.32) and gender (4/10 vs. 1/5 female, p=0.60) were similar in patients with and without ARDS. Septic shock was present in 4 of the 5 patients with ARDS and only 1 without. Patients with ARDS had longer ICU length of stay (median 9 vs. 1 days, P=0.03). Only one patient (from the ARDS group) died in the hospital. **Conclusion.** In this retrospective study ARDS occurred in one third of patients with microbiologically proven *Legionella* pneumonia and was associated with prolonged length of ICU stay.

**Key words:** Pneumonia, Legionella, ARDS.

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## Introduction

Legionella is a Gram negative pathogen that causes legionellosis or Legionnaires' disease. Legionella has 50 species and 70 serogroups identified, most commonly L. pneumophila. Since the first breakout of Legionella in July 1976 that affected 221 persons, resulting in 34 deaths

(1), there have been numerous reported breakouts worldwide (2-9), often associated with severe presentations and substantial mortality. The incidence of Legionella pneumonia ranges from 2 to 15 percent of all community-acquired pneumonias (CAP) that require hospitalization (10) and is the second cause of severe CAP requiring ICU admission (11). So far, several case reports have described the development of ARDS in patients with Legionella pneumonia. In addition, a recent report demonstrated an increased risk for ARDS in pulmonary vs. non-pulmonary infection, as well as the relationship between Legionella infection and the development of ARDS in critically ill patients (12). To our knowledge, there is no study that has looked specifically at the features of hospitalized patients with Legionella pneumonia and its relation to ARDS.

The objective of this study was to determine the frequency, characteristics and outcome of ARDS among patients with sporadic Legionella pneumonia at Mayo Clinic Rochester over a five year period.

## Patients and methods

In this retrospective cohort study, we reviewed electronic medical records of patients admitted to two Mayo Clinic Rochester hospitals between 01/01/2003 and 12/31/2007 with microbiologically confirmed Legionella pneumonia based on positive cultures and/or urinary antigen test. Patients were identified by using the Mayo Clinic Life Sciences System (MCLSS) database. Pneumonia was defined as new or progressive infiltrate as seen on a chest X-ray or CT scan along with a high clinical suspicion of pneumonia defined as at least one of the following: fever (>38°C or >100.4°F), leucopenia (<4000 WBC/mm$^3$) or leukocytosis (>12,000 WBC/mm$^3$), altered mental status with no other recognized cause (for adults >70 years old) and at least two of the following: new onset of purulent sputum, or change in character of sputum, or increased respiratory secretions, or increased suctioning requirements, new onset or worsening cough, or dyspnea, or tachypnea, rales or bronchial breath sound and worsening gas exchange, increased oxygen requirements, or increased ventilation demand (13). The outcome measures were: development of ARDS, the length of ICU stay and overall mortality. ARDS was diagnosed based on the criteria by the North American/European consensus conference definition as acute hypoxemic respiratory failure ($PaO_2$/$FIO_2$<300) and development of bilateral pulmonary infiltrates in the absence of left atrial hypertension (14).

## Statistical analysis

Continuous data are presented as median and interquartile range (IQR) and categorical data as counts and percentages. Continuous variables were compared using the Wilcoxon rank-sum test for non-normally distributed variables. The Fisher's exact tests were used to compare categorical variables. JMP statistical software (JMP Version 7, SAS Institute Inc., Cary, NC) was used for all statistical analyses.

## Results

We identified 15 patients, 5 women, median age 46 years (interquartile range (IQR) 42-57) with microbiologically proven Legionella pneumonia, of whom 11 were admitted to the intensive care unit (ICU), 6 required mechanical ventilation and 5 met the criteria for ARDS. The median duration of mechanical ventilation was 5 days (IQR 4-8). The diagnosis was made by culture in 2, urinary antigen in 10 and both in 3 cases. Two patients had positive bronchoalveolar lavage by direct fluorescent antibody staining (DFA). Only 4 patients had no previous

medical history. The most frequent comorbidities were hypertension and diabetes. The majority of patients presented with bilateral chest infiltrates at the time of hospital admission, and four patients met the criteria for ARDS at the time of hospital admission. Figure 1 presents a portable chest radiograph appearance in patient with ARDS due to Legionella pneumonia. The mean time from symptom onset to appropriate antibiotic treatment was 6 days (2-10) (Table 1).

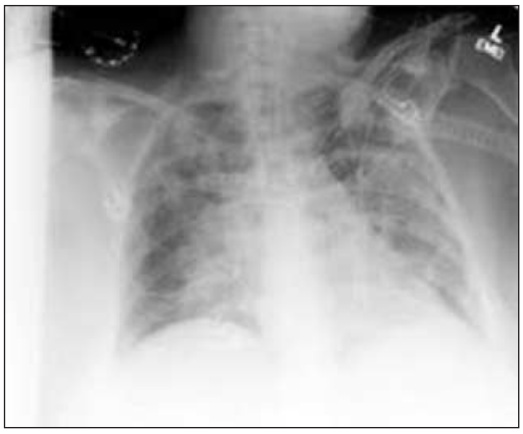

Figure 1 Acute respiratory distress syndrome in patient with Legionella pneumonia. Dense right middle lobe consolidation and bilateral alveolar infiltrates, endotracheal tube 2 cm above the carina

Age (median 42 vs. 50 years, p=0.32) and gender (4/10 vs. 1/5 female, p=0.60) were similar in patients with and without ARDS. ARDS developed in 4 patients with no comorbidities and 1 with past medical history of COPD and diabetes mellitus. Median time from symptom onset to initiation of appropriate antibiotic therapy was similar in patients with and without ARDS (6 vs.4 days, p=0.17). Septic shock was present in 4

Table 1 Characteristics of patients with Legionella pneumonia

| Number of patient | Age (Years) | Gender | Comorbidities | Chronic Immuno-suppression | Symptom to AB (days) | Chest X ray (on admission) | ARDS |
|---|---|---|---|---|---|---|---|
| 1 | 75 | F | CAD, HTN, DM, PMR | Yes | 2 | Bilateral | No |
| 2 | 46 | M | HTN | No | 14 | Unilateral | No |
| 3 | 51 | M | None | No | 14 | Bilateral | Yes |
| 4 | 42 | F | HTN, DM | No | 1 | Bilateral | No |
| 5 | 42 | F | None | No | 5 | Bilateral, ARDS | Yes |
| 6 | 10 | F | IPH | Yes | 6 | Bilateral | No |
| 7 | 68 | M | Multiple myeloma | Yes | 1 | Unilateral | No |
| 8 | 39 | M | None | No | 6 | Bilateral, ARDS | Yes |
| 9 | 74 | M | Ulcerative colitis, CAD, DM | Yes | - | Unilateral, cavitary mass | No |
| 10 | 57 | M | COPD, DM | No | 6 | Bilateral, ARDS | Yes |
| 11 | 50 | M | Crohn's disease | Yes | 2 | Unilateral | No |
| 12 | 54 | M | Alcohol abuse | No | 4 | Bilateral | No |
| 13 | 46 | F | Bilateral lung transplant for emphysema | Yes | 12 | Unilateral | No |
| 14 | 46 | M | Alcohol abuse | No | 6 | Unilateral, pleural effusion | No |
| 15 | 25 | M | No | No | 9 | Bilateral, ARDS | Yes |

AB-Antibiotics, CAD-Coronary artery disease, HTN-Hypertension, DM-Diabetes mellitus, PMR-Polymyalgia rheumatica, IPH-Idiopathic pulmonary hemosiderosis

of the 5 patients with ARDS and only 1 without. All ARDS patients were treated per protocol with lung protective (tidal volume 4-8 mL/kg predicted body weight) mechanical ventilation. Patients with ARDS had longer ICU length of stay (median 9 vs. 1 days, P=0.03). Only one patient (from the ARDS group) died in the hospital.

## Discussion

The results of this study demonstrated the high frequency of ARDS among hospitalized patients with confirmed Legionella pneumonia and substantially lower mortality than previously reported. Comorbidities were common, including diabetes mellitus and immunologic diseases. This is in line with previous studies that showed increased risk for Legionnaires' disease in patients 50 years and older, with comorbidities including diabetes mellitus, chronic obstructive pulmonary disease, renal disease and immuno suppression (15, 16).

The mortality from Legionnaires' disease ranges from 5-17% in hospitalized patients (17, 18), up to 30% in patients requiring ICU admission. Considering most patients in this study had severe forms of Legionella pneumonia, mortality was considerably lower than previously reported (19, 20). Over the past decade there has been a substantial decline in mortality from Legionella infections in the US (21). Early initiation of empirical antimicrobial coverage for atypical bacteria, as well as the recent developments in critical care medicine, including the standardized protocols for lung protective ventilation(22), sedation (23), weaning (24), early goal directed therapy in sepsis (25) and conservative strategy of fluid management in patients with acute lung injury (26) could explain the lower mortality in the observed patients. Several factors have been shown to independently influence survival in critically ill patients with Legionella pneumonia, including the baseline severity of illness and hyponatremia (20). The majority of patients in our study presented with bilateral chest infiltrates and required ICU care, almost half of them were mechanically ventilated, and 30% developed septic shock. Although these features have been previously recognized to be associated with mortality in severe pneumonia patients, and are often associated with ARDS, the relationship between Legionella and ARDS has been mostly described in case reports. Interestingly, severe forms of Legionella pneumonia associated with ARDS were recorded not only in patients at risk (27-29), but also in previously healthy young adults (30, 31). In our study, majority of ARDS patients had no past medical history.

Experimental studies have shown that in macrophages, *L. pneumophila* inhibits phagolysosome fusion, multiplies and causes lysis of the host cell. Both, in macrophages and alveolar epithelial cells the virulent strain of *L. pneumophila* induces programmed cell death, DNA fragmentation and activation of various caspases (32, 33).

Delay in appropriate antibiotic treatment (34) has been associated with increased mortality in patients with Legionnaires' disease. In our study, patients who developed ARDS had longer time from symptom onset to initiation of appropriate antibiotic treatment although the difference was not statistically significant.

There are several limitations of the present study. Due to retrospective design and the fact that hospital database was used to select patients, it is likely that the frequency of Legionnaires' disease is largely underestimated, resulting in confirmed infections mostly in severely ill patients who underwent detailed microbiological evaluation. Legionella is rarely identified in pneumonia patients since the diagnosis requires the use of specific tests and the isolation is difficult due to organisms' fastidious nature. In addi-

tion, the study includes relatively low number of patients and was underpowered to detect all relevant differences between patients with and without the ARDS.

## Conclusion

In conclusion, in this retrospective study ARDS occurred in one third of patients with microbiologically proven Legionella pneumonia and was associated with prolonged length of ICU stay. Only one patient with ARDS due to Legionella pneumonia died.

**Authors' contributions:** Conception and design: MK and OG; Acquisition, analysis and interpretation of data: MK and GL; Drafting the article: MK; Revising it critically for important intellectual content: GL and OG.

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### References


1. Fraser DW, Tsai TR, Orenstein W, Parkin WE, Beecham HJ, Sharrar RG, et al. Legionnaires' disease: description of an epidemic of pneumonia. N Engl J Med. 1977;297(22):1189-97.
2. Fernandez JA, Lopez P, Orozco D, Merino J. Clinical study of an outbreak of Legionnaire's disease in Alcoy, Southeastern Spain. Eur J Clin Microbiol Infect Dis. 2002;21(10):729-35.
3. Sonder GJ, van den Hoek JA, Bovee LP, Aanhane FE, Worp J, Du Ry van Beest Holle M, et al. Changes in prevention and outbreak management of Legionnaires disease in the Netherlands between two large outbreaks in 1999 and 2006. Euro Surveill. 2008;13(38).
4. Kirrage D, Reynolds G, Smith GE, Olowokure B. Investigation of an outbreak of Legionnaires' disease: Hereford, UK 2003. Respir Med. 2007;101(8):1639-44.
5. Joseph C. New outbreak of legionnaires' disease in the United Kingdom. BMJ. 2002;325(7360):347-8.
6. From the Centers for Disease Control and Prevention. Update: outbreak of Legionnaires' Disease associated with a cruise ship, 1994. JAMA. 1994;272(12):915.
7. Monforte R, Cayla J, Sala M, Estruch R, Vidal J, Plasencia A, et al. Community outbreak of Legionnaires' disease in Barcelona. Lancet. 19896;1(8645):1011.
8. Zumla A, Weyell R, Tettmar RE. Legionnaires' disease: early lessons from 1988 London outbreak. Lancet. 1988;1(8597):1275.
9. Joseph CA, Ricketts KD. Legionnaires disease in Europe 2007-2008. Euro Surveill. 2010;15(8): 19493.
10. Stout JE, Yu VL. Legionellosis. N Engl J Med. 1997;337(10):682-7.
11. Rello J, Bodi M, Mariscal D, Navarro M, Diaz E, Gallego M, et al. Microbiological testing and outcome of patients with severe community-acquired pneumonia. Chest. 2003;123(1):174-80.
12. Sheu CC, Gong M, Zhai R, Bajwa EK, Gallagher DC, Chen F, et al. Infection-Related Acute Respiratory Distress Syndrome: Epidemiology, Microbiology, Risk and Prognostic Factors. Am J Respir Crit Care Med; 2009. p. A4637.
13. Bartlett JG, Dowell SF, Mandell LA, File Jr TM, Musher DM, Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis. 2000;31(2):347-82.
14. Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, et al. The American-European Consensus Conference on ARDS: Definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med. 1994;149(3 I):818-24.
15. England AC, 3rd, Fraser DW, Plikaytis BD, Tsai TF, Storch G, Broome CV. Sporadic legionellosis in the United States: the first thousand cases. Ann Intern Med. 1981;94(2):164-70.
16. Marston BJ, Lipman HB, Breiman RF. Surveillance for Legionnaires' disease. Risk factors for morbidity and mortality. Arch Intern Med. 1994;154(21):2417-22.
17. Mykietiuk A, Carratala J, Fernandez-Sabe N, Dorca J, Verdaguer R, Manresa F, et al. Clinical outcomes for hospitalized patients with Legionella pneumonia in the antigenuria era: the influence of levofloxacin therapy. Clin Infect Dis. 2005;40(6):794-9.
18. Fang GD, Fine M, Orloff J, Arisumi D, Yu VL, Kapoor W, et al. New and emerging etiologies for community-acquired pneumonia with implications for therapy. A prospective multicenter study of 359 cases. Medicine (Baltimore). 1990;69(5):307-16.
19. Moine P, Vercken JB, Chevret S, Chastang C, Gajdos P. Severe community-acquired pneumonia. Etiology, epidemiology, and prognosis factors. French Study Group for Community-Acquired

Pneumonia in the Intensive Care Unit. Chest. 1994;105(5):1487-95.

20. el-Ebiary M, Sarmiento X, Torres A, Nogue S, Mesalles E, Bodi M, et al. Prognostic factors of severe Legionella pneumonia requiring admission to ICU. Am J Respir Crit Care Med. 1997;156(5):1467-72.
21. Benin AL, Benson RF, Besser RE. Trends in legionnaires disease, 1980-1998: declining mortality and new patterns of diagnosis. Clin Infect Dis. 2002;35(9):1039-46.
22. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. N Engl J Med. 2000;342(18):1301-8.
23. Kress JP, Pohlman AS, O'Connor MF, Hall JB. Daily interruption of sedative infusions in critically ill patients undergoing mechanical ventilation. N Engl J Med. 2000;342(20):1471-7.
24. Ely EW, Bennett PA, Bowton DL, Murphy SM, Florance AM, Haponik EF. Large scale implementation of a respiratory therapist-driven protocol for ventilator weaning. Am J Respir Crit Care Med. 1999;159(2):439-46.
25. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345(19):1368-77.
26. Wiedemann HP, Wheeler AP, Bernard GR, Thompson BT, Hayden D, deBoisblanc B, et al. Comparison of two fluid-management strategies in acute lung injury. N Engl J Med. 2006;354(24):2564-75.
27. Chang CC, Chung CL, Huang CL, Wang FC. Legionnaires' disease in a patient with rheumatoid arthritis. J Microbiol Immunol Infect. 2001;34(1):76-8.
28. Kakeya H, Ehara N, Fukushima K, Seki M, Izumikawa K, Yamamoto Y, et al. Severe legionnaires' disease successfully treated using a combination of fluoroquinolone, erythromycin, corticosteroid, and sivelestat. Intern Med. 2008;47(8):773-7.
29. Marques AS, Estrada MH. Legionella pneumonia-a case report. Rev Port Pneumol. 2005;11(2):165-73.
30. Da Broi U, Pasqualucci A, Savron F. ARDS in a severe case of primary pulmonary infection caused by Legionella pneumophila. Minerva Anestesiol. 1993;59(9):455-8.
31. Demello D, Kierol-Andrews L, Scalise PJ. Severe sepsis and acute respiratory distress syndrome from community-acquired legionella pneumonia: case report. Am J Crit Care. 2007;16(3): 317-20.
32. Furugen M, Higa F, Hibiya K, Teruya H, Akamine M, Haranaga S, et al. Legionella pneumophila infection induces programmed cell death, caspase activation, and release of high-mobility group box 1 protein in A549 alveolar epithelial cells: inhibition by methyl prednisolone. Respir Res. 2008;9(1):39.
33. Gao LY, Abu Kwaik Y. Apoptosis in macrophages and alveolar epithelial cells during early stages of infection by Legionella pneumophila and its role in cytopathogenicity. Infect Immun. 1999;67(2):862-70.
34. Heath CH, Grove DI, Looke DF. Delay in appropriate therapy of Legionella pneumonia associated with increased mortality. Eur J Clin Microbiol Infect Dis. 1996;15(4):286-90.

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# Psychological consequences of war-traumatized children and adolescents in Bosnia and Herzegovina

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Research into the psychosocial consequences of war and political violence on children's and adolescent's developmental wellbeing has shown a steady increase over the last decades. Numerous studies, from differing cultures in different war zones around the world, have documented the effect on children of exposure to war atrocities. The war in Bosnia and Herzegovina (BH) 1992-1995, at the end of 20th century found the citizens of BH and the world mental health professionals and scientists unprepared to deal with the adverse consequences for the entire BH population and especially for its most vulnerable part, children and adolescents, to be able to take adequate measures of sufficient mental health care to prevent devastating consequences of severe multiple traumas. Only a few research studies were done during and after this war in BH, the United States, Sweden, Norway, the UK and Germany focusing on the relationship between war trauma, Posttraumatic stress disorder (PTSD), depression, suicidal thoughts, acculturation, repatriation, poverty, behavioral problems, school adjustment, relational problems of children and their mothers after deployment of war PTSD veterans and war prisoners, and treatment of psychological consequences in examined children and adolescents from BH. The major part of this paper reviewed available literature on Medline that reported national and international studies which investigated the psychological consequences of war on BH children and adolescents and several papers about children and adolescents from Srebrenica, that were not indexed on Medline, but showed very crucial results for the issue described.

**Key words:** Children, Adolescents, PTSD, Depression, Bosnia and Herzegovina.

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## Introduction

The investigation of psychosocial consequences in children and adolescents from living through war and political violence has shown a steady increase over the last decades. Numerous studies, from differing cultures in different war zones around the world, have documented the effect on children of exposure to war atrocities (1). Dur-

ing the 1992-1995 war in Bosnia and Herzegovina (BH), the whole social structure in the country was destroyed, many families were shattered, and an individual was often left without adequate family and social system support (2). More than two million Bosnian Muslims were ethnically cleansed in the Balkan region; of these, 200,000 were killed while the others were forced to flee their homes and become refugees. This war in BH has had a tremendous impact on civilians; thousands of inhabitants were left with numerous traumatic experiences (3). Large parts of the population were exposed to extreme threats and intense feelings of helplessness (4). This war has caused a large amount of psychic and social breakdowns. The consequences of the war which caused stress on mental health are of importance, as well as influences on the emotional functioning of individuals caused by changes in the social structure of the population and economic potential of the society (5). After the Dayton Peace Accord was signed in Paris in November 1995 , the war ended the following month. The war in Bosnia lasted approximately 3 and 1/2 years, and although military casualties were high on all sides (6), an estimated 100,000 to 250,000 people were killed and 200,000 were wounded (7), by far the greatest impact of the fighting was on civilians. It is estimated that >1.3 million Bosnians were displaced within the country, more than 800 000 became refugees to other nations, and up to one quarter of 1 million were killed (6), 90% of whom were civilian (8). These long-term traumatic and stressful experiences leave an imprint upon people's inner lives and take a long time to heal (4). Children were the most vulnerable. Many of them suffered or witnessed horrifying acts of violence and aggression (4). It is known that massive exposure to wartime trauma overwhelms most psychological defense mechanisms in children, and that children may mitigate war effects in low-to-moderately intense wartime settings, which allows effective functioning after acclimatization (9). Although young trauma victims are often resilient, many experience mental health difficulties, including PTSD (4) depression and suicidal thoughts (2). Between 25%-30% of Bosnian refugee children lost their fathers in the war in BH (4, 10). Husain et al. (11) reported that two-thirds of 521 Sarajevo children had lost a member of the family, similar to 52.6%-89.6% of children in our previous studies (2, 4). Most children develop strong attachments to their parents (12), which become even stronger in adverse situations. Separation and loss increase the likelihood of various difficulties in children's psychological development. This trauma, in addition to various other types of trauma, explains the high frequency of serious psychological disorders among these children (2).

Whereas there is extensive clinical literature about the developmental challenges facing children and issues of war trauma exposure and postwar adjustment, there is little systematic research about the situation in BH. This review summarizes the findings of the existing publications.

## Exposure to war trauma experiences

The use of violence against civilians in times of war has been one of this century's most alarming military developments, creating increasing numbers of displaced persons and refugees in the wake of regional and tribal conflict. Many refugees were exiled to different European countries, where they faced different acculturation processes (4). 'Loss of place', acute and chronic trauma, family disruption and problems of family reunification have become issues of concern. When BH was thrown into war in 1992-1995, it was clear from the beginning that the extent of the trauma would be enormous and evident for generations to come (13).

The war in BH was characterized by massive displacement, disruption and loss of life,

relatives and property. Health and psychosocial well-being were affected in a number of ways. The findings show that there may be more serious longer-term psychological problems in people who are forced to leave their country during wartime (14) even after the war ended. Due to life-threatening danger BH children and adolescents experienced: "wounding of one's father", "father loss because of killing or missing and loss of immediate or extended family member(s)", "unwillingly leaving home", "separation from family and friends", "forced expulsion from home", "living in collective refugee settlements", "refugee problems and life in a small room in foreign country(ies)". Also, they experienced: "severe fear", shelling and firing very close to children and firing their houses, devastation and ruins" "killing of close relatives and other people", "enemies maltreating father", "running away, hiding and crawling from life threatening danger", "mother's crying and severe sadness", "staying in the concentration camp together with family". Together with their families, during the war in BH children experienced: "non-adequate residence", "living in collective settlements", "non-adequate and non-safe neighborhood", "separation from parent/s", "poor financial means", "extreme poverty and lack of social support" (4, 14). Besides these directly life threatening experiences, children witnessed "dead bodies", "enemy soldiers killing people", "watching people being slaughtered on TV", "wounding and wounded people", "beating and torturing of men and women" (14).

A growing body of research has increased knowledge of the after-effects of trauma in children, including the development of PTSD. Several factors increase children's risk for development of PTSD or PTSD symptoms after trauma. Such information is potentially useful for identifying children who may benefit from more thorough or ongoing assessment after trauma. With regard to assessment, an array of increasingly sophisticated and clinically useful measures is being developed; however, currently there is poor high quality data concerning the diagnostic use of different assessments. A significant amount of evidence indicates that trauma can produce diverse reactions in children, including a general increase in internalizing and externalizing symptoms. Clinically useful measuring instruments allow PTSD to be differentiated from this general reaction to traumatic events, much of which may reflect a non-pathological response (15). Self-report checklists may be useful as a public health measure to assess the prevalence of psychological distress in war affected areas, but they are not an adequate means of clinical screening. Checklists used in combination with other qualitative approaches make it possible to identify those in need and avoid unnecessary pathologization (16). Limited information suggests that PTSD can have a cascading negative effect on children's development and functioning (15). Differences in child exposure during war and exile could be understood in relation to identifiable socio-demographic factors, particularly ethnic background, social class, child age and family size (17). According to most studies, more than half of children exposed to war meet the criteria for PTSD.

Although we know that children are adversely affected by atrocities of war, we do not know which specific war events are associated with children's stress reactions. For example, it is unclear whether differences exist in response to violent versus nonviolent war-trauma experiences (18). Parent and clinician reports show marked group similarities but they often differ with regard to the individual child. Clinician scores reveal a social class gradient not visible on the Harvard Trauma Questionnaire (HTQ) (19, 20). Parent and teenager assessments correlate strongly in total exposure but diverge markedly on specific events. Discrepancy

derives as frequently from events affirmed by teenagers alone as by parents alone. Primary school children, on the other hand, systematically offer a less-detailed account of their own war exposure. In summary, original HTQ functions "quite well" as a standardized questionnaire, but a Bosnian-specific version would expectedly afford greater validity and capture social class differences in child exposure. For teenagers, the value of multiple informants appears evident; for primary school children, a more adequately age-adjusted procedure remains the first priority (21). Alwood et al. (18) examined the relationship of violent and nonviolent war experiences to children's trauma reactions and adjustment in a group of children from the Bosnian capital, Sarajevo. In their sample of 791 children, 41% had clinically significant PTSD symptoms. Children were adversely affected by exposure to both violent and nonviolent war-traumas. They found an additive effect of trauma exposure on trauma reactions; however, many war experiences were not associated with the children's adjustment and trauma reactions. They concluded that additive effects of violence and deprivations during war may overwhelm the coping skills of children and leave them vulnerable to externalizing and internalizing adjustment difficulties and symptoms of PTSD (18). Angel et al. (22), in their study of 99 school-aged Bosnian refugee children living in Sweden, who were analyzed to reveal the patterns of war stress experienced and the relationship between these stressors and their current psychological problems, found that when children had experienced much stress, talking about their experiences seemed to exacerbate the negative effects (22).

Papageorgiou et al. (10) found on the Impact of Event Scale (IES) (23) a significant association between the number of war traumatic experiences and the intrusion and avoidance scores (10). Brennen et al. (24) in their study provided evidence that BH adolescents who were exposed to trauma in childhood have difficulty retrieving specific autobiographical memories (24). To evaluate the psychometric properties of the IES scale in children Dyregrov et al. (25) conducted a study on 1787 children exposed to warfare in Croatia and BH. The study group comprised 877 girls and 910 boys ranging in age from 6 to 15 years. High levels of post-traumatic reactions were found in that group of children. The pattern of endorsement and the factor structure of the IES scale were similar to that found in other samples. As in other studies, both the overall IES score as well as the intrusion and avoidance score were significantly higher in girls than boys. The factor structure for boys and girls were very similar, and the reliability of the scale was adequate across different age groups and for subgroups of displaced and refugee children (25).

In the BH war 1992-95, the citizens of Sarajevo and Srebrenica were exposed to extremely severe war trauma. There are only a few studies that have researched the psychosocial consequences on war traumatized children and adolescents from these two places.

## Sarajevo

The Siege of Sarajevo was the longest siege of a capital city in the history of modern warfare (26). The capital city of BH was besieged from April 5, 1992 to February 29, 1996 during the BH war, with a siege force of 18,000 stationed in the surrounding hills, from which Serb soldiers assaulted the city with weapons that included artillery, mortars, tanks, anti-aircraft guns, heavy machine-guns, multiple rocket launchers, rocket-launched aircraft bombs, and sniper rifles (27). From May 2, 1992, the Serbs blockaded the city. The defense forces of the BH government inside the besieged city were poor-

ly equipped and unable to break the siege. It is estimated that nearly 10,000 people were killed or went missing in the city, including over 1,500 children. An additional 56,000 people were wounded, including nearly 15,000 children. The 1991 census indicates that before the siege the city and its surrounding areas had a population of 525,980. There are estimates that prior to the siege the population in the city proper were 435,000. The current estimates of the number of persons living in Sarajevo range between 300,000 and 380,000 residents (28). Among the children and adolescents there was an increase in neurotic and psychotic disorders in the very beginning of the first year of the war, and a decrease in the same diagnoses during the second year. This might be explained by the particular adaptation of the youngsters to the war conditions. When we are talking about the invalidity of neurological and psychiatric disorders, the investigations showed that illness is the mostly caused by invalidity (85.1%) among the global invalidity during the war in Sarajevo (5). The effect of long-standing siege on children is less researched because of the rare occurrence of siege conditions these days and the difficulties those conditions present for research. Husain et al. (11) did a unique study in that the participants lived in prolonged siege conditions and were deprived of the basic necessities of life for over 1 year. They were exposed to random sniper fire from the hills surrounding the city of Sarajevo, and many lost family members and close friends. In the completely unusual circumstances that the siege of Sarajevo presented at the time of the study, data were collected in February and March 1994 from 791 children studied, aged 7–15 years; the mean age was 11.0 years (SD=2.3) in one school district. They could not determine how their sample differed from children in other school districts in Sarajevo. Data on socioeconomic status were not included because of the widespread deprivation resulting from the war. Of 719 subjects, eighty-five percent of the samples had experienced sniper fire, but there was no significant relationship between experiencing sniper fire and the development of PTSD symptoms. A total of 521 (66%) of the children had lost a member of their immediate or extended family; children who had experienced such a loss had more symptoms of PTSD. Twenty-six percent of our sample experienced food deprivation, 48% experienced clothing deprivation, 29% reported shortage of water, and 10% reported lack of shelter. The children who experienced lack of water and shelter were significantly more likely to manifest avoidance and re-experiencing symptoms than those who did not. Girls reported significantly higher mean scores for avoidance cluster, Impact of Event Scale, and cluster of re-experiencing symptoms than boys. The loss of a family member and deprivation of food, water, and shelter had a severe adverse impact on the children. They identified the needs for food and clothing more frequently than the needs for water and shelter. In general, deprivation was associated with significantly increased symptoms of avoidance and hyper-vigilance. Results of this study imply that proximity to war atrocities and personal losses are highly correlated with the development of symptoms of PTSD (11). To examine risk and protective factors of postwar adjustment among adolescents from Sarajevo who had been exposed to war traumas during the war in Bosnia and Herzegovina Durakovic-Belko et al. (2003) specifically examined differential linkages between PTSD symptoms and depression (as outcomes) and (a) war traumas, (b) individual and socio-environmental factors, and (c) cognitive appraisals and coping mechanisms. Their results of hierarchical multiple regression analyses indicated that the dimensions of war traumas, individual characteristics, and cognitive appraisals and coping mechanisms play a sig-

nificant role in determining who will have more serious PTSD symptoms. Although individual and socio-environmental factors are the strongest predictors of depression, the dimensions of war traumas are also significantly correlated with depressive symptoms. Common risk factors for more serious depression and PTSD symptoms in postwar adjustment were female gender and low optimism. While the strongest predictor of posttraumatic stress reactions (PTSR) was trauma experience in the category of loss, the strongest predictor of depressive symptoms was female gender (29). In their conceptualized an overview of the clinically verified psychological consequences of war-stress exposure in children and adolescents in BH, Daneš and Horvat (30) presented data that related to the city of Sarajevo, as their sample was in a particular way very specific in comparison with other areas of BH. They found that all types of stressors affecting the population could be found there, and had particular significance in the light of the status of the city of Sarajevo as a war target. The delivered data were presented as a comparison of the clinical phenomena during the war and those several years afterwards. It was evident from the data that the war has left psychological consequences in the developing population, primarily in quantitative terms, i.e., in the increase of prevalence of psychological disorders by approximately 15% compared to the pre-war circumstances (30).

## Srebrenica

During the recent BH war, Srebrenica was a United Nations safe haven, but besieged citizens suffered because of a systematic blockade of relief convoys which disrupted access to many foodstuffs (31). Many children, more than 1,000 between October 1992 and May 1993, died there from hunger and disease (32). Children in Srebrenica lived in a horrible environment and each of them was physically and psychologically abused and terrorized by the various disastrous war events. Tahirović (33) found in his study that all children from Srebrenica have had high IES scores and a high degree of distress, consequently this study showed that young children who had suffered the horror of war presented suboptimal growth (33). The darkest moment in the international involvement in Bosnia was the fall of the Bosniak-populated, eastern Bosnian enclave of Srebrenica on July 11$^{th}$, 1995. It became a significant event that fundamentally changed the conflict dynamics and enhanced the prospect for peace (7). On that day about 15,000 people attempted to walk from the enclave of Srebrenica to free territory in Bosnia. Two-thirds were captured or killed. Many of the remainder experienced hallucinations on the march, leading them to believe they were the victims of chemical weapons. After the literature on the likeliest CW agent, 3-quinuclidinyl benzilate (BZ), and on stress as a cause of hallucinations was reviewed, CW exposure could not be ruled out. It was concluded that the hallucinations can be ascribed to the consequence of multiple stress: artillery attacks, exhaustion due to lack of sleep, starvation, thirst and the effects of drinking unpurified water (34). Despite the fact that Srebrenica was a United Nations (UN) safe zone, the murder of 7,000-8,000 Bosniak men was not prevented. Some were killed after surrendering, believing the UN would protect them. Others were hunted down while attempting to escape into Bosnian government-held territory. Some committed suicide, unable to endure the harrowing trek to safe ground (7). Hasanović et al. (14, 36) in their study, analyzed acculturation and psychological characteristics of Bosnian refugee and internally displaced adolescents who survived the Bosnian war and the Srebrenica enclave disaster. All of them survived a very difficult

humanitarian disaster, being besieged without a normal life together with the threat of war they were exposed to together with their families. After separation from the men, adolescents who participated in this study reported that they were transported in crowded trucks and buses together with their mothers, very old men and other children to Dubrave airport close to Tuzla town. Among the more than 15, 000 displaced persons arriving from the Srebrenica enclave, the field emergency services found various pathologies in these exhausted persons. Among children there was a predominance of cases of respiratory disease and untreated skin infections (35). As Srebrenica is one of five regions of BH previously identified as iodine deficient, continual prophylaxis was realized with iodized salt over the twenty years prior to the war in the former Yugoslavia. Thus, Tahirović et al. (31) investigated the current state of iodine nutrition in Srebrenica children who had undergone the siege, and found that urinary iodine concentrations were significantly lower than those for controls from Tuzla. The obvious cause was the interruption of the availability of iodized salt by the blockade of relief convoys while Srebrenica was a United Nations safe haven (31). During the research (14, 36) in May 1999, some of the adolescents were in the process of repatriation to their home country after a certain period of life in exile (1995-1998), and others from the investigated group stayed during and after the war in BH as internally displaced persons, without the possibility of returning to their place of origin despite the end of the war. Adolescents from Srebrenica were compared with their peers from Janja and Bijeljina (Semberija plain), who survived different catastrophic war conditions at the very beginning; and some were exiled (1992-1998) and some were internally displaced, but did not go through the Srebrenica disaster (14, 36). This study showed that in both groups, participants who had been internally displaced or/and refugee adolescents had been exposed to severe war trauma and had reported high levels of traumatization more than three years after the war. The prevalence and severity of trauma experiences were significantly related to their region of residence, with adolescents from the region of Srebrenica having a significantly higher prevalence and severity of experiences. We found that adolescents from Srebrenica, after surviving the initial war experiences in 1992, stayed in their homeland significantly more and survived additional trauma exposure to war catastrophes as internally displaced persons in the so called "free territory", while a significantly lower number of adolescents from the same region were exiled from BH during and after the war, compared with their peers from Semberija. Being forcedly expelled from private property and homes, 56.6% of Srebrenica participants were resettled as displaced or exiled persons in collective settlements significantly more than those from Semberija. Srebrenica participants survived significantly more war related catastrophic experiences, while participants from Semberija reported significantly more frequently trauma experiences related to the process of exile and repatriation. In the Srebrenica group the severity of traumatic experiences was not related to age and gender, but was related to refugee status and father loss. The internally displaced and those who lost their father reported significantly more severe trauma experiences. In the Semberija group, the severity of trauma experiences did not relate to refugee status or father loss. Being out of the war did not help exiled adolescents to diminish the severity of many trauma experiences. Girls and older participants had significantly more severe trauma experiences similar to other studies (2, 4, 11,14).

Amongst 143 participants from the Srebrenica region 36.4% experienced loss of their father which is significantly more than

those from Semberija, IDP experienced the loss of their father much more than refugees; also, 89.4% of 143 Srebrenica adolescents experienced separation from family and friends, which is significantly more than their peers from Semberija. Adolescents from Srebrenica were significantly less active, less sociable, in other words they were more introverted (14, 36). Psychological trauma, physical injury and low socioeconomic status provoked by the events of war delayed menarchal age among girls who survived the Srebrenica disaster (37).

## Acculturation

As stated previously, together with adults, many children witnessed or experienced traumatic events, and many families were forced to leave their homes. Many found refuge in European countries, where they faced different acculturation processes (4). There are only two studies that have investigated the acculturation problems of BH refuges (4, 14).

Hasanović et al. (4) found returnees reported significantly fewer acculturation problems, such as: "thought about native place after arriving in refugee/displaced environment," "wanted to return to homeland/hometown," "was sad after arriving in refugee/displaced environment," "listened to news from homeland/hometown," "had digestion problems after being exiled/displaced," and "missed friends during exile/displacement". Younger returnees significantly more often "thought about hometown after arriving in a foreign country" than older ones. Older non-repatriated adolescents had "digestion problems in displacement" significantly more often than younger ones. Older returnees and older internally displaced pupils "were forbidden to play outside" significantly more than younger ones. Internally displaced girls were significantly more often "sad after arriving in the place of displacement" than boys. Repatriated girls were significantly more often "forbidden to play outside in place of exile" than boys. Repatriated boys were "prevented from accepting the new environment" significantly more often than girls. Internally displaced girls were significantly more often prevented from accepting their new environment (milieu) than boys. Returnees who had lost their father were significantly more often "Sad after arriving in the place of exile" than those who had both parents. They experienced "sleeping disturbances" more often than those who had complete families. Internally displaced adolescents in BH who had lost their fathers had significantly more "sleeping disturbances" and "digestion problems" than those with both parents. On the other hand, returnees who had both parents "were forbidden to play outside" significantly more often than those who had lost a father. Internally displaced adolescents who had lost a father wanted to "return to homeland" significantly more often than those who had both parents. During exile, returnees who lost their father "listened to news from their home country" significantly more often than their peers who did not lose their father (4). In the other study regarding acculturation problems, participants from Srebrenica reported significantly more that they: "missed friends during exile/displacement", "wanted to return to native place/homeland", "listened to news from hometown/homeland" and "had digestion problems after being exiled/displaced". Acculturation problems were highly correlated with occurrence of school-emotional/behavioral problems (14, 36).

## Repatriation

In 1995, after the end of the war in Bosnia, a number of refugee families were repatriated, often against their whish, and returned to a completely changed, unsafe, and devastated environment. During this process, children

were exposed to further stress caused by dislocation and the process of acculturation. This has left long-term emotional scars and mental health problems, including PTSD, in children and the adolescent population. In the study of Hasanović et al. (4) on 120 refuge adolescents who returned to their homeland, but not to their own prewar property, after return to their home country, 79% returnees had no social support; 58% of them had unemployed parents; 10% were living in extreme poverty and only 10.8% had properly resolved health care insurance. For 87% of returnees, the school system was completely different than in the exile country, and for 77% their economic situation was worse than in exile, 73% preferred watching exile country programs on satellite TV, and 48% were sad after return. Older returnees reported significantly more often "a different school system in home country than in country of exile". They also reported significantly more "missing being with their old friends," whereas younger returnees reported significantly more "communication problems with friends, relatives and neighbors" and "digestion difficulties". Returnee girls reported significantly more "a different school system in the home country than in the country of exile," and "missing being with old friends," whereas returnee boys reported difficulties with school studies more often after their return. Returnees who had both parents, reported significantly more "worsening of economic situation than before exile" and "missing being with old friends," whereas returnees who had lost their father reported significantly more "difficulties with teacher/s" (4). In the study about Srebrenica and Semberija adolescents (14, 36) according to difficulties experienced during repatriation, Semberija students reported significantly more: "economic situation is worse than before the exile", "preferring to watch programs from exile country on satellite TV" and "missed meeting with friends from pre-war period". In both groups the participant were "sad after return" 45-48% with no significant difference between the groups. Returnees from the Srebrenica group who "listened to news from their home country" and who "missed friends in exile" had more "sleeping difficulties" after return to their home country. Those who "were thinking about relatives" in exile were more sad in their home country and had more "sleeping disturbances" after return. Those returnees who preferred to "watch exile country programs on satellite TV" in home country, had less "thinking about native place in exile". Semberija returnees who preferred to "watch exile country programs on satellite TV" in home country, had less sadness during exile, less "sleeping difficulties" in exile, less "digestive problems", they "missed friend(s) from home country" less during exile, wanted to "return to home country" less and "listened to news from home country" less during exile. Adolescents who stayed in their home country during the whole war and postwar period were faced with different acculturation processes in the new environments but in the same culture and their mother tongue. They, together with other family members remained exposed to continual life-threatening situations, sometimes very often. They had more problems to adapt to their new life environment than those who were in exile, probably because they experienced more discomfort and life threatening conditions in the war zone of their own homeland (14, 36). The process of acculturation after returning to one's home country is a long-lasting and very complex process in view of all the emotional, socio-political and financial obstacles they had to face and go through with minimal harm. The findings of this survey of Bosnia-Herzegovina internally displaced and refugee adolescents living in post war conditions after surviving fighting, expulsion from home, displace-

ment, exile, acculturation, repatriation, loss of fathers, loss of close family members, loss of property, lack of safety and normal conditions for life suggest that today and in the future the impact of the war was both severe and widespread (14, 36).

## Post-traumatic stress disorder

As part of a United Nations Children's Fund (UNICEF) psychosocial program during the war in BH, data were collected from a community sample of 2,976 children aged between 9 and 14 years. Children completed standardized self-report measures of posttraumatic stress symptoms, depression, anxiety, and grief, as well as a report of the amount of their own exposure to war-related violence. Results showed that children reported high levels of posttraumatic stress symptoms and grief reactions. However, their self-reported levels of depression and anxiety were not raised. Levels of distress were related to children's amount and type of exposure. Girls reported more distress than boys, but there were few meaningful age effects within the age band studied (37).

According to previously established cut-off scores on self-report measures, in a sample of 95 children of 8-13 years, who had experienced the war in Bosnia 65 (28%), scored within the clinical range on the IES measuring PTSD reactions (11). The level severity of re-experience cluster symptoms, were significantly higher among adolescents from Srebrenica. The prevalence of PTSD in the Srebrenica group (67.8%) was significantly higher than 52.4% in the Semberija group of respondents. PTSD prevalence was higher in both groups than in other studies (14, 35). Eytan et al. in their study about Albanian Kosovar found out 23.5% PTSD prevalence (38), but it was less than 94% in 364 IDP children from central Bosnia in the study of Goldstein et al. (6). In the study on 186 children/adolescents (2) PTSD was present in 51.6% of them, with the highest prevalence among children in the non-governmental Children's Village. PTSD prevalence was higher among children who lost a parent but lived with the surviving parent than among children in the governmental orphanage or children living with both parents. Loss of a parent was associated with higher frequency of PTSD. The loss of both parents was associated with high prevalence of PTSD. Prevalence of PTSD was positively correlated with the prevalence of depression. The children/adolescents who survived war traumas but were living with both parents reported the lowest rate of psychological disturbances (2). In the study of IDP and returnees (4) the whole sample, 149 (62%) of 239 adolescents reported symptoms that met DSM-IV criteria for PTSD; the repatriated group had 57% of 120 and non-repatriated group had 68% of 119 participants with PTSD. The prevalence of PTSD was significantly greater among older returnees than among younger ones. PTSD prevalence among younger returnee boys was significantly higher than among their girl peers, whereas PTSD prevalence among older returnee girls was significantly higher than among boys. Internally displaced adolescent girls had significantly greater prevalence of PTSD than boys. Father loss had no substantial influence on the prevalence of PTSD among returnees, either in the whole group or in age subgroups. However, more internally displaced adolescents who had lost their father had PTSD both in the whole group, and in age subgroups (4). Hasanović et al. (29) in their study about adolescents from rural (Teočak) and urban (Tuzla) settings found that adolescents experienced an extremely high number of traumatic events, as described elsewhere (2, 4). Of 366 tested adolescents, 40.4% reported symptoms that met PTSD criteria. Adolescents from the rural area demonstrated PTSD significantly more frequently 53.9% than their urban

peers 25.4%. We found that significant predictor variables for development of PTSD symptoms were rural settings, being female, actual lack of money in family, experiences of coldness and lack of food during the war. The prevalence of PTSD was higher amongst adolescents from the rural areas. In rural areas age and gender did not influence the prevalence of PTSD, but in urban areas girls and older participants presented significantly more frequently with PTSD. Adolescents who experienced forced expulsion from their homes, home destruction, shelling, firing, and killing of close family member(s) reported significantly more PTSD. Adolescents who developed PTSD expressed a higher level of desire not to be born in BH and a lack of prospects in BH (39). Furthermore, rural adolescents had significantly higher scores on individual PTSD items. Adolescents who grow up in vulnerable families may have different difficulties (2, 4, 9). In this study we found that between 28-62% of adolescents lost close family member(s) in the 1992-1995 war in BH. This was similar to previous studies about BH children and adolescents (2, 4, 10, 11, 14, 39). In this study, in the rural area, more than half the adolescents lost close family member(s). This one event, in association with other reported traumas, may explain such a high frequency of serious psychological disorders among rural adolescents. It is possible that these children continue to live in vulnerable families whose members have not yet finished the mourning process (2, 10, 11, 40).

## Depression and suicidal thoughts

There are only a few studies that have investigated depressiveness and suicidal thoughts in the period after the BH war amongst children and adolescents who experienced war. One of these was the study of Papageorgiou et al. (2000), who according to previously established cut-off scores on self-report measures, found 45 children (47%) scored within the clinical range on the Depression Self-Rating Scale for Children in a sample of 95 children of 8-13 years, who had experienced the war in Bosnia (10).

Among orphan children and adolescents in the study of Hasanović et al. (11) all participants experienced war trauma, many had psychological consequences, and depression was presented in 42 of 186 (22.6%) children, but with no statistical difference among the groups. The prevalence of depression was similar in boys and girls. Loss of a parent was associated with a higher frequency of depression. The loss of both parents was not associated with a high prevalence of depression. The highest prevalence of depression, often comorbid with PTSD, was found among children who lost one or both parents. The children with the lowest rate of psychological disturbances were those living with both parents (2). In the study about rural and urban adolescents, the rural group had statistically more depression symptoms than their peers from the urban region.

68 of 366 (18.6%) tested adolescents met the Children's Depression Inventory (CDI) criteria for depression. There was no difference between rural and urban adolescents in the frequency of depression. The number of adolescents who reported suicidal thoughts but would not commit suicide was higher in the rural group (40.9%) than in the urban group (27.8%). Amongst those who would kill themselves there was no difference (15.0%) in rural and (15.6%) in urban areas.

Forcible expulsion from home, home destruction, separation from family longer than three weeks, lack of money for everyday needs, worse financial situation than before the war and extreme poverty increased the prevalence of depression. Those who developed depression expressed a desire not to be born in BH and an opinion of having no prospects in BH. There was a positive as-

sociation between age, frequency of trauma experiences, PTSD, depression, hopelessness, and suicidal thoughts (40). In the study about Srebrenica/Semberija adolescents, we found significantly more severe anxious/depressed behavior, withdrawn/depressed behavior, and significantly more severe internalizing problems in the Srebrenica group. They had significantly more severe thought problems and attention problems. In the Srebrenica group IDP adolescents had more anxious/depressed behavior, withdrawn/depressed behavior and internalizing behavior than refuges. Girls were more withdrawn/depressed than boys. In the Srebrenica group, participants who met new friends had somatic complaints, those who continued education with their peers had less anxious/depressed behavior. Participants who had "learning difficulties" after return had withdrawal/depression problems, but reported more somatic complaints (14, 36, 38).

## Behavioral problems

In their study, Hasanović et al. (14) found that in the Srebrenica group IDP adolescents had more social problems, thought problems, attention problems, "other problems" and "everything listed - total problems" compared with refugees. Girls had more thought problems, while boys had more aggressive behavior. Older Srebrenica participants had more of all the school behavioral problems, except attention problems, aggressive behavior and externalizing behavior than younger participants. Participants who lost fathers had more attention problems, but externalizing problems than those who had both parents. Participants who developed PTSD had more thought problems and had less aggressive behavior. Participants who smoke cigarettes had more attention problems. Participants with withdrawn/depressed behavior, somatic complains, internalizing behavior, social problems, thought problems, attention problems and with "total problems" misused illicit drugs more than those who did not have contact with it. Semberija participants smoked cigarettes more than Srebrenica ones. Srebrenica participants who lost fathers misuse illicit drugs, smoke cigarettes and drink alcohol more than those who have both parents. Among Srebrenica adolescents those who reported "sleeping disturbances, alimentary problems, prevented from playing outside in the refugee environment and acceptance of new environment" reported the most school/behavioral problems reported by school teachers. Only "listening to news from homeland" highly negatively correlated with the aggressive behavior of these participants. Those who had trouble with teachers had more social and "other problems" (14, 36). With the hypothesis that school bullying was more frequent in Stolac than in Posušje, due to the greater level of aggression in children caused by recent war events Cerni Obrdalj and Rumboldt (2008) compared the prevalence and characteristics of bullying between these two towns in BH: Stolac, which was exposed to firearm conflict and Posušje, which was outside the active combat zone during the 1992-1995 war in BH. They found that every sixth pupil (16.4%) experienced at least one form of bullying almost every day, while 34 (7.0%) pupils constantly bullied other children. Sixth-eighth graders were more often bullies than 4th-5th graders. Girls were most often victims of bullying, while boys were most often bullies. The expected difference in bullying between the two towns was not observed, except for older pupils in Posušje, who were more violent than their peers in Stolac. Among the analyzed variables of sex, age, town, and school achievement, only male sex was a significant predictor of bullying, increasing the relative risk by 3,005 times. They concluded that bullying among primary school pupils did not differ between areas that experienced war activities in 1992-

1995 (41). Their results differed in relation to the results of a study conducted in Croatia, which showed that primary school children from a town exposed to war perceived themselves as more aggressive in their early adolescence than their peers from a town that was not exposed to war (42). Zalihić et al. (43) reported that children of fathers who had PTSD, experienced stomach pain, eating problems and breathing problems more often than children of fathers who did not have PTSD. Children of fathers with PTSD were more easily upset and worried more often; they were also more aggressive towards other children. The group of children of no PTSD fathers who wanted to help with the house work was larger than the group of children whose father(s) had PTSD (43).

## Military and concentration camp related PTSD in war veterans and war prisoners and family relationships

Almost all children and adolescents and their families experienced different numerous personal trauma experiences and the stress of the deployment of a family member(s) during the recent 1992-1995 war in BH. Some veterans developed post-traumatic stress disorder as a consequence of their experiences. This condition drives many of the adverse changes in the families of returning veterans, through the effects on intimacy and nurturance in their families of withdrawal, numbing and irritability that are components of PTSD. War related PTSD, as well as the intimate relationship problems that accompany the disorder, can influence the course of veterans' trauma recovery. This became the targeted interest of research. The association between PTSD and intimate relationship problems confirmed (44). The surviving family members may have war-related PTSD and depression, thus are unable to create a protective atmosphere for the child's development (2). It is known that the children of depressed parents are at higher risk of psychopathology (2, 45). Zalihić et al. (43) analyzed the frequency of depression and anxiety and children's behavior in families where the head of the family (father) suffered from military related PTSD, in participants living in Mostar. They found that a significantly larger number of women, whose husbands had PTSD, were depressed, unlike women whose husbands were not ill. There was no significant difference in depression manifestation in a group of children older than 18, as well as in the behavior of a group of children younger than 18, but significant differences were found in some answers provided, that indicate differences between the no-PTSD and PTSD groups (42). Among 209 displaced women attending a Women's Center in a war zone in BH in 1994, who had survived the most severe traumas (concentration camps or other kinds of detention) the proportion of PTSD cases was highest: 71% compared to 47% of the women without this kind of traumatic background. High numbers of traumas, having children, being over 25 years of age, and the reporting of an absent husband, were characteristics associated with being a PTSD-case. In the multivariate analysis, severe trauma and reporting of an absent husband remained significantly associated with PTSD-cases (46). In terms of the satisfaction of children in BH whose parents suffered from PTSD, there was a distinction in contacts with their families, relatives, schoolmates and formal contacts. For children of parents who have symptoms of PTSD, the most important persons that they communicate with were schoolmates and they had problems communicating with fathers and males (47, 48). There is the more general challenge that all families and children face when a partner/parent deploys of role ambiguity consequent on anxiety that is provoked by the threat that deployed family members experience.

A study of Kuwaiti military showed that mothers' anxiety had the greatest impact on the children of deployed fathers, although absence of posttraumatic stress disorder in mothers could mitigate the effects of their fathers' posttraumatic stress disorder (49). As part of a UNICEF-sponsored Psychosocial Program in Bosnia, data were collected from a representative sample of 339 children aged 9-14 years, their mothers and teachers in order to investigate risk and moderating factors in children's psychological reactions to war. Self-reported data from children revealed high levels of post-traumatic stress symptoms and grief reactions, but normal levels of depression and anxiety. The mothers' self-reports also indicated high levels of post-traumatic stress reactions, but normal levels of depression and anxiety. Child distress was related to both their level of exposure and to maternal reactions. There is also a substantive association between maternal mental health and children's adjustment following the war (50).

Prevalence rates are also likely to be affected by issues related to the course of PTSD, chronicity, and comorbidity; symptom overlap with other psychiatric disorders; and sociopolitical and cultural factors that may vary over time and by nation. The disorder represents a significant and costly illness to veterans, their families, and society as a whole (51). Green et al. (52) evaluated the association of resilience and trauma exposure with the main effects of PTSD revealed from combat exposure, lifetime trauma exposure, and resilience. They found a significant interaction between combat exposure and resilience in that higher levels of resilience were particularly protective among individuals with high combat exposure. After controlling for age, gender, minority status, trauma exposure and PTSD diagnosis, resilience was uniquely associated with decreased suicidality, reduced alcohol problems, lower depressive symptom severity, and fewer current health complaints and lifetime and past-year medical problems. These results suggest that resilience is a construct that may play a unique role in the occurrence of PTSD and the severity of other functional correlates among deployed veterans (52). Berk found that those individuals who were not able to incorporate the resilience-promoting factors may have been able to cope in the short term with the immediate threat, but then had difficulties following the cessation of the armed conflict. Over the course of his subsequent visits to Bosnia, Berk found a higher rate of alcoholism and suicide among teenagers than anticipated. They had apparently survived the most imminent threat and then succumbed to long-term stress. Some of these problems were due to the demobilization from the army of those teenagers who had been in the military. They were now left with no defined role after having had an important one, which had given them considerable power. The demobilization of their fathers caused problems as well. Friction between the parents arose as the father took authority back from the mother who had been in charge of the family in the father's absence. He also found considerable turnover among humanitarian aid workers. Those who had been able to stay on the job and be effective in the long run were those who had truly internalized resilience strategies (53).

Amongst 149 male survivors from "ethnic cleansing" camps in Bosnia during 1992 who were examined within two weeks from arriving in Sweden as refugees, Björn et al. (54) found that they had an extremely traumatic background: 76% cent had a history of physical abuse, 17% had survived systematical physical torture and 89% had undergone psychic torture, but few serious somatic problems were found. The most important factor for their present well-being was if their family and other relatives had been reunited and if they knew what had happened

to other members of their family. The authors suggested that by early identification of a high risk for developing PTSD and by focusing the general risk that everyone in the investigated group faced to develop this symptomatology, relevant contact could be established before severe symptoms developed (54).

Despite the mental health service reform performed in BH, in the treatment of war traumatized veterans, their spouses and children today, important obstacles are still present today on both social and political levels in the organization of the health care system in postwar BH. The stigmatization of mental health issues is an important problem in the treatment of traumatized individuals, especially among war veterans. The lack a single Center for psycho trauma in postwar BH shows the absence of political will in BH to resolve the problem of war veterans with trauma related psychological disorders (55).

## Poverty

It is known that often the direct effects of war trauma tend to be more easily seen and investigated than indirect effects, such as poverty. Poverty has been described as an economic state that does not allow the provision of the family's and children's basic needs, such as adequate food, clothing, and housing. General poverty often exerts the strongest impact on children (56, 57), followed by school- and health-related risks. Poor children are particularly vulnerable to the effects of poverty because of the environment they live in. Investigations among the refugee camps and the population of Sarajevo shows that "life equipment" among the displaced persons was lower than in local people. That shows that after the phase of surviving, this part of the population was at risk of many psycho-social problems. Also, the very low socio-economical level of the inhabitants of Sarajevo leads to the potential of absolutely poverty. The indicators of this trend are: low level of education, very low living standard, unemployment, bad health conditions etc. Among refugees all these indicators are worse. Criminality in Sarajevo increased during the war, particularly among adolescents (5). Lack of money for essential life needs, a worse financial situation compared to the prewar period, extreme poverty, lack of income and parental unemployment increased PTSD prevalence (42). Adolescents from rural areas more frequently reported a more difficult financial situation currently than before the war, the situation of no salary in the family at all, parent(s) who lost their employment after the war, unemployed mothers, and no health insurance in comparison to their peers from urban areas. Adolescents from rural areas reported more frequent traumas due to war circumstances and more destruction of material and human resources than their peers from urban areas (42).

## School adjustment

After the war, many multinational communities witnessed ethnic segregation, which was particularly visible in schools ("two schools under one roof"). In many such schools, Bosniak and Croatian children, as well as their teachers, have no mutual contact. Pupils often enter these schools through different entrances, take separate breaks, and the teachers have segregated common-rooms (41). Hasanović et al. (3) found in the research that the severity of PTSD symptoms and cluster symptoms of re-experience and avoidance were significantly reduced amongst students where the members of the School project team worked in compliance with their plan and program of psycho-social help to lessen prevailing prejudices and acceptance of differences as well as lessening aggressive features among children by

connecting children, parents, teachers and school management in both entities, as well as the establishment of Students' clubs as a legal part of the schools in which they work. This can be explained by the fact that the war in BH destroyed interpersonal relationships between citizens in terms of ethnical affiliation , so the traditional trust and confidence that were cultivated amongst neighborhoods had vanished. The current school system based on ethnical school programs has differing points of view on the history of the BH war; the issue of who is guilty for the war increases mistrust and gaps in interethnic dialogue. This comprehensive project involved students, their teachers and school directors and particularly their parents spending a certain amount of time together, it helped them to go over the artificial and tragic ethnical division and enmity, and to reestablish trust and confidence, which help in decreasing the severity of PTSD symptoms (3). On the other hand, age, frequency of trauma experiences, PTSD, depression, hopelessness and suicidal thoughts were negatively associated with the level of the academic achievements of the study participants (40).

## Treatment or how to help to war traumatized children and adolescents

The war in Bosnia was the first time the United Nations had been faced with mass rape as a weapon of war. In fact, many of the estimated 20,000 women and men who were raped or sexually abused during the war, often by gangs and often repeatedly over months, have not seen a doctor or any sort of mental health expert since. Bosnia's gynecologists in Sarajevo worked without electricity, anesthetics, or oxygen and with only a skeleton staff, to keep delivering babies at Sarajevo's University Hospital while the city was under siege. The number of pregnancies dropped dramatically, and the numbers asking for abortions rose. Then in the late summer of 1992, some months after the war began, the women who had been raped started to appear. Most of them came alone, at night, so no one would see them. They were silent and full of shame and hatred. Often while they were treated, they would not speak. Some asked for abortions. Others gave birth and then rejected the child (53). The majority of BH residents were exposed to cumulative traumatic events during the 1992-1995 war, which demanded emergency organization of psychosocial support as well as psychiatric-psychological treatment of psycho-traumatized individuals (58). At the beginning of the war (1992) knowledge about the psychological consequences of war and therapeutic approaches to PTSD in BH was rather poor. The therapeutic approach was based on the experience of psychiatrists and their receptiveness to the ideas suggested by foreign literature and the many foreign workers (59, 60). At the end of the war, various psychosocial programs were organized by the government and international nongovernmental organizations (61, 62).

In Bosnia and Herzegovina today there are no children who experienced the war 1992-1995, because they have grown up. Some of them are still adolescents now who need our competences to help those who have mental health problems. During the war, because of the life threatening circumstances, the first task was to survive successfully. Those who stayed in BH in their original homes or who were internally displaced persons met the same problems; the lack of an organized structure for the mental health care of citizens and human resources adequate and competent to organize a sufficient network for mental health care in war conditions. Practically, mental health care was organized in psychiatry departments in existing BH clinical centers and only by a few nongovernmental organizations (NGO). The psychosocial approach to trauma aimed

to reduce not only the risk of serious mental disorders but also stigma, through mass education about the psychological consequences of trauma. The therapy methods applied have had a significant effect in increasing children's willingness to take part in the dialogue, in increasing the interest to work in a group and a greater contribution from each one within the group. It has been confirmed that the effect of methodical procedure and therapy treatment at children is successful and applicable in future activities (63). Working with traumatized people during the war, we perceived that religious people coped more successfully with difficulties than those who were not religious. In selected cases, spirituality and religion are therefore used in the process of healing, and so they have found their place in educational programs and psychotherapeutic treatment. In hospitals, appropriate rooms were allocated for the spiritual and religious needs of patients (64).

For refugees there were some attempts made in the different countries where they were living. We have some evidence in scientific literature. Snyder et al. (65) focused on the influence of societal and cultural values coupled with wartime experiences on the transition of Bosnian refugee families to their new countries. They suggested that social workers can use culturally competent theoretical frameworks and practice principles to assist Bosnian Muslim children and families in their adaptation process within their resettlement communities (65).

Brunvatne et al. (66) stated that Bosnian "war refugees" who sought shelter in Norway after the first two years of the war in Bosnia (1992-93) had experienced extreme traumas, qualifying them for help from expert psychotherapists, but no such help was available. Most of them were former prisoners from Serbian concentration camps, together with their families. Authors investigated whether useful psychosocial preventive work could be done within the primary health services during a six month period in 1993-94. They concluded that all the refugees reported that they experienced deep sorrow, and the majority had difficulty in sleeping and concentrating. A pessimistic view of their future was common. Most of the refugees were positive towards participating in the interviews. Several of them reported that the interviews were of direct help. In the majority of cases the authors have experienced that ordinary health contacts later were meaningful (66).

After assessing war violence exposure and behavioral symptoms in 31 Bosnian refugee children in 1996 at the International Clinic of Boston Medical Center, in Massachusetts and behavioral screening of refugees during the Refugee Health Assessment (RHA), the state's largest contracted provider of the RHA, Geltman et al. (67) emphasized that children were also offered referrals to appropriate mental health services. Authors found that 68% experienced long-term separation from a parent, 81% were directly exposed to armed combat, 71% experienced the death of a close friend or relative, 52% experienced economic deprivation. Families reported behavioral symptoms in 77% of children. Only one family expressed an interest in psychosocial services of any kind. Finally they concluded that large numbers of Bosnian refugees were likely to have experienced traumatic war violence and are at risk of behavioral symptoms, but the RHA affords opportunities to screen for behavioral problems but not intervention. Additionally they recommended that primary care providers and other clinicians should be aware of likely recurrences of symptoms in high-risk children such as these (67).

In their review article, Ehntholt and Yule (68) found that young refugees were frequently subjected to multiple traumatic events and severe losses, as well as ongoing stressors within the host country. Although

young refugees are often resilient, many experience mental health difficulties, including PTSD, depression, anxiety and grief. They underlined that an awareness of relevant risk and protective factors is important, and that a phased model of intervention is often useful, as well as the need for a holistic approach crucial. Promising treatments for alleviating symptoms of war-related PTSD include cognitive behavioral treatment (CBT), testimonial psychotherapy, narrative exposure therapy (NET) and eye movement desensitization and reprocessing (EMDR). Knowledge of the particular needs of unaccompanied asylum-seeking children (UASC), working with interpreters, cross-cultural differences, medico-legal report writing and the importance of clinician self-care is also necessary (68).

Murray et al. (69) in their article focuses on the mental and behavioral health component of services for refugee youth, suggesting that the diverse clinical presentation of refugee children and adolescents after their traumatic experiences requires a treatment model that can mitigate a number of internalizing and externalizing symptoms. Refugee populations also require interventions that can adjust to the wide-ranging experiences likely encountered during preflight, flight, and resettlement. There is some evidence that immigration stressors or social stressors, such as discrimination, are associated with symptoms of posttraumatic stress disorder in refugee youth. Therefore refugee youth may benefit from multiple levels of services ideally integrated (69).

After a year's experience (from August 1997 until July 1998) of being psychotherapist supervisor and trainer in Bosnia, Wintsch (70) declared that even though it was sometimes really difficult, looking back it seems to him that it was a very meaningful and helpful work in the service of children and adults in order to provide new hope and to help them to cope with their war trauma. As he had really good results, he wanted to encourage other psychotherapists and psychiatrists to dare to contribute their skills in similar work. He emphasized all general conditions and the need for building up sufficient therapeutic and training project conditions with local partners in a poor, neglected and remote area, to build up trust; work on trauma; training for local professionals; results, evaluation and continuation of eventual project(s) (70).

## Conclusion

The young BH population who survived the 1992-1995 war is a multi-traumatized group. Traumatic symptoms are related to harm avoidant personality traits. Certain war experiences were also associated with greater symptomatology. The findings show that there may be more serious longer-term psychological problems in young people who were forced to leave their property, original homes and country during wartime, and who lost fathers and/or other family members. There were regional variations in warfare. The most difficult war trauma was in surviving children and adolescents from Sarajevo and Srebrenica. The adolescents examined revealed a variety of serious psychological manifestations, including PTSD, depression, suicidal thoughts, somatic and behavioral problems, that influenced their academic achievements. Children who had fled their homes together or without complete families were likely to have had particularly harsh war experiences, which were additionally complicated by the processes of acculturation and repatriation and lack of basic means for life. The wide spread the fighting and the large numbers of families displaced by the war suggests that the findings from these studies are likely to reflect the experience of a large proportion of the young Bosnian population. This might be considered as a general characteristic of a

society in war. The treatment of war-traumatized children and adolescents was rare, insufficient and of differing characteristics, depending on where the children and adolescents were settled after surviving trauma, in their homeland or in a foreign country. In the organizing of the health care system in postwar BH, meaningful obstacles are still present today on both the social and political levels, despite the mental health service reform performed in BH. The stigmatization of mental health issues is an important problem in the treatment of traumatized individuals, especially among war veterans, their spouses and children. The lack of a single Center for psycho trauma in postwar BH shows the absence of political will in BH to resolve the problem of war veterans with trauma related psychological disorders. Psychosocial work with elementary and secondary school students, giving them psychosocial support within the School Project resulted in a significant reduction of PTSD symptom severity. More research is required to expand our limited knowledge base and improve our understanding of disorder prevalence, as well as associated information on course, phenomenology. More research is also needed to delineate the factors that reflect a risk for PTSD after trauma, factors that reflect the consequences of PTSD, and mediating variables. Future studies in this area would benefit from a prospective design, the evaluation of possible protective processes (e.g. social support), and specific examination of particular aspects of resilience and how resilience may be increased. This may help in establishing a network of resources for adequate treatment, and decreased economic costs.

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## References

1. Smith P, Perrin S, Yule W, Rabe-Hesketh S. War exposure and maternal reactions in the psychological adjustment of children from Bosnia-Hercegovina. J Child Psychol Psychiatry. 2001;42(3):395-404.
2. Hasanović M, Sinanović O, Selimbašić Z, Pajević I, Avdibegović E. Psychological Disturbances of War-traumatized Children from Different Foster and Family Settings in Bosnia and Herzegovina. Croat Med J. 2006;47(1):85-94.
3. Hasanović M, Srabović S, Rasidović M, Sehović M, Hasanbasić E, Husanović J, Hodzić R. Psychosocial assistance to students with posttraumatic stress disorder in primary and secondary schools in post-war Bosnia Herzegovina. Psychiatr Danub. 2009;21(4):463-73.
4. Hasanovic M, Sinanovic O, Pavlovic S. Acculturation and psychological problems of adolescents from Bosnia and Herzegovina during exile and repatriation. Croat Med J. 2005;46:105-15.
5. Loga S, Cerić I, Stojak R, Kantardzić D, Danes V, Trograncić Z, Fiseković S, Stanić D, Termiz D, Kulenović F, Licanin I, Papović S, Resić D. Psychosocial research during the war in Sarajevo. Med Arh. 1999;53(3):139-44.
6. Goldstein RD, Wampler NS, Wise PH. War experiences and distress symptoms of Bosnian children. Pediatrics. 1997;100(5):873-8.
7. Bull D. Operation joint forge veterans - Balkan tour broadens scope. Vet. Center Voice. 2004;25(4):12-16.
8. Weinberg J, Simmonds S. Public health, epidemiology and war. Soc Sci Med. 1995;40:1663.
9. Jensen PS, Shaw J. Children as victims of war: current knowledge and future research needs. J Am Acad Child Adolesc Psychiatry. 1993;32:697-708.
10. Papageorgiou V, Frangou-Garunovic A, Iordanidou R, Yule W, Smith P, Vostanis P. War trauma and psychopathology in Bosnian refugee children. Eur Child Adolesc Psychiatry. 2000;9:84-90.
11. Husain SA, Nair J, Holcomb W, Reid JC, Vargas V, Nair SS. Stress reactions of children and adoles-

cents in war and siege conditions. Am J Psychiatry. 1998;155:1718-9.

12. Rutter M. Maternal deprivation reassessed. 2nd ed. London: Penguin Books; 1991. p. 19.
13. Röper G, Gavranidou M. Capacity building in trauma therapy and trauma research in Bosnia-Herzegovina. New Dir Youth Dev, 2003;(98):99-110.
14. Hasanović M, Pavlović S, Sinanović O. Acculturation and Psychological Characteristics of Bosnian Internally Displaced and Refugee Adolescents from Srebrenica Region after the War 1992-1995 in Bosnia and Herzegovina. In: Rene S. Grenell (ed.) Adolescent Behavior Research Studies. New York: Nova Publishers; 2007 - 3rd Quarter. p. 155-91.
15. Lonigan CJ, Philips BM, Richey JA. Posttraumatic stress disorder in children: diagnosis, assessment, and associated features. Child Adolesc Psychiatr Clin N Am. 2003;12(2):171-94.
16. Jones L, Kafetsios K. Assessing adolescent mental health in war-affected societies: the significance of symptoms. Child Abuse Negl. 2002;26(10):1059-80.
17. Goldin S, Levin L, Persson LA, Hägglof B. Stories of pre-war, war and exile: Bosnian refugee children in Sweden. Med Confl Surviv. 2001;17(1):25-47.
18. Alwood MA, Bell-Dolan D, Husain SA. Children's trauma and adjustment reactions to violent and nonviolent war experiences. J Am Acad Child Adolesc Psychiatry. 2002;41(4):450-7.
19. Allden K, Cerić I, Kapetanović A, Lavelle J, Loga S, Mathias M, & McInnes K. Merhamet Medical Team-Rijeka, Mollica RF, Puratić V, Ruke Team-Zagreb, Sarajlić N (alphabetical order). Harvard Trauma Manual: Bosnia-Herzegovina Version. Cambridge (MA): Harvard Program in Refugee Trauma; 1998. (Available in English and Bosnian).
20. Allden K, Frančišković T, Lavelle J, Mathias M, McInnes K, Mollica RF & Moro L. (alphabetical order). Harvard Trauma Manual: Bosnia-Herzegovina Version. Cambridge (MA): Harvard Program in Refugee Trauma; 1998. (Available in English and Bosnian).
21. Goldin S, Levin L, Persson LA, Hägglof B. Child war trauma: a comparison of clinician, parent and child assessments. Nord J Psychiatry. 2003;57(3):173-83.
22. Angel B, Hjern A, Ingleby D. Effects of war and organized violence on children: a study of Bosnian refugees in Sweden. Am J Ortopsychiatry. 2001;71(1):4-15.
23. Horowitz MJ, Wilner N, Alvarez W. Imact of Event Scale: a measure of subjective stress. Psychosom Med. 1979;41:209-18.
24. Brennen T, Hasanović M, Zotović M, Blix I, Skar AM, Prelić NK, Mehmedović I, Pajević I, Popović N, Gavrilov-Jerković V. Trauma exposure in childhood impairs the ability to recall specific autobiographical memories in late adolescence. J Trauma Stress. 2010;23(2):240-7.
25. Dyregrov A, Kuterovac G, Barath A. Factor analysis of the impact of event scale with children in war. Scand J Psychol. 1996;37(4):339-50.
26. Connelly C. The new siege of Sarajevo. The Times. 2005 Oct 8.
27. Strange H. Serb general Dragomir Milosevic convicted over Sarajevo siege. The Times. 2007 Dec 12.
28. Bassiouni C. Final report of the United Nations Commission of Experts established pursuant to Security Council resolution 780. United Nations. 1994 May 27.
29. Duraković-Belko E, Kulenović A, Dapić R. Determinants of posttraumatic adjustment in adolescents from Sarajevo who experienced war. J Clin Psychol. 2003;59(1):27-40.
30. Daneš V, Horvat V. Psychological consequences of war stress in the developing population in Bosnia and Herzegovina. Psychiatr Danub. 2005;17(3-4):225-9.
31. Tahirović FH, Tulumović T, Uzeirbegović M, Hadžibegić N, Hasanović A. How war disrupts iodine prophylaxis: An example from Bosnia. IDD Newsletter. 1995:13.
32. Uvin P, Cohen MJ, Snyder AC, Hoehn RA, Harris M. Overview of world hunger. Causes of hunger. Fifth annual report on the state of world hunger. Bread for the World Institure; 1994. p. 10-20.
33. Tahirović H. The influence of abuse and terrorism on somatic growth of children. Arabisches Arzteblatt. 1996:56-9.
34. Hay A. Surviving the impossible: the long march from Srebrenica. An investigation of the possible use of chemical warfare agents. Med Confl Surviv. 1998;14(2):120-55.
35. Pavlović D, Bijedić S. Personal experience in the organization of mass admission of traumatized refugees. Med Arh. 1997;51(1-2 Suppl 1):21-2.
36. Hasanović M, Pavlović S, Sinanović O. (2008) Acculturation and Psychological Characteristics of Bosnian Internally Displaced and Refugee Adolescents from Srebrenica Region after the War 1992-1995 in Bosnia and Herzegovina. In: Monica K. Zimmermann (ed.) Political Refugees: Social Conditions, Health and Psychological Characteristics. New York: Nova Publishers; 2008 - 3rd Quarter. p. 139-75.
37. Tahirovič FH. Menarchal age and the stress of war: an example from Bosnia. Eur J Pediatr. 1998;157:978-80.
38. Smith P, Perrin S, Yule W, Hacam B, Stuvland R. War exposure among children from Bosnia-Her-

cegovina: psychological adjustment in a community sample. J Trauma Stress. 2002;15(2):147-56.

39. Eytan A, Gex-Fabry M, Toscani L, Deroo L, Loutan L, Bovier PA. Determinants of postconflict symptoms in Albanian Kosovars. J Nerv Ment Dis. 2004;192(10):664-71.
40. Hasanović M, Haračić E, Ahmetspahić Š, Kurtović S, Haračić H. Poverty and Psychological Disturbances of War-Traumatized Adolescents from Rural and Urban Areas in Bosnia and Herzegovina. In: Fiedler A, Kuester I (Eds) Child Development and Child Poverty. New York: Nova Publishers; 2010. p. 229-55.
41. Cerni Obrdalj E, Rumboldt M. Bullying among school children in postwar Bosnia and Herzegovina: cross-sectional study. Croat Med J. 2008;49(4):528-35.
42. Kerestes G. Children's aggressive and prosocial behavior in relation to war exposure: testing the role of perceived parenting and child's gender. Int J Behav Dev. 2006;30: 227–39.
43. Zalihić A, Zalihić D, Pivić G. Influence of posttraumatic stress disorder of the fathers on other family members. Bosn J Basic Med Sci. 2008;8(1):20-6.
44. Monson CM, Taft CT, Fredman SJ. Military-related PTSD and intimate relationships: from description to theory-driven research and intervention development. Clin Psychol Rev. 2009;29(8): 707-14.
45. Beardslee WR, Gladstone TR, Wright EJ, Cooper AB. A family-based approach to the prevention of depressive symptoms in children at risk: evidence of parental and child change. Pediatrics. 2003;112:119-31.
46. Dahl S, Mutapcic A, Schei B. Traumatic events and predictive factors for posttraumatic symptoms in displaced Bosnian women in a war zone. J Trauma Stress. 1998;11(1):137-45.
47. Selimbasic Z, Sinanovic O, Avdibegovic E, Kravic N. Contact network and satisfaction with contacts in children whose parents have post traumatic stress disorder. Med Arh. 2009;63(3):124-7.
48. Selimbasić Z, Avdibegović E, Sinanović O. Posttraumatic stress reaction in children. Med Arh. 2000;54(3):177-80.
49. McFarlane AC. Military deployment: the impact on children and family adjustment and the need for care. Curr Opin Psychiatry. 2009;22(4):369-73.
50. Smith P, Perrin S, Yule W, Rabe-Hesketh S. War exposure and maternal reactions in the psychological adjustment of children from Bosnia-Hercegovina. J Child Psychol Psychiatry. 2001;42(3):395-404.
51. Richardson LK, Frueh BC, Acierno R. Prevalence estimates of combat-related post-traumatic stress disorder: critical review. Aust N Z J Psychiatry. 2010;44(1):4-19.
52. Green KT, Calhoun PS, Dennis MF. Mid-Atlantic Mental Illness Research, Education and Clinical Center Workgroup, Beckham JC. Exploration of the resilience construct in posttraumatic stress disorder severity and functional correlates in military combat veterans who have served since September 11, 2001. J Clin Psychiatry. 2010;71(7): 823-30.
53. Berk JH. Trauma and resilience during war: a look at the children and humanitarian aid workers of Bosnia. Psychoanal Rev. 1998;85(4):640-58.
54. Björn A, Eriksson T. Released Bosnian prisoners of war. High risk of posttraumatic symptoms. Lakartidningen. 1993;90(24):2305-8.
55. Arie S. Conflict and Rape. After war, what next? BMJ. 2010;341:c6910.
56. Cairns E, Dawes A. Children: Ethnic and political violence – A commentary. Child Development. 1996;67:29-139.
57. Dybdahl R. Children and mothers in war: An outcome Study of a psychosocial intervention program. Child Development. 2001;72(4),1214-30.
58. Avdibegović E, Hasanović M, Selimbasić Z, Pajević I, Sinanović O. Mental health care of psychotraumatized persons in post-war Bosnia and Herzegovina--experiences from Tuzla Canton. Psychiatr Danub. 2008;20(4):474-84.
59. Sinanović O, Avdibegović E, Hasanović M, Pajević I, Sutović A, Loga S, Cerić I. The organisation of mental health services in post-war Bosnia and Herzegovina. International Psychiatry. 2009;6(1):10-12.
60. Hasanović M, Sinanović O, Pajević I, Avdibegović E, Sutović A. (2006) Post-war mental health promotion in Bosnia-Herzegovina. Psychiatria Danubina. 18(1-2):74-78.
61. de Jong K & Stickers R. Early psychosocial interventions for war-affected populations. In: Orner R & Schnyder U (eds). Early interventions in emergencies. Oxford University Press; 2003.
62. Nelson BS. Post war trauma and reconciliation in Bosnia and Herzegovina: observations, experiences, and implications for marriage and family therapy. Am J Fam There. 2003;31(4):305-316.
63. Selimbasić Z, Pavlović S, Sinanović O, Vesnić S, Petrović M, Ferković V, Cipurković-Mustacević A. Posttraumatic stress disorder--effects of psychosocial treatment in children. Med Arh. 2001;55(1 Suppl 1):25-9.
64. Pajević I, Sinanović O, Hasanović M. Religiosity and mental health. Psychiatria Danubina. 2005;17(1-2):84-9.

65. Snyder CS, May JD, Zulcic NN, Gabbard WJ. Social work with Bosnian Muslim refugee children and families: a review of the literature. Child Welfare. 2005;84(5):607-30.

66. Brunvatne R, Lysgård KH, Hjortdahl P. Psychosocial preventive work among war refugees. A task for primary health care? Tidsskr Nor Laegeforen. 1995;115(1):23-6.

67. Geltman PL, Augustyn M, Barnett ED, Klass PE, Groves BM. War trauma experience and behavioral screening of Bosnian refugee children resettled in Massachusetts. J Dev Behav Pediatr. 2000;21(4):255-61.

68. Ehntholt KA, Yule W. Practitioner review: assessment and treatment of refugee children and adolescents who have experienced war-related trauma. Journal of Child Psychology and Psychiatry. 2006;47(12):1197-210.

69. Murray LK, Cohen JA, Ellis BH, Mannarino A. Cognitive behavioral therapy for symptoms of trauma and traumatic grief in refugee youth. Child Adolesc Psychiatr Clin N Am. 2008;17(3):585-604.

70. Wintsch H. Spreading hope: therapeutic teams with war-traumatized children and adolescents in Bosnia. Prax Kinderpsychol Kinderpsychiatr. 2000;49(3):209-30.

Review article

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# Affective disorders in childhood and adolescence

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Affective disorders in childhood have been more intensively studied in the last three decades. They can be recognized among the children of all ages, but are more frequent among the older children. The main characteristics of mood disorders are similar among children, adolescents and adults, although development factors affect their clinical features. Development factors affect the manifestation of all symptoms. Two main criteria for these disorders in childhood are mood disorders, such as reduced or elevated mood and irritability. These symptoms may result in social or academic damage. Depression among children is a wide-spread, family and recurrent condition, which continues episodically in adulthood. Depression is frequently associated with other psychiatric disorders, increasing the risk of suicidal behaviour, misuse of psychoactive substances and behavioural disorders. Depression in childhood brings about worse psychosocial, academic and family functioning. Family, social and environmental factors have a significant role in affective disorders of children and young people.

**Key words:** Mood disorders, Children, Adolescence, Depression, Bipolar disorder.

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## Introduction

Mood disorders among children and adolescents have been more intensively studied in the last three decades, and more comprehensive clinical study is a result of the increase of suicidal behaviour and realized suicides among young people (1). These disorders are characterized by mood swings in the form of a reduced or excessively good mood, which is followed by difficulties in the family, academic and social functioning. The majority of these disorders show the tendency towards recurrence, and the beginning of individual episodes is often connected with certain stressful events. Depressive and bipolar affective disorders are most frequent in the development period. The existing classification systems do not classify mood disorders in the development age into separate catego-

ries, but they use the same diagnostic criteria both for children and adults. Two basic criteria for affective disorders in childhood and adolescence are mood disorder and irritability. The symptomatology with younger children is less clearly differentiated and is more difficult to recognize, while it becomes clearer and more specific with adolescents and even takes a clinical picture of a disorder in adults (2). These disorders are often associated with other psychiatric disorders, increasing the risk of suicidal behaviour, misuse of psychoactive substances and behavioural disorder (3).

## Depressive disorders

Although there were individual descriptions of depression in childhood in literature, most experts have doubted the possibility of the existence of depression in children considering that children do not have a psychic apparatus mature enough for them to be depressive, or that it is a matter of overemphasis in the normal development processes in childhood (4). However, sensitizing psychiatrists to psychic difficulties among children and adolescents, taking development components into account, has increased awareness of the importance and spread of this group of disorders. Depression at this age represents to a certain degree a contradiction, since that age in life is "predetermined for happiness and life joy". Depression in the development period has been more intensively studied in the last twenty or so years. Depressive symptomatology is not a normal aspect of child and adolescent development, but rather a psychological disorder, which, unless recognized, may persist for months and years and may seriously endanger their functioning.

## Epidemiology and causes

Epidemiological studies on depression in children and adolescents are still difficult to interpret, they differ from one author to another and depending on the criterion for assessment, the manner of forming samples, the examiner's objectivity, and social, cultural and other factors. However, most authors present the data that 2.5% of children and 8.3% of adolescents suffer from depressive disorders (5). The presence of a depressive mood is much more widespread and about one third of adolescents report depressive moods, the girls more often. The disorder is equally represented among girls and boys, while a significantly higher number of girls suffer from depressive disorders in adolescence (6). It is considered that hormonal and environmental factors contribute to the increased frequency of depression among female adolescents (7). The rate of depression increases significantly as they move into adolescence (8).

It is considered that genetic factors play an important role in creating a predisposition for development of depression in the development period, while numerous external factors contribute to the expression of depression, as the initiators of the first episode of depression. Depressive disorders in children are also often associated with a major psychic and physical burden with no sufficient rest, great expectations, a weak emotional relationship with their mother and other important persons, psychic traumas because of a real or threatened loss of a beloved person or object (9).

The research into neuroendocrine changes has shown that depressive pre-puberty children had a lower secretion of cortisol during the first 4 hours of sleep (10), and the study of adolescents with depression showed a reduction of perfusion in the left-side frontal and temporal cortical region (11). Molecular genetic studies discovered some abnormalities in the neurotransmitter system: the MAOA gene responsible for functioning of monoamine oxidase and serotonin transporter gene (5-HTT), which is involved in the process of serotonin production and represents a marker for vulnerability to depression in children (12).

The risk factors for depressive expressions among children are: family history of depression, family conflicts, exposure to violence, development incapacities, chronic diseases, comorbid conditions, stress, traumatic events, break-up of a romantic relationship, etc. Numerous studies on stressors associated with war-time events and loss of parents show that in children they are especially manifested in the affective-emotional area (13). Research among the war-traumatized children showed that children experienced many psychological consequences and a high prevalence of PTSD, often in comorbidity with depression, in children who had lost one or both parents (14). A higher frequency of PTSD was also noticed in adolescent refugees even a few years after the end of the war (15). The latest studies show that the death of parents represents more than a four-fold risk for developing depression in children and adolescents (16).

## Clinical manifestations of depressive disorders

The diagnostic criteria and main characteristics of depression are the same as in adults, but the manner in which children manifest them is different, which makes them more difficult to recognize. The manner in which children manifest depression is different at individual stages of development, and small children find it difficult to recognize and describe internal emotions or moods; or they do not have a vocabulary to speak about such feelings and they manifest them through their behaviour. The expression of depression differs according to the cognitive level. Pre-school children are not cognitively equipped to present subjective expressions of depression; but, they may complain about somatic symptoms. Children often express sorrow through irritability and frustrations, fits of anger and behavioural problems. That is why it is necessary to decide on depressive symptoms based on behaviour, including apathy, withdrawal, postponement or regression of development benchmarks or insufficient growth that has no organic cause. These children may be less mobile, enthusiastic or spontaneous; they may seem serious, absent or sick; they can spontaneously show conditions of crying or irritability.

School children are cognitively capable of internalizing environmental stressors (e.g. family conflict, criticism, failure at school...) and they express low self-respect and guilt. However, they express much of this internal unrest through somatic complaints, especially headaches and stomach problems; anxiety and irritability (fits of ill humour, rage and other problems in behaviour). Depression may primarily exist as a behavioural disorder, misuse of alcohol and substances or as a state of revolt and turmoil in the family. Children's complaints of being bored often hide depressive feelings.

Depression in children and adolescents may be manifested as:

- Frequent unclear and undetermined corporal complaints (headache, stomach pains, tiredness, pains in muscles...);
- Being frequently absent from school and poor achievement in school;
- Bursts of screaming, protests, complaints, inexplicable irritability or crying;
- Being bored;
- Decreased interest in playing with children of the same age;
- Social isolation, poor communication; difficulties in establishing relations;
- Fear of death;
- Distinct over-sensitivity to rejection or failures;
- Abuse of alcohol and other problems.

Child and adolescent psychopathology is characterized by the presence of many disorders, and this is especially the case with depression. With regard to depression we may say that comorbidity is rather a rule than an expectation. It is considered that the major-

ity of young people with major depression have at least one comorbid disorder, and also 30% to 50% of them have two or more psychiatric disorders (17).

The two most frequent diagnosed disorders are dysthymic and anxiety disorder, and then disruptive disorders in behaviour and abuse of substances (18). Comorbid disorders are similar in girls and boys; although boys have more frequent disruptive disorders and depression and the girls have disorders in diet and depression.

*Suicidal behaviour*

One of the significant risks following depressive disorders in children and adolescents is the risk of suicidal behaviour, which becomes an important mental-hygiene problem in many countries (19). There is a constant tendency of growth of both attempts and committed suicides. The risk of suicidal behaviour is increased among the depressive adolescents with the comorbidity of behavioural disorder and abuse of substances. Suicidal activity is generally associated with a significant acute crisis in the life of a teenager. Poor grades in school, a negative warning or reprimand by important persons, especially by parents and teachers, the break-up of a romantic relationship or loss may precipitate a suicidal action (20). Suicidal ideas and actions are more frequent among the children who have already gone through an important stress in their lives (divorce of parents, parental or family disagreements, corporal or sexual abuse, misuse of psychoactive substances, etc.). The highest number of attempted suicides belongs to the group of mixed disorders (affective disorders and behavioural disorders).

*Assessment, diagnosis and consequences of depressive disorders*

The assessment of depression in children may be difficult depending on their development phase. These children often resist or withdraw, and many of them refuse to talk. Some of them deny sorrow, but often have fits of irritability, sleep problems, frequently complain of being bored, and have persistent problems in their behaviour at home and at school. Parents and teachers notice that the child withdraws, spends a lot of time alone and does not make friends with children of the same age as before. The diagnosis of a depressive disorder is established through a medical and psychiatric evaluation. Numerous medical conditions may hide depression, like infections (hepatitis, influenza, infectious mononucleosis, virus infections, immunodeficiency disorder), Cushing's disease, hypothyroidism, hyperparathyroidism, porphyria, etc. Some conditions incline to mood disorders: diabetes mellitus, epilepsy, anxiety disorders, eating disorders, abuse of alcohol and other substances, electrolyte imbalance, use of medicaments (barbiturates, benzodiazepines, corticosteroids, anticonvulsants...), behavioural disorder, physical abuse, etc.

After recovering from an episode of serious depression, many children show sequels in the form of poor self-respect, damaged interpersonal relations, subclinical depressive symptoms and a decrease in global functioning. The most serious complication of depression is suicide.

*Treatment of depressive conditions*

Depressive disorders in the development age are inclined to be recurrent, therefore the approach and treatment have to be extended. It is important to help the child and the parents in recognizing the signs of disease recurrence at an early stage and encourage them to ask for help as soon as the first symptoms appear. Reduction of depression is the main focus of treatment, in which the treatment of comorbid difficulties also has to be taken into account. Some of these difficulties, like the problem with regard to

relationships with children of the same age, can result in prolonged depression, and that is why depression in the development period requires a series of therapeutic interventions. These children feel lonely and the fear of loneliness is often more painful than depression itself, and the feeling of loneliness and fear of abandonment are pervasive for the child. The optimal sequence of treatment means establishing a warm and caring relationship that both the child and the family can accept, with the initiation of emotional growth and independence and the gradual decrease of dependence on the therapist, along with building better relationships with the parents and peers.

In treating depressive conditions in the development age we use pharmacotherapy along with various psychosocial and psychotherapeutic interventions. Psychotherapy can be useful as an initial therapy for children and adolescents with mild to moderate depression or as a supplement to medication therapy in more serious and grave forms of depression. The appropriate psychotherapy approach will be applied depending on the emotional and cognitive development level. Play therapy and parental training are applied with depressive pre-school children, while psychodynamic or cognitive-behavioural therapy will be applied with older children and adolescents (21, 22). The basis of this treatment is to help depressive children in changing their negative cognition about themselves and the surrounding world.

Pharmacological treatment most often represents an integral part of the general therapeutic program. Medicaments cannot either build or change patterns of behaviour, moral standards, positions about oneself and others; but they can alleviate some symptoms and help in establishing relationships between the child and his/her family group and group of peers. When applying psycho-pharmaceuticals it is important to pay attention to the specifics of the child's or adolescent's age, i.e. the physiological characteristics of the child's organism, liver metabolism, kidney filtration, body weight and other pharmacodynamic factors. A special problem may be presented by the parents who often, on the basis of their free will, terminate the therapy, reduce the proposed dose, etc. Tricyclic antidepressants were the first accessible pharmacotherapy for depressive children (23). Because of the anticholinergic and potential cardiotoxic effect of these medications, especially in the case of overdosing, tricyclic antidepressants become the second line in the therapy, yielding the first place to serotonin reuptake inhibitors. The recent studies on the successfulness of serotonin reuptake inhibitors indicate positive results in treating depression in the development age (24, 25). These antidepressants have a secure profile and carry less risk of cardiotoxicity and lethal overdose, which is especially important in treating impulsive adolescents. A few randomized, placebo-controlled studies, which encompassed 1619 children and adolescents, have indicated that fluoxetine, paroxetine, sertraline and citalopram showed safety and efficiency in treating depression in children and adolescents (26). Due to the fewer undesired side effects and safer profile, these antidepressants represent the most often used antidepressants in treating children and adolescents. Depression in the development age is recurrent and has a high tendency of relapse, therefore antidepressants are generally and continuously administered for a period of 6 to 12 months after the remission of the symptoms of an acute episode of depression. After this period the dose is gradually reduced over the course of six weeks, along with continuous observance of recurrent symptoms.

## Bipolar affective disorder

Bipolar disorders in children and adolescents have been unrecognized and neglected

for a long time. Only in the 80-ies of the last century did the awareness rise of the need to define and diagnose this group of disorders in children and adolescents. This disorder is characterized by extreme changes in mood, energy level and behaviour, and the symptoms may occur in early childhood, but the occurrence in adolescence is more typical. In the last twenty or so years, bipolar disorders are diagnosed more often, including in pre-school children (27). Children with bipolar disorder usually have quick swings from an extremely elevated mood (mania) to a reduced mood (depression). These quick swings of mood may cause irritability with periods of good condition between the episodes, or the young people may feel both extremes at the same time.

*Epidemiology and causes of bipolar disorder*

Studies of the school population in the community have established life prevalence in 1%, and mania alone in 0.15% among older adolescents. The majority of discovered cases had hypothymic or cyclothymic disorder. Around 5.7% of them had symptoms below the threshold (28). Although this disorder affects both sexes equally, an earlier beginning is more frequent in boys, especially under 13 years of age. The new studies indicate that mood swings (most often depression) start in childhood, and some experts suggest that there is a prevalence of 1% in young people (29). The aetiology of bipolar disorder is still unknown, and it is believed that it is a result of the interaction of a series of biological, psychological and social factors. It is considered that the pathogenesis of bipolar disorder has a great deal in common with depression, with regard to the abnormality of neurotransmitter and neuroendocrine functions. Nevertheless, it seems that the genetic component of bipolar disorder is stronger than in depression, with a higher number of close relatives affected. It is known that psychosocial stress often precedes the first manic episode, but its influence on the occurrence of subsequent episodes has not been observed.

*Clinical manifestation of bipolar disorder*

The basic characteristic of bipolar disorder is the clinical course, characterized by one or more manic or mixed episodes. Mania is defined by the period during which there is an abnormal and permanently elevated or irritable mood and it may last for one week or shorter, if it is of such an intensity that it requires hospitalization and is associated with at least three of the following symptoms:

- Serious mood swings from unusual joy or senselessness to irritability, anger or aggressiveness;
- Unrealistically high self-respect, feeling of invincibility;
- Major increase in energy levels, sleeping little without feeling tired;
- Excessive involvement in multiple projects and activities, moving easily from one activity to the other and easily getting distracted;
- Increased talking, speaking too much, quickly, frequently changing topics, cannot be interrupted;
- Risky behaviour, such as abuse of drugs, alcohol, attempts at amazing escapades, starting sexual activities;
- Exaggerated involvement in pleasant activities potentially resulting in unpleasant, painful consequences.

A depressive episode of bipolar disorder in the development period is characterized by:

- Frequent sorrow, sadness or crying;
- Withdrawal from friends and activities;
- Reduction of energy levels, lack of enthusiasm or motivation;
- Feeling of worthlessness or excessive guilt;
- Extreme oversensitivity to rejection or failure;

– Major changes in habits, like excessive sleeping or eating too much;
– Frequent corporal complaints, like headaches and stomach pains;
– Repeated thoughts about death, suicide or self-destructive behaviour.

Many teenagers with bipolar disorder misuse alcohol and drugs. If addiction develops, it is necessary to treat both mental disorders at the same time. One of the main sources of diagnostic confusion for the children with bipolar disorder is comorbidity with other psychiatric disorders, especially with ADHD, behavioural and anxiety disorders. One of the reasons for the huge agreement of bipolar disorder with ADHD is that they share many diagnostic criteria, including absent-mindedness, hyperactivity and verbosity (talking too much).

Some corporal conditions and use of psychoactive substances may produce symptoms similar to mania, like neurological disorders (brain tumours, CNS infections, including HIV, multiple sclerosis, temporal epilepsy, Klein-Levin syndrome), systemic conditions like hyperthyroidism, porphyria, uraemia, Wilson disease; and use of antidepressants, stimuli, steroids, as well as use of cocaine, amphetamines, phencyclidine and inhalants.

The presence of these disorders complicates diagnosis and recognition of this disease in children. It is considered that the early occurrence of this disorder often has a chronic course, including a high incidence of suicide and frequent mood swings. Many children, and especially adolescents, consider mood swings as a normal part of growing up, but when these feelings persist and disturb the capability of the child to function on a daily basis, bipolar disorder may be the cause.

*Treatment of bipolar disorder with children*

Treatment of bipolar disorder in the development period includes a biopsychosocial, multifactoral and multidisciplinary approach. An overall, comprehensive diagnostic assessment is necessary, with a special assessment of suicidal risk and the presence of comorbid diseases, and identification of other problems, like dysfunctional family and difficulties at school. The fundamental psychopharmacological therapy is the use of mood stabilizers, but it is possible to add antipsychotics, highly potent benzodiazepines and other medication depending on the clinical picture and speed of exchange of episodes (30). Psychotherapeutic interventions are applied in the course of treatment, and specifically cognitive-behavioural psychotherapy and interpersonal psychotherapy. The psycho-educational work with family members is of special importance with the goal of improving cooperation during treatment, a better understanding of disease and preventing relapse.

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**Reference**

1. Birmaher B, Ryan ND, Williamson DE, Brent DA, Kaufman J, Dahl, et al. Childhood and adolescent depression: a review of the past 10 years. Part I. J Am Acad Child Adolesc Psychiatry. 1996;35(11):1427-39.
2. Birmaher B, Brent D, Bernet W, Bukstein O, Walter H, Benson RS, et al. Practice parameter for the assessment and treatment of children and adolescents with depressive disorders. J Am Acad Child Adolesc Psychiatry. 2007;46(11):1503-26.
3. Gunnell DJ. The epidemiology of suicide. Int Rev Psychiatry. 2000;12(1):21-6.
4. Burgić-Radmanović M. Depresija u djece i mladih. Medicinski fakultet Banja Luka: Atlantik; 2007.
5. Lewinsohn PM, Rohde P, Klein DN, Seeley JR. Natural course of adolescent major depressive disorder: I. Continuity into young adulthood. J Am Acad Child Adolesc Psychiatry. 1999;38(1):56-63.
6. Birmaher B, Brent DA, Benson RS. Summary of the practice parameters for the assessment and treatment of children and adolescents with depressive disorders. J Am Acad Child Adolesc Psychiatry. 1998;37:1234-8.

7. Saluja G, Iachan R, Scheidt PC, Overpeck MD, Sun W, Giedd JN. Prevalence of and risk factors for depressive symptoms among young adolescents. Arch Pediatr Adolesc Med. 2004;158:760-5.
8. Costello EJ, Mustillo S, Erkanli A, Keeler G, Angold A. Prevalence and development of psychiatric disorders in childhood and adolescence. Arch Gen Psychiatry. 2003;60:837-44.
9. Kelly J. Parental Death Has Major Impact on Depression Risk in Youth. Am J Psychiatry. 2009;166: 786-94.
10. De Bellis MD, Dahl RE, Perel JM, Birmaher B, al-Shabbout M, Williamson DE, et al. Nocturnal ACTH, cortisol, growth hormone, and prolactin secretion in prepubertal depression. J Am Acad Child Adolesc Psychiatry. 1996;35(9):1130-8.
11. Tutus A, Kibar M, Sofuoglu S, Basturk M, Gönül AS. A technetium-99m hexamethylpropylene amine oxime brain single-photon emission tomography study in adolescent patients with major depressive disorder. Eur J Nucl Med. 1998;25(6):601-6.
12. Nobile M, Begni B, Giorda R, Frigerio A, Marino C, Molteni M, et al. Effects of serotonin transporter promoter genotype on platelet serotonin transporter functionality in depressed children and adolescents. J Am Acad Child Adolesc Psychiatry. 1999;38(11):1396-402.
13. Daneš V. Dijete, vanjski svijet i psihički poremećaji: psihopatološka interakcija ličnosti u razvoju sa njenim vanjskim okruženjem. Zenica: Dom štampe; 2003.
14. Hasanović M, Sinanović O, Selimbašić Z, Pajević I, Avdibegović E. Psychological Disturbances of War-traumatized Children from Different Foster and Family Settings in Bosnia and Herzegovina. Croat Med J 2006;47:85-94.
15. Hasanović M, Sinanović O, Pavlović S. Acculturation and Psychological Problems of Adolescents from Bosnia and Herzegovina during Exile and Repatriation. Croat Med J. 2005;46(1):105-115.
16. Kelly J. Parental Death Has Major Impact on Depression Risk in Youth. Am J Psychiatry. 2009;166:786-94.
17. Zalsman G, Brent DA, Weersing VR. Depressive disorders in childhood and adolescence: an overview: epidemiology, clinical manifestation and risk factors. Child Adolesc Psychiatr Clin N Am. 2006;15(4):827-41.
18. Harrington R, Rutter M, Weissman MM, Fudge F, *Groothues C, Bredenkamp D,* et al. Psychiatric disorders in the relatives of depressed probands. I. Comparison of prepubertal, adolescent and early adult onset cases. J Affect Disord. 1997;42(1):9-22.
19. Shaffer D, Craft L. Methods of adolescent suicide prevention. J Clin Psychiatry. 1999;60(Suppl 2):70-4; discussion 75-6,113-6.
20. Brown J, Cohen P, Johnson JG, Smailers EM . Childhood abuse and neglect: specificity of effects on adolescent and young adult depression and suicidality. J Am Acad Child Adolesc Psychiatry. 1999;38(12):1490-6.
21. Reinecke MA, Ryan NE, DuBois DL. Cognitive-behavioral therapy of depression and depressive symptoms during adolescence: a review and meta-analysis. J Am Acad Child Adolesc Psychiatry. 1998;37(1):6-34.
22. Mufson L, Weissman MM, Moreau D, et al. Efficacy of interpersonal psychotherapy for depressed adolescents. Arch Gen Psychiatry. 1999;56(6):573-9.
23. Geller B, Reising D, Leonard HL, Riddle MA, Walsh BT. Critical review of tricyclic antidepressant use in children and adolescents. J Am Acad Child Adolesc Psychiatry. 1999;38(5):513-6.
24. Emslie GJ, Rush AJ, Weinberg WA, Kowatch RA, Hughes CW, Carmody T, et al. A double-blind, randomized, placebo-controlled trial of fluoxetine in children and adolescents with depression. Arch Gen Psychiatry. 1997;54(11):1031-7.
25. Donnelly CL, Wagner KD, Rynn M, Ambrosini P, Landau P, Yang R, et al. Sertraline in children and adolescents with major depressive disorder. J Am Acad Child Adolesc Psychiatry. 2006;45(10):1162-70.
26. Sharp S, Hellings J. Efficacy and Safety of Selective Serotonin Reuptake Inhibitors in the Treatment of Depression in Children and Adolescents. Clin Drug Invest. 2006;26(5):247-55.
27. Luby J, Belden A. Defining and validating bipolar disorder in the preschool period. Dev Psychopathol. 2006;18:971-88.
28. Geller B, Tillman R, Craney JL, Bolhofner K. Four-year prospective outcome and natural history of mania in children with a prepubertal and early adolescent bipolar disorder phenotype. Arch Gen Psychiatry. 2004;61:459–67.
29. Perlis RH, Miyahara S, Marangell LB, Wisniewski SR, Ostacher M, DelBello MP, et al. Long-term implications of early onset in bipolar disorder: data from the first 1000 participants in The Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD). Biol Psychiatry 2004; 55:875-81.
30. Kowatch RA, Fristad MA, Birmaher B, Wagner KD, Findling RL, Hellander M. Treatment guidelines for children and adolescents with bipolar disorder. J Am Acad Child Adolesc Psychiatry. 2005;44:213-25.

Case report

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# Fulminant hepatic failure in a neonate with systemic echovirus infection

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We report the fatal case of neonatal fulminant hepatic failure due to Echovirus infection mimicking an acute metabolic decompensation. After exclusion of several metabolic disorders, the diagnosis of the infectious etiology was confirmed by Polymerase Chain Reaction of Echovirus in liver and spleen tissue. Establishment of etiological diagnosis and exclusion of inherited metabolic disease helped the family counseling regarding risk for future pregnancies. Fulminant hepatic failure in the neonate is a diagnostic and therapeutic challenge that requires a multidisciplinary management. Our case illustrates the broad differential diagnosis, the common final pathway leading to severe liver injury, and the multidisciplinary approach to diagnosis and treatment.

**Key words:** Hepatic Failure, Neonate, Echovirus.

Abbreviations: ECHO: entero-cytopathic human orphan

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## Introduction

The diagnosis and management of fulminant hepatic failure requires a multidisciplinary approach for the evaluation of congenital, inflammatory, metabolic, infectious, and systemic conditions that may present with primary or secondary liver failure (1). Certain conditions, such as neonatal iron storage disease, affect the liver in utero since the placental feto-maternal unit does not protect the infant from a prenatal insult (2, 3), while other conditions manifest after birth as a result of toxic, metabolic, or infectious injury.

Careful attention to the perinatal history and physical examination in combination with biochemical parameters can help direct diagnostic and therapeutic interventions in a critically ill baby. Combinations of clinical and biochemical parameters and the timing of the presentation can fit into one of several patterns of liver injury

and help in the rapid determination of the cause. This allows for the timely initiation of therapy. Determination of the exact cause of the liver injury can be immensely helpful to the family in coping with the infant's illness and determine the risk to siblings and future offspring. However, establishing the diagnosis may be a challenge and despite extensive diagnostic investigations, the etiology of hepatic failure in neonates often remains unknown.

We report the case of fatal neonatal fulminant hepatic failure due to Echovirus infection presenting initially with hyperammonemia, and as such, mimicking a metabolic decompensation.

## Case report

A seven-day old girl presented with acute onset of lethargy, hypotonia and hypothermia (which is a typical onset of urea cycle disorders). She was the product of a full-term pregnancy complicated by mild abdominal pain in the third trimester, born as a second child to a 27-year-old female without risk factors. The birth weight was 2630 gr, the length was 48 cm and head circumference was 33 cm. Apgar scores were 8 and 9 after the first and fifth minute, respectively. The child was dismissed from the hospital in good condition, on the third day of life, being breast-fed. Three days later she was noted to be uninterested in eating. Within a few hours she became cold to the touch and lethargic. She was admitted to the newborn intensive care unit.

At admission, the child was unresponsive, with core temperature of 32 Celsius and heart rate of 50/beats per minute. Her resuscitation was immediate and included intubation with positive pressure ventilation, chest compressions, and central lines placement with both umbilical venous and arterial catheter. The bradycardia resolved rapidly, but the infant remained lethargic. The examination was otherwise unremarkable, the child was nondysmorphic, normocephalic and well nourished. The skin had no bruises or birth marks, there was no rash. The neck was supple, without nodules. The chest was normal and the cardiac sounds were normal. There were no murmurs, clicks, or rubs. The lungs were clear. There was no organomegaly, jaundice, ascites, edema, and the baby had normal female genitalia.

Laboratory tests showed profound metabolic acidosis (with mild elevation of anion gap), which was corrected with bicarbonate administration. The patient had initial blood ammonia of 466 ug/dl, which rose to 852 within the next 12 hours of hospitalization. She had marked hyperkalemia and mild hyponatremia. Electrolyte imbalance raised the possibility of congenital adrenal hyperplasia, and appropriate evaluation was performed before the administration of dexamethasone. Her 17-hydroxyprogesterone and cortisol blood levels were normal. Blood glucose level was normal. Additional laboratory investigations performed at that time are summarized in Table 1. It became clear that the baby had evidence of liver disease with severe hepatocyte lysis and coagulopathy as well as disseminated intravascular coagulation (DIC).

The presence of hyperammonemia prompted further evaluation for metabolic disorders. Plasma amino-acid analysis showed markedly elevated proline, glycine, tyrosine, glutamine and alanine, normal citrulline and undetectable arginine. Argininosuccinic acid and orotic acid were normal in a urine sample. Urine organic acid showed massive amounts of lactate but no other abnormal metabolites. The urine sample was negative for succinylacetone, excluding tyrosinemia. The relatively low ferritin and the high elevation of the aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase

Table 1 Initial laboratory evaluation in infant

| Analysis | Parameters | The patient's range | Normal range |
|---|---|---|---|
| Serum | Ammonia | 466-852 | <50 μg N/dl |
| | Lactic acid | 14.5-16.7 | <2.2 mmol/l |
| | Pyruvic acid | 0.30 | 0.08-0.16 mmol/l |
| | Osmolality | 330 | 275-295 mosm/kg |
| | Potassium | 7.3-8.2 | 3.7-5.0 mEq/l |
| | Sodium | 115-128 | 135-145 mEq/l |
| | AST | 7896-11308 | 26-48 U/l |
| | ALT | 828-1104 | 12-31 U/l |
| | pH | 7.16 | 7.35-7.45 |
| | Bicarbonate | 7-17 | 21-25 mEq/l |
| | Base | (-20) – (17) | 0-(-2) mEq/l |
| | D-dimer | >500 | < 250 ng/ml DDU |
| | Fibrinogen | 84-104 | 175-350 mg/dl |
| | Prothrombin time | 32-19.7 | 8.4-12.0 sec |
| | APTT | 96-225 | 21-33 sec |
| | Ferritin | 581 | 25-200 ug/l |
| | Acylcarnitine | Normal profile | - |
| Urine | Generalized aminoaciduria | Massive | Negative |
| | Lactic acid | Massive amount | Negative |
| | 4-hydroxy phenyllactic | Massive amount | Negative |
| | 4-hydroxy phenylpyruvic | Massive amount | Negative |
| | Lactose | Large amount | Negative |
| | Galactose | 211 mg/dl | <30 mg/dl |
| | Acylglycines | Normal profile | - |
| | Succinylacetone | Negative | Negative |

(GGT) excluded the diagnosis of iron storage disease. Blood counts and bilirubin were normal. The exclusion of inborn error of bile acid synthesis was performed by Fast Atom Bombardment Spectroscopy on a urine sample. Mitochondrial beta-oxidation defects and carnitine deficiency were also excluded.

The patient remained unresponsive despite therapy that normalized her hyperammonemia, electrolyte abnormalities, and acidosis with continuous veno-venous hemofiltration. After samples for microbiologic studies were collected (blood, urine, rectal and pharyngeal swab), broad-spectrum antibiotic therapy was initiated. Fresh frozen plasma, packed red blood cells, maintenance fluid with appropriate glucose and electrolytes achieved normal homeostasis of the infant from a biochemical point of view but she continued to show evidence of severe liver failure with persistent bleeding from puncture sites. The patient developed an increased requirement for inotropic support with poor cardiac contractility. Persistent coagulopathy and poor perfusion resulted ultimately in her demise early on the third hospital day.

Immediately post mortem, tissue was obtained with the family's consent to determine the possibility of an inborn error of metabolism with particular emphasis on the possibility of a respiratory chain defect. An exhaustive metabolic evaluation was conducted on available tissues, and remained negative (Table 2). Reduced activity of CPSI,

Table 2 Postmortem evaluations

| Analysis | Parameters | The patient | Normal range |
|---|---|---|---|
| Erythrocytes | Galactose-1-phosphate uridyl transferase | Normal | |
| Fibroblasts | Chromosomes | 46, XX | N/A |
| | Electron microscopy | Normal | N/A |
| Skeletal muscle | Histology | Normal | N/A |
| | Respiratory chain enzyme activities[1] | All reduced, no abnormality detected | N/A |
| Spleen tissue | PCR for enteroviruses[2] | Positive | Negative |
| Liver tissue | CPS I activity[3] | 0.6 | 5.6±0.3 mmol/g liver/min |
| | OTC activity[3] | 8.3 | 70.1±9.7 mmol/g liver/min |
| | PCR for enteroviruses[2] | Positive | Negative |
| Urine | Succinylacetone | Negative | Negative |
| | Fast Atom Spectroscopy[4] | Normal Bile Acids | Normal Profile |

N/A= not applicable; PCR= polymerase chain reaction
Measured activities of fumarase, citrate synthase, cytochrome oxidase, NADH cytochrome C reductase, NADH dehydrogenase, succinate cytochrome reductase and succinate dehydrogenase
[1] Performed in Clinical Genetics Laboratory, The Children's Hospital of Buffalo
[2] Performed at the Center for Disease Control in Atlanta
[3] Performed in Biochemical Genetics and Metabolism Laboratory, Children's National Medical Center, Washington DC
[4] Performed at the University of Cincinnati, The Children's Hospital Medical Center

OTC activity and respiratory chain analysis reflected the analysis performed on the sample collected postmortem. A limited autopsy disclosed bilateral adrenal hemorrhage and massive hepatic coagulative necrosis with little evidence of viable hepatocytes.

Blood, urine, nasopharyngeal, rectal, and CSF fluid were collected for bacterial, fungal and viral cultures. Viral cultures and serum titers for congenital infections were obtained. The culture from the spleen and the liver yielded echovirus. The typing of the virus was performed at the Center for Disease Control in Atlanta by polymerase chain reaction (PCR) and showed 90% homology to type 11, the most common type involved in neonatal fulminant hepatic failure.

## Discussion

Fulminant hepatic failure in the neonatal period is rare and the presentation can be devastating with rapid decompensation by multiorgan failure (1-10). The classic definition of fulminant hepatic failure, i.e. the onset of encephalopathy within two weeks of jaundice, is problematic in the neonate due to the difficult assessment of encephalopathy in this age group. In addition, the neonatal period precludes the characterization of liver failure into acute and subacute forms, which carry prognostic implications in the adult.

Therefore the definition of fulminant hepatic failure in the neonate involves a combination of clinical and biochemical parameters that reflect severe primary liver injury. This may include lethargy, jaundice, petechiae, bruising, bleeding, as well as ascites and organomegaly. These clinical findings are of course not specific for primary liver disease and occasionally reflect severe circulatory compromise.

A biochemical profile of elevated transaminases, coagulopathy, hyperammonemia, and hypoglycemia are important in this diagnosis. The degree and pattern of biochemical abnormalities and time of onset of liver injury may help in the differential diagnosis of the liver failure (1, 3-12). For example, disproportionate prolongation of the pro-

thrombin time in relation to mild elevation of the transaminases is indicative of a toxic hepatopathy, as seen in tyrosinemia, or the association of liver disease and persistent diaper dermatitis as the original manifestation of Langerhans cell histiocytosis.

Recently, the recognition of several metabolic diseases affecting the liver in the early neonatal period has shifted the emphasis of the evaluation of an ill neonate with liver failure towards this group of disorders (4-9, 11, 12). Various inborn errors of bile acid metabolism, urea cycle defects, mitochondrial oxidation and respiratory chain defects can present with liver failure. Making a correct diagnosis often allows efficient therapy for the infant and can help family planning by predicting risk for recurrence in future children.

Enteroviruses are one genus of the Picornaviridae; they comprise coxsackieviruses, echoviruses, enteroviruses and polioviruses (13). The group of enteric cytopathic human orphan viruses or echoviruses comprises several viruses that were initially found to lack animal pathogenicity and hence were termed "orphan" viruses. Viral transmission of echovirus in the newborn period is either transplacental or perinatal. The factors that affect pathogenesis include viral load, virulence, and the subtype of the virus (10, 13-15). Over the last decade or so, fatal newborn infections have been reported with echovirus (11, 15-28). In the majority of cases, massive hepatic necrosis was present. Other findings included adrenal hemorrhage, renal tubular necrosis, and myositis. Massive hepatic necrosis was reported with types 3, 6, 9, 14, 19, 20 and 21. The range of severity of enteroviral illness is variable. Mild febrile illness occurs in about 10% of cases. Hepatitis occurs in about 2% of enteroviral infections. The newborn is particularly susceptible to overwhelming infection with multiorgan failure and death. The combination of hepatitis, disseminated intravascular coagulation, apnea, lethargy, poor feeding, and jaundice were described in several cases of neonatal echovirus infection.

This case represents another echovirus 11 infection resulting in liver failure and multiorgan dysfunction that mimicked an inborn error of metabolism. The identification of the virus in secretions may not be sufficient to establish a causal relationship to liver injury and may be an incidental finding (23, 24). Nursery outbreaks have been described without overt liver failure. However, knowing that the virus has been implicated in other liver failure cases prompted us to evaluate the presence of the virus in multiple tissues in this baby. The likelihood of the enterovirus being an innocent bystander was eliminated with the use of PCR tissue samples from the spleen and the liver. The exclusion of metabolic disorders, including disorders of bile acid metabolism, urea cycle defects, and mitochondrial oxidation and respiratory chain defect was performed as part of this evaluation.

It is important to consider a metabolic cause of liver disease, where therapeutic intervention might be available to prevent irreversible liver damage, neurologic damage and other complications. Another important aspect in the identification of a potential metabolic disease is genetic counseling and evaluation of the parents for carrier states, which has implications in counseling regarding risk for future pregnancies.

## Conclusion

Increased awareness of a number of metabolic disorders and improved diagnostic capabilities for detection of rare inherited conditions may cause a bias toward neglecting other etiologies of neonatal liver failure. The possibility of perinatal infection as a cause of severe hyperammonemia and fulminant hepatic failure should be kept in mind when evaluating a sick neonate. The team ap-

proach in the evaluation and management of this complex entity is necessary. Establishment of the correct diagnosis is essential for appropriate management. Elimination of metabolic liver disease and the presence of multiorgan infectious process preclude liver transplantation. Correct diagnosis helps identification of other family members at risk and allows counseling for the family regarding disease recurrence in subsequent pregnancies.

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## References

1. Schneider B. Neonatal Liver Failure. Curr Opin Pediatr. 1996;8:495-501.
2. Schafer DF, Shaw BW. Fulminant Hepatic Failure and Orthotopic Liver Transplantation. Semin Liver Dis. 1989;9(3):189-94.
3. Rand EB, Karpen SJ, Kelly S, Mack CL, Malatack JJ, Sokol RJ, Whitington PF. Treatment of neonatal hemochromatosis with exchange transfusion and intravenous immunoglobulin. J Pediatr. 2009;155(4):566-71.
4. Siafakas CG, Jonas MM, Perez-Atayde AR. Abnormal Bile Acid Metabolism and Neonatal Hemochromatosis: A Subset with Poor Prognosis. J Pediatr Gastroenterol Nutr. 1997;25(3):321-6.
5. Shneider BL, Setchell KD, Whitington PF, Neilson KA, Suchy FJ. Delta 4-3-oxosteroid 5 beta-reductase deficiency causing neonatal liver failure and hemochromatosis. J Pediatr. 1994;124:234-8.
6. Brusilow SW, Maestri NE. Urea Cycle Disorders: Diagnosis, Pathophysiology, and Therapy. Adv Pediatr. 1996;43:127-70.
7. Mazzella M, Cerone R, Bonacci W, Caruso U, Munnich A, Rustin P, et al. Severe Complex I Deficiency in a Case of Neonatal-Onset Lactic Acidosis and Fatal Liver Failure. Acta Paediatr. 1997;86(3):326-9.
8. Tóth T, Bókay J, Szönyi L, Nagy B, Papp Z. Detection of mtDNA Deletion in Pearson Syndrome by Two Independent PCR Assays from Guthrie Card. Clin Genet. 1998;53(3):210-3.
9. Bakker HD, Van den Bogert C, Scholte HR, Zwart R, Wijburg FA, Spelbrink JN. Fatal Neonatal Liver Failure and Depletion of Mitochondrial DNA in Three Children of One Family. J Inherit Metab Dis. 1996;19(2):112-4.
10. Shanmugam NP, Bansal S, Greenough A, Verma A, Dhawan A. Neonatal liver failure: aetiologies and management-state of the art. Eur J Pediatr. 2010; Epub ahead of print.
11. Mbida AD, Pozzetto B, Gaudin OG, Grattard F, Le Bihan JC, Akono Y, et al. A 44,000 Glycoprotein is Involved in the Attachment of Echovirus 11 Onto Susceptible Cells. Virology Monographs. 1992;189(1):350-3.
12. Saudubray JM, Nassogne MC, de Lonlay P, Touati G. Clinical approach to inherited metabolic disorders in neonates: an overview. Semin Neonatol. 2002;7(1):3-15.
13. Pruekprasert P, Stout C , Patamasucon P. Neonatal Enterovirus Infection. J Assoc Acad Minor Phys. 1995;6(4):134-8.
14. Shafren DR, Dorahy DJ, Thorne RF, Kinoshita T, Barry RD, Burns GF. Antibody Binding to Individual Short Consensus Repeats of Decay-Accelerating Factor Enhances Enterovirus Cell Attachment and Infectivity. J Immunol. 1998;160(5):2318-23.
15. Wang J, Atchison RW, Walpusk J, Jaffe R. Echovirus hepatic failure in infancy: report of four cases with speculation on the pathogenesis. Pediatr Dev Pathol. 2001;4(5):454-60.
16. Gitlin N, Visveshwara N, Kassel SH, Byock IR, Rajani KB, Weinberg HM. Fulminant Neonatal Hepatic Necrosis Associated with Echovirus Type 11 Infection. West J Med. 1983;138(2):260-3.
17. Piraino FF, Sedmak G, Raab K. Echovirus 11 Infections of Newborns with Mortality During the 1979 Enterovirus Season in Milwaukee, Wisconsin. Public Health Rep. 1982;97(4):346-53.
18. Mostoufizadeh M, Lack EE, Gang DL, Perez-Atayde AR, Driscoll SG. Postmortem Manifestations of Echovirus 11 Sepsis in Five Newborn Infants. Hum Pathol. 1983;14(9):818-23.
19. Mertens T, Hager H, Eggers HJ. Epidemiology of an Outbreak in a Maternity Unit of Infections with an Antigenic Variant of Echovirus 11. J Med Virol. 1982;9(2):81-91.
20. Mulcahy DL, Hanlon MG, de Silva LM. A Report of a Spring-Summer Outbreak of Echovirus 11 Infection. J Infect. 1983;6:61-6.
21. Bose CL, Gooch 3rd WM, Sanders GO, Bucciarelli RL. Dissimilar Manifestations of Intrauterine Infection with Echovirus 11 in Premature Twins. Arch Pathol Lab Med. 1983;107(7):361-3.
22. Nagington J, Gandy G, Walker J, Gray JJ. Use of Normal Immunoglobulin in an Echovirus 11 Outbreak in a Special-Care Baby Unit. Lancet. 1983;2(8347):443-6.
23. Speer ME, Yawn DH. Fatal Hepatoadrenal Necrosis in the Neonate Associated with Echovirus

Types 11 and 12 Presenting as a Surgical Emergency. J Pediatr Surg. 1984;19(5):591-3.

24. Gillam GL, Stokes KB, McLellan J, Smith AL. Fulminant Hepatic Failure with Intractable Ascites Due to an Echovirus 11 Infection Successfully Managed with a Peritoneo-Venous (LeVeen) Shunt. J Pediatr Gastroenterol Nutr. 1986;5:476-80.
25. Chuang E, Maller ES, Hoffman MA, Hodinka RL, Altschuler SM. Successful Treatment of Fulminant Echovirus 11 Infection in a Neonate by Orthotopic Liver Transplantation. J Pediatr Gastroenterol Nutr. 1993;17:211-4.
26. Chambon M, Delage C, Bailly JL, Gaulme J, Dechelotte P, Henquell C, et al. Fatal Hepatic Necrosis in a Neonate with Echovirus 20 Infection: Use of the Polymerase Chain Reaction to Detect Enterovirus in Liver Tissue. Clin Infect Dis. 1997;24(3):523-4.
27. Schwarer AP, Opat SS, Watson AM, Spelman D, Firkin F, Lee N. Disseminated Echovirus Infection after Allogeneic Bone Marrow Transplantation. Pathology. 1998;29(4):424-5.
28. Ward T, Powell RM, Evans DJ, Almond JW. Serum Albumin Inhibits Echovirus 7 Uncoating. J Gen Virol. 1999;80:283-90.

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# Ultrasound diagnosis of oropharyngeal teratoma in the 17th week of pregnancy

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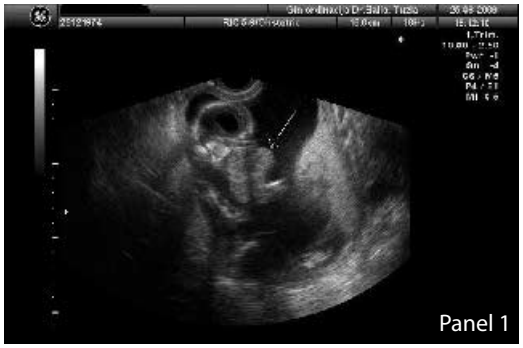
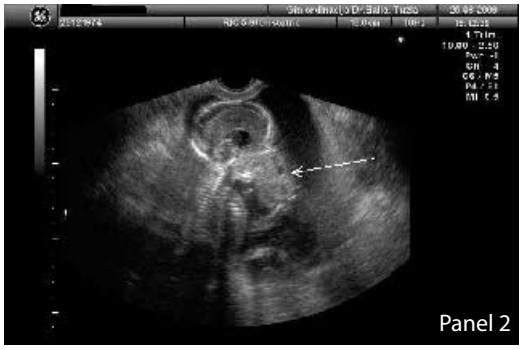
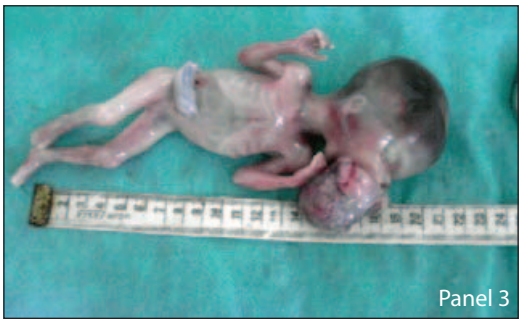

Panel 1


Panel 2

In the 17th week of pregnancy a woman aged 35 years came for an ultrasound examination due to her risky age and two previous miscarriages. By conventional transvaginal (two-dimensional – 2D) ultrasound examination it was established that the foetus was of appropriate size for the length of amenorrhea, and all vital organs had normal structure. The face could not be shown, except the forehead and orbits, due to an oval formation sized 30 x 40 mm, whose ultrasound structure was reminiscent of the tissue of the placenta (Panel 1). Shown from another angle, on the profile image of the fetus, the formation appeared as a solid tumour, with its base located in the mouth, with most of it protruding in front of the face (Panel 2). The pregnancy was terminated two days later. The aborted fetus was 22 cm long, with a solid tumour 4 cm in length protruding from the mouth (Panel 3). No other anomalies were noticed on the fetus. The ultrasound examination undertaken in the 13th week of gestation was morphologically and biometrically normal, and the thickness of the nuchal translucency was 1.3 mm. Oropharyngeal teratoma (epignathus) are very rare benign congenital tumours (0.5-1:10000) which grow in the mouth, mostly from the palate (1). The high mortality (80-100%) in the early neonatal period is caused by obstruction of the airways, although cases have been described of a positive outcome after surgery undertaken immediately after birth by an "exit procedure" (EX utero intrapartum technique).


Panel 3

1. Tsitouridis I, Sidiropoulos D, Michaelides M. Sonographic evaluation of epignathus. Hippokratia. 2009;3(1):55-7.

**Conflict of interest**: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

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# The hypertension caused by stenosis of renal arterie

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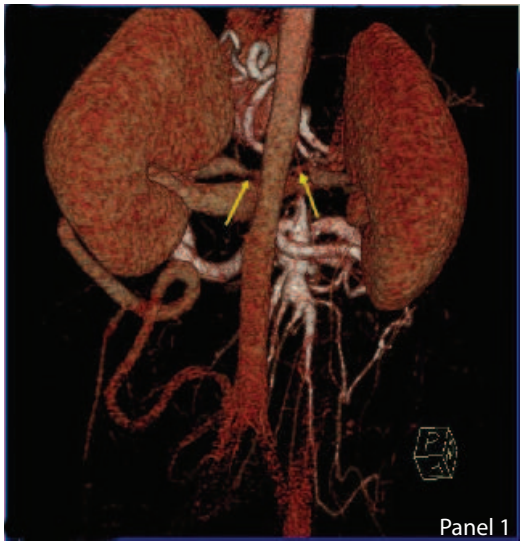
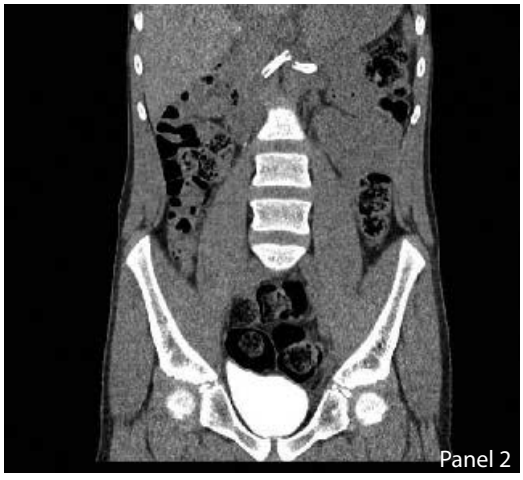


Panel 1



Panel 2

An otherwise healthy, 12.5-year-old male presented with sudden onset chest pain, was admitted to hospital for elevated blood pressure. Physical examination showed systolic heart murmur grade 2/6 in the aortic area with abdominal vascular murmur and normal peripheral pulses. Blood pressure was elevated: (left arm) 170/110 mmHg, (left leg) 185/115 mmHg. The plasma renin activity (6.5 nmol/l/h) was elevated. Renal function was normal. Doppler blood flow from both renal arteries showed a suspected mild obstruction. Magnetic resonance imaging of the kidneys and suprarenal glands were normal. Computerised tomography angiography demonstrated the normal dimension of the abdominal aorta with narrowed origins and proximal segment of both renal arteries with "string-of-beads" appearance (range occlusion of right renal artery was 90% and of the left renal artery 95%, with poststenotic dilatation) (Panel 1). Several occlusions of the origin of the celiac trunk and the origin of the superior mesenteric artery were identified. Extensive collateralization from the inferior mesenteric artery and Riolan arcus existed. The diagnosis was renovascular hypertension caused by several stenosis of both renal arteries. Medical treatment of arterial hypertension was started, and the patient underwent interventional revascularisation. Bilateral percutaneous transluminal angioplasty of the renal arteries was performed. Follow-up computerised tomography showed a good stent position (Panel 2). At two months follow-up, Doppler ultrasound examination showed a great improvement in the blood flow in both renal arteries. Medical antihypertensive therapy was gradually reduced, after three months he had an uneventful recovery, his blood pressure remained normal with no drug treatment.

**Conflict of interest**: The authors declare that they have no conflict of interest. This study was not sponsored by any external organisation.

# Corruption in Croatian health care. 1st edition

Gorjanski D, editor and associates Gajski L, Škaričić N, Sladoljev S, Marušić M, Osijek (Croatia): Fond Hipokrat – Zaklada Slagalica; 2010. 309 pages; ISBN 978-953-56131-0-7; price: kn 110.00

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**Field of medicine:** social medicine, deontology.

**Format:** Paperback monograph.

**Audience:** Undergraduate and postgraduate students of medicine, general practitioners, specialists in all specializations, teachers at Schools of Medicine, and journalists who would like to understand current policies in the health care services. Also it could be a helpful tool to policy makers and users of health care services and particularly for basic research in the field of social medicine, health care policy.

**Purpose:** To fill the gap in literature on corruption in the health care system – a major and frequent late complication of privatization in the Croatian health care system, characterized by signs of destruction of the existing health care system. The final result of this phenomenon will be the decreased survival of patients whose money, collected in health care funds on the basis of the solidarity of all citizens for the sick, goes astray and ends up in the pockets of individuals and small groups. The book provides a reference that will be valid in many settings, including teaching about corruption in medical schools and colleges. It will be helpful in the process of reestablishing the integrity of the health care system, in the process of establishing non-profit principles, a publicly available operating system, in the separation of public and private health care, in the creation of the legal preconditions for fighting corruption in the health care sector, relieve the health system from politicization, and the implementation of easily accessible medical services, in applying the medical Code of Ethics in everyday life, and in converting corruption from a conspiracy of silence to a public issue for all citizens.

**Content:** Corruption in the Croatian health sector is seen in the legalization of unnecessary costs that allow immoral (but legal) enrichment of small groups or individuals in the health care system, generated by the theft of patients' money, and is found in the majority of Croatian health institutions. Corruption as a phenomenon of the health care system is associated with irregularities in the way in which some parts of the system operate, failing to fulfill their main mission, and so destroy the system itself.

Corruption in the health care system behaves like an organic malignant disease. Using again the same image of malignant behavior – part of the system (malignant cells) does not perform the work for which it was intended, and so destroys the entire body (the host). This book is divided into 11 sections: Introductory knowledge, Corruption in health care systems, Further findings on corruption in health care, The corrupt behavior of patients, Consequences of corruption in health care, Combating corruption, Matko Marušić: My story, Supplement, Index, Literature and About the authors. The first seven sections consist of several parts. The first section, entitled "Introductory knowledge," comprehensively explores general aspects about corruption, general aspects of health care systems, the appearance of negative objectives in profitable health care systems. This section also includes consideration of the nature of non-profit health care systems, then about conflicts of interests in the Croatian health care system, about those who fight against corruption, and finally about most frequent nonsense's about health care systems. The second section is entitled "Corruption in Health Care Systems." This section focuses on corruption as an illness of the system, comparing it with an organic malignant disease. Corruption is described as a misunderstanding of the archetypal role of medicos, as a misunderstanding of their social role, as the collapse of the ideology of a profitable health care system. This section provides chapters that will be an especially relevant and helpful resource for physicians who treat patients, emphasizing the importance of the recently developed profitable criteria for organizing the Croatian health care system, the separation of pharmaceutical production from the system, separation of other organizing units from the health system, the networking of the health system with private medical practices. Also the consequences of a corruptive health care system and solutions for the difficulties produced by the corruptive health system are considered. Further chapters include the behavior of political parties in power, the feudalization (pseudo privatization) of the health care system and the concept of soft corruption in health. Finally in this section other forms of corruption are described: corruption in the patient-doctor relationship, corruption between profitable institutions and organizational groups, corruption between profit institutions and doctors, corruption between suspects of various crimes and doctors, and direct service to groups or individuals in power which is detrimental for citizens. The third section, entitled, "Further findings on corruption in health care", reveals the most recent knowledge about corruption in schools of medicine, about conflicts of interests in medicine (author Lidija Gajski), about corruption in the privatization of the Immunological Institute (author Srećko Sladoljev) and about corruption in the Croatian health system (author Nataša Škaričić). Lidija Gajski gives readers very important information abut achievements in modern pharmacotherapy, about conflicts of interests in medicine in general and particularly about conflicts of interests of patients, journalists, doctors and pharmacists, professionals in all fields, politicians, and institutions. Finally she writes about solving conflicts of interests. Nataša Škaričić gives five stories: about the budget, doctors, patients, politics and about the disciplinary responsibility of doctors and compensation. Therefore, it is obvious that this is of interest to a wide variety of physicians, politicians, patients and citizens, students and their teachers. The fourth part of the book, entitled "Corruptive behavior of patients", includes an introduction, the basis of corruptive behavior of patients, the objectives of corruptive behavior of patients, corruptive behavior techniques used by patients and the consequences of patients' corruptive

behavior. The fifth section, entitled "Consequences of corruption in health", describes the final outcomes of health care corruption. This a very educational part for readers interested in corruption in health and its consequences: impoverishment of the health system, preventing the equal and fair distribution of medical services and goods, stifling the development of private health facilities, and encouraging the development of private health facilities. Furthermore, in this section the detrimental impact on the macro economy, then the negative influence on the motivation and moral of health workers, and the negative influence on the working morals in society as a whole are described. At the end of this section there is a description of how to redirect the goals of the health system and the consequences of the butterfly effect. In the sixth section, ways of combating corruption are described through teaching about corruption in medical schools and faculties, establishing health system integrity, organizing health system on non-profit principles, making the health system a completely public enterprise. There is also a description of how to separate the private and public health systems, creating the regulative preconditions for combating health corruption, relieving the health system from politicization, creating easy access to medical services, implementation in everyday life of the Code of Medical Ethics and finally changing corruption into the public problem of all citizens. There are also stories about specific academic forms of corruption and scandals in the seventh section entitled "Matko Marušić: My story". Matko Marušić tells the story about attacks on the editors of the Croatian Medical Journal (CMJ), the chronology of attacks on CMJ editors, about the trial and attempt at defamation through psychiatric expertise. Also he describes restriction of the freedom of speech to conceal corruption and scandals, and attacks by the management of the Medical Faculty in Zagreb against the editors of the CMJ. Furthermore he tells the story of dishonorable conduct by the court of honor of the Medical Faculty in Zagreb and attempts by the management of Zagreb Medical Faculty to take over CMJ in 2009. Finally in his notes he underlines that his story was not a testimony of personal suffering, or persecution of certain people but an experience that illustrates the finesse, depth and deleterious effects of academic corruption. Additionally, the book has a Supplement, Index, Literature and About the Authors sections that help readers to read the book more easily. In the supplement, the journalist Nataša Škaričić, has kindly made available to readers the first research project, "KIT-Corruption and Transparency in the health care system of the Republic Croatia", which is a chance to examine the extent of corruption in the Croatian health care systematically.

The list of authors is quite distinguished and includes national experts in the area of corruption in Croatian health: Dr Dražen Gorjanski is an employee of the Croatian Institution for Health Insurance, and he deals with the realization of the rights of patients on the basis of current regulations and supervision of the health system; Dr Lidija Gajski, an internist, is the author of the book "Drugs or a story of deception," who began a new era in thinking about medications and treatment; Nataša Škaričić, journalist, whose name has become a trademark for investigative journalist in Croatian healthcare; Srećko Sladoljev, Ph.D., a courageous and persistent fighter against corruption, despite being exposed to threats of all kinds; and finally professor Matko Marušić, whose story in this book reveals in detail all the horrors through which not only the individual who dares to oppose corruption but also his / her family have to pass.

**Highlights and limitations:** Giving readers in one place the present state of corruption in postwar Croatia, this book pro-

vides a comprehensive, up-to-date, and relevant resource for a wide range of medical professionals, politicians, patients, students and almost all citizens, by using an interdisciplinary approach to this serious and concerning public problem. The unique feature of this book is that it was not written with the intention of accusing anyone specific for corruption; it was written with the intention of accusing all of us. By presenting the details of known corruptive mechanisms in health systems that lead to the development of the destruction of the national resources for keeping health system sustainable in the future, this book also addresses some possible solutions in combating corruption in the health system that require further investigation, the open engagement of all responsible persons and institutions to stop this negative trend. This is also very interesting for all health systems organizers dealing with this topic in the region of the former Yugoslavia after the catastrophic war from 1991 to 1995. The editor Gorjanski D, and associates Gajski L, Škaričić N, Sladoljev S, Marušić M, are pioneers in the recognition of the multi-disciplinal complexity of this public disease, and have included in writing of this book a refreshing variety of current and real resources. The book includes contributions with 210 references from different fields of medicine, journalism, and health care policy, which makes it the very first textbook dedicated to corruption in health systems. We may predict that it will find its place in the medical history of deontology and social medicine and have a strong influence on the future understanding of corruption as a public malignant disease. If research into the problem of corruption in health care systems continues to be active in the region, we may expect more brave and dedicated individuals and groups to publish new data and studies, which could update the book with new chapters and, hopefully, we can expect a further edition of this excellent book.

**Related reading:** Readers interested in corruption in health care systems unfortunately cannot find textbooks dedicated especially to this topic. However, for those who want additional reading, the book "Lijekovi ili priča o obmani," edited by Gajski L (Pergamena, Zagreb; 2009) is available, along with internet resources about corruption from Transparency International.

# International publications of authors from Bosnia and Herzegovina in Current Contents indexed publications in the second half of 2010*

**1. Bašić B, Beganović A, Skopljak-Beganović A, Samek D. Occupational exposure doses in interventional procedures in Bosnia and Herzegovina. Radiat Prot Dosimetry. 2011; 144(1-4):501-4. Epub 2010 Dec 7.**

*Radiation Protection Centre, Institute of Public Health of Federation of Bosnia and Herzegovina, Maršala Tita 9, Sarajevo, Bosnia and Herzegovina.*

Monitoring of occupationally exposed workers in Bosnia and Herzegovina started in 1960s and it was interrupted in 1992. Dosimetry service resumed in 1999 when the International Atomic Energy Agency provided Radiation Protection Centre with Harshaw 4500 Thermoluminescence dosemeter (TLD)-reader and the first set of TLDs. The highest doses are received by professionals working in interventional procedures (radiology, cardiology, gastroenterohepatology etc.). Number of these procedures is increasing each year (just in cardiology this increase is 24 % per year). Results from two TLDs are used to estimate effective dose. One is worn under the apron (chest level), and the other above (neck level). Calculation is performed using Niklason's methodology. Total number of occupationally exposed persons in interventional radiology is 90. The collective dose they receive is 67 person mSv, while the mean dose is 0.77 mSv (based on 12-month period). Highest doses are received by physicians (3.7 mSv), while radiographers and nurses receive 2.1 and 1.9 mSv respectively. This occurs due to the fact that physicians stand closer to the source (patient). The lead apron is proven to be the most efficient radiation protection equipment, but, also, lead thyroid shield and glasses can significantly lower doses received by professionals. The use of this equipment is highly recommended.

**2. Brennen T, Hasanović M, Zotović M, Blix I, Skar AM, Prelić NK, Mehmedović I, Pajević I, Popović N, Gavrilov-Jerković V. Trauma exposure in childhood impairs the ability to recall specific autobiographical memories in late adolescence. J Trauma Stress. 2010 Apr;23(2):240-7.**

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J. M. G. Williams (1996) predicted that exposure to potentially traumatizing events at an early age would give rise to overgeneral recall from autobiographical memory, i.e., recall of general rather than specific events, and that in adolescence this tendency would be uncorrelated with psychopathological symptoms, e.g., depression. This was supported by two studies where war-exposed Bosnian adolescents produced significantly fewer specific autobiographical memories than a Norwegian control group, as did bombing-exposed Serbian adolescents compared to nontrauma-exposed Serbians. No significant correlations were found between autobiographical memory specificity and measures of depression, anxiety, dissociation or impact of trauma, which is consistent with Williams' idea that an overgeneral memory retrieval strategy is at first protective, and a risk factor for depression only upon reaching adulthood.

**3. Čengić B, Resić H, Spasovski G, Avdić E, Alajbegović A. Quality of sleep in patients undergoing hemodialysis. Int Urol Nephrol. 2010 Dec 9. [Epub ahead of print]**

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*Data for this survey were collected from PubMed database using the keywords Bosnia and Herzegovina and 2010.

BACKGROUND: Sleeping problems are common in hemodialysis (HD) patients. The aim of this study was to determine sleep quality (SQ) in HD patients and to evaluate its relationship with patient's quality of life (QoL), sociodemographic and laboratory data. METHODS: Two hundred HD patients from the Hemodialysis Clinic, Bosnia and Herzegovina, were enrolled in the study. There were 122 men and 78 women with a mean age of 56.8 ± 14.3 (range 20-85) years and a mean HD duration of 62.6 ± 57.0 months. We used the Pittsburgh Sleep Quality Index (PSQI) and the Health Survey for Dialysis Patients (SF-36). RESULTS: Seventy-three percent of patients showed a poor SQ response. The average sleep latency of patients was 48.2 min, and the average sleep duration was 4.9 h. Ninety-eight percent of patients experienced some sort of sleep disturbances on weekly basis. The most common sleep disturbances were insomnia (84.5%), day and night sleep reversal (39.0%), excessive daytime sleepiness (EDS) (34.0%), nightmares (25%) and restless legs syndrome (RLS) (20.5%). The most frequent causes of sleep disorders were snoring (47%), pain (35%), daytime napping (34%), breathing problems (30%) and pruritus (28%). Ninety-three percent of patients experienced daytime dysfunction and 46.5% of them were taking sleep medications. Younger patients, employed patients and patients in 3rd HD shift showed significantly better SQ compared to the others. Compared with good sleepers, poor sleepers were more frequently on conventional HD and had higher serum phosphate and PTH and significantly lower Hb. CONCLUSION: The poor SQ in our HD population significantly correlated with their QoL.

**4. Ćavar I, Kelava T, Vukojević K, Saraga-Babić M, Čulo F. The role of prostaglandin E2 in acute acetaminophen hepatotoxicity in mice. Histol Histopathol. 2010 Jul;25(7):819-30.**

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Prostaglandin E2 (PGE2), which is synthesized by many cell types, has a cytoprotective effect in the gastrointestinal tract and in several other tissues and cells. On the other hand, overdose or chronic use of a high dose of acetaminophen (Paracetamol, APAP) is a major cause of acute liver failure in the western world. These observations prompted us to investigate whether PGE2 plays a role in host defence to toxic effect of APAP. (CBAT6T6xC57Bl/6)F1 hybrid mice of both sexes were intoxicated with a single lethal or high sublethal dose of APAP, which was administered to animals by oral gavage. Stabile analogue of PGE2, 16,16-dimethyl PGE2 (dmPGE2), or inhibitor of its production, CAY10526, were given intraperitoneally (i.p.) 30 minutes before or 2 hours after APAP administration. The toxicity of APAP was determined by observing the survival of mice during 48 hours, by measuring concentration of alanine-aminotransferase (ALT) in plasma 20-22 hours after APAP administration and by liver histology. The results have shown that PGE2 exhibits a strong hepatoprotective effect when it is given to mice either before or after APAP, while CAY10526 demonstrated mainly the opposite effect. Immunohistochemical or immunofluorescent examinations in the liver tissue generally support these findings, suggesting that PGE2 inhibited APAP-induced activation of nuclear factor kappa B (NF-kappaB). Similarly, PGE2 down regulated the activity of inducible nitric oxide synthase (iNOS), which was up regulated by APAP. Thus, by these and perhaps by other mechanisms, PGE2 contributes to the defence of the organism to noxious effects of xenobiotics on the liver.

**5. Ćenanović M, Pojskić N, Kovačević L, Džehverović M, Čakar J, Musemić D, Marjanović D. Diversity of Y-short tandem repeats in the representative sample of the population of Canton Sarajevo residents, Bosnia and Herzegovina. Coll Antropol. 2010 Jun;34(2):545-50.**

*Institute for Genetic Engineering and Biotechnology, Sarajevo University, Sarajevo, Bosnia and Herzegovina.*

In our previous population study, we have used twelve Y-chromosomal short tandem repeats loci incorporated in the PowerPlex Y System to determine Y-STR diversity in B&H human population. With intent to obtain additional verification of the previously obtained results as well as to establish specific reference for a local B&H population, we have decided to test DNA samples collected from 100 unrelated healthy male Canton Sarajevo residents (from Sarajevo region) for the same twelve Y-linked short tandem repeats loci. Qiagen DNeasy Tissue Kit (Qiagen, GmbH, Hilden, Germany) was used for DNA extraction from buccal swabs and PowerPlex Y System (Promega Corp., Madison, WI) has been used to simultaneously amplify Y-STR loci by PCR. PowerPlex Y System includes 12 STR loci: DYS19, DYS385a, DYS385b, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439. The total PCR reaction volume was 5 microL. PCR amplifications were carried out in PE GeneAmp PCR System Thermal Cycler (ABI). Electrophoresis of the amplification products was preformed on an ABI PRISM 310 genetic analyzer (ABI, Foster City, CA) according to the manufacturer's recommendations. The raw data were compiled and analyzed using the accessory software: ABI PRISM Data Collection Software and Genemapper version 3.2. In addition, we have compared the obtained "Sarajevo" dataset with the data previously generated for the en-

tire Bosnian and Herzegovinian population, as well as with the available data on geographically close (neighboring) European populations. The results of this study will be used as guidelines in additional improving of research into genetic relationship among recent local B&H populations, both isolated and open, which is a long-term project in our country.

**6. Dujić T, Ostanek B, Marc J, Čaušević A, Mlinar B. Optimization of high-resolution melting analysis for simultaneous genotyping of two 11β-hydroxysteroid dehydrogenase type 1 gene polymorphisms. Genet Test Mol Biomarkers. 2011 Jan-Feb;15(1-2):43-9. Epub 2010 Nov 30.**

*Department of Biochemistry and Clinical Analysis, Faculty of Pharmacy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.*

BACKGROUND: Polymorphisms in HSD11B1, the gene encoding 11β-hydroxysteroid dehydrogenase type 1 enzyme, have been associated with obesity, metabolic syndrome, and type 2 diabetes. In this study, we present an optimized high-resolution melting (HRM) method for genotyping two common polymorphisms of the HSD11B1 gene: rs846910: G>A and rs45487298: insA. METHODS: One hundred DNA samples from patients with polycystic ovary syndrome and healthy controls were genotyped by HRM. The results were compared with those obtained with classic polymerase chain reaction followed by restriction fragment length polymorphism analysis. RESULTS: Various approaches were used during HRM specificity optimization. With the optimized method, genotyping accuracy of 100% was achieved. CONCLUSIONS: HRM analysis is a fast, simple, and cost-effective method compared with the alternative genotyping approaches. The work required for optimizing the method (improvement of specificity) is minor compared to the advantages.

**7. Hukić M, Valjevac A, Tulumović D, Numanović F, Heyman P. Pathogenicity and virulence of the present hantaviruses in Bosnia and Herzegovina: the impact on renal function. Eur J Clin Microbiol Infect Dis. 2011 Mar;30(3):381-5. Epub 2010 Oct 23.**

*Institute of Clinical Microbiology, Clinical Center University of Sarajevo, Sarajevo, Bosnia and Herzegovina.*

Dobrava (DOBV) and Puumala (PUUV) viruses are endemic throughout the Balkans and cause haemorrhagic fever with renal syndrome (HFRS). The aim of this study was to assess the impact of two different hantaviruses on renal function in HFRS patients during the acute stage of illness. We also aimed to assess the DOBV and PUUV distribution between symptomatic HFRS patients and asymptomatic hantavirus antibody-positive subjects. The study included 264 symptomatic HFRS patients and 63 asymptomatic hantavirus antibody-positive healthy subjects. In our study, 131 (49.6%) HFRS patients were regarded as PUUV- and 69 (26.1%) as DOBV-infected patients, while in 64 (24.2%) of HFRS patients that showed all clinical and biochemical signs of HFRS, the causal hantavirus could not be determined with commercially available tests. DOBV-infected patients were associated with more requirements for haemodialysis treatment, lower diuresis and higher serum creatinine and urea values compared to PUUV-infected patients. PUUV was significantly predominant in asymptomatic hantavirus antibody-positive subjects (69.8%) compared to HFRS patients. DOBV was present in 17.5% of asymptomatic subjects and, interestingly, the preferential hantavirus serotype could not be determined in 12.7% of the asymptomatic antibody-positive subjects.

**8. Imamović G, Zerem E, Osmanović E. Kidney graft outcomes in living-related transplantation have improved with substitution of basiliximab and mycophenolate mofetil for antithymocyte globulin and azathioprine: a single center retrospective cohort study. Eur J Intern Med. 2010 Dec;21(6):524-9. Epub 2010 Oct 12.**

*University Clinical Center Tuzla, Department of Nephrology and Dialysis, Tuzla, Bosnia and Herzegovina.*

BACKGROUND: Despite significant reduction in acute rejection rates and improvements in one year kidney allograft outcomes over the past decade, there is an overall lack of improvement in long-term allograft outcomes. We conducted this study to evaluate whether immunosuppressive regimens involving basiliximab and mycophenolate mofetil improved allograft outcomes in living-related kidney transplantation beyond the first year. METHODS: In a retrospective cohort study we analyzed kidney graft survival, acute rejection-free survival, kidney function, delayed graft function, and primary non-function in patients receiving an immunosuppressive regimen that included basiliximab and mycophenolate mofetil (group A), and compared to patients receiving antithymocyte globulin and azathioprine (group B). The rest of the treatment protocols remained the same, including cyclosporine A and steroids in both groups. RESULTS: Seven-year graft survival rates in groups A and B were 83% and 44%, respectively (p=0.005), 7-year acute rejection-free survival rates were 82% and 53%, respectively (p=0.03), kidney function was better (p=0.004) and its deterioration rate was lower (p=0.006) in patients receiving regimen A. In group A 1 primary non-function event was observed in con-

trast to 4 composite events of delayed graft function and primary non-function in group B (p>0.05). CONCLUSION: Long-term graft outcomes in living-related kidney transplantation have improved with substitution of basiliximab and mycophenolate mofetil for antithymocyte globulin and azathioprine in immunosuppressive protocols.

**9. Latić F, Zerem E. Phytobezoar of the stomach - laparoscopic approach. Dig Surg. 2010;27(4):338. Epub 2010 Aug 3.**

No abstract available.

**10. Muratbegović AA, Marković N, Zukanović A, Kobašlija S, Dragaš MS, Jurić H. Oral health related to demographic features in Bosnian children aged six. Coll Antropol. 2010 Sep;34(3):1027-33.**

*Department of Preventive and Paediatric Dentistry, Faculty of Dentistry, Sarajevo University, Sarajevo, Bosnia and Herzegovina.*

The main aim of this paper is to present epidemiological indicators of oral health among six-year olds in Bosnia and Herzegovina (BH) and to analyze values of dmft index and dental treatment needs in order to identify differences in parts of the country. Another aim is to identify the needs from the public oral health care system in Bosnia and Herzegovina related to early permanent dentition by analyzing the condition of first permanent molars (FPM) as an indicator of oral health of permanent dentition. Survey was carried out in 2004 in 8 cantons of the Federation of BH (FBH) and in the Republic of Srpska (RS). Final sample included 560 participants aged six (mean 6.2, SD +/- 0.87). One dental team clinically examined all participants according to WHO methodology and criteria. The parameters used were: dmft index, DMFT index of first permanent molars (FPM), presence of sealants and treatment needs. A questionnaire about oral health habits had been administered. Dmft was 6.71 in that the d-component constituted the major part of the index. DMFT index of FPM was 0.61 (SD +/- 1.08). Percentage of caries free participants aged 6 was 6.8%. Average number of FPM with fissure sealants in BH was 0.25 (SD +/- 0.78). Significant demographic differences in dmft index, DMFT FPM and treatment needs were identified. Most participants (48.5%) had their first dental visit between the ages of five and seven. National oral health goal for Bosnia and Herzegovina should be to develop and implement disease prevention programs based on education of both parents and dental practitioners. It is necessary to improve access to dental care and shift focus from curative to preventive procedures. It is also necessary to set real goals for improvement of oral health which can be achieved within a desired time frame, as well as to precisely define measures to be taken.

**11. Obradović Z, Velić R. Epidemiological characteristics of brucellosis in Federation of Bosnia and Herzegovina. Croat Med J. 2010 Aug 15;51(4):345-50.**

*Public Health Institute, Canton Sarajevo, Sarajevo, Bosnia and Herzegovina.*

AIM: To analyze the frequency and distribution of human brucellosis in the Federation of Bosnia and Herzegovina in the period 2001-2008, and measures and activities undertaken for prevention and control of the disease. METHOD: In this descriptive, retrospective study, we used official reports on infectious diseases from public health institutes at the federal and cantonal level, as well as epidemiological surveys. For comparison with animal brucellosis cases, we used the distribution data from veterinary surveillance. RESULTS: Since 2001, the number of infected people has rapidly increased and brucellosis has become a very important public health problem. In the period 2001-2008, there were 1639 human brucellosis cases and the number of cases increased every year. The morbidity rate over the study period ranged from 3.8 to 33.4 per 100,000 inhabitants. According to epidemiological surveys, in villages human brucellosis was transmitted mostly by contact with infected animals and their products, and in cities by consumption of dairy products made from contaminated, unpasteurized milk. When test-and-slaughter control approach was used, the prevalence of seropositive livestock was 4.6% and approximately 70,000 animals were slaughtered after testing between 2001 and 2008. From 1 June 2009, this approach was replaced with mass vaccination of sheep and goats. CONCLUSION: The large number of human brucellosis cases and seropositive livestock poses a very serious problem for Bosnia and Herzegovina. The solution may be the introduction of mass vaccination.

**12. Odobašić A, Pašić A, Iljazović-Latifagić E, Arnautalić L, Odobašić A, Idrizović E, Dervišefendić M, Dedić L. Perineal endometriosis: a case report and review of the literature. Tech Coloproctol. 2010 Nov;14 Suppl 1:S25-7.**

*Surgery Clinic, University Clinical Center Tuzla, Trnovac bb, Tuzla, Bosnia and Herzegovina.*

Endometriosis is the presence of endometrial-like tissue outside the uterine cavity, which most commonly affects peritoneal surfaces, ovaries and uterine ligaments. Even it is quite rare, endometriosis may

affect the vulva, vagina, rectovaginal septum or perineal region, generally secondary to obstetric or surgical trauma. In this case report, we present a patient with perineal endometriosis in an episiotomy scar. Diagnostic tools used included transvaginal and endorectal ultrasonography (USG), magnetic resonance imaging (MRI) and biopsy. The endometriotic mass was wide-excised together with episiotomy scar. The recovery was uneventful with excellent functional and esthetic results. Six months after operation, woman is asymptomatic. According to the literature and our own experience, wide excision of endometriotic tissue seems to be the best chance of cure with satisfactory functional results and should be recommended.

**13. Omeragić J. Ixodid ticks in Bosnia and Herzegovina. Exp Appl Acarol. 2011 Mar;53(3):301-9. Epub 2010 Oct 22.**

*Department of Parasitology and Invasive Diseases, Veterinary Faculty Sarajevo, University of Sarajevo, Zmaja od Bosne 90, Sarajevo, Bosnia and Herzegovina.*

Ticks of the Ixodidae family represent an enormous threat to human and animal health. From January to December 2004, a total of 10,050 ixodid ticks were collected from 26 areas in Bosnia and Herzegovina and determined to the species level. Ticks were collected from dogs, sheep, cows, goats, rodents, humans and plants. Ixodes ricinus was the most prevalent species, followed by Dermacentor marginatus marginatus, Rhipicephalus bursa, Hyalomma marginatum marginatum, Rhipicephalus sanguineus, Haemaphysalis punctata, Dermacentor reticulatus and Ixodes hexagonus.

**14. Petričević J, Forempoher G, Ostojić Lj, Mardešić-Brakus S, Anđelinović Š, Vukojević K, Saraga-Babić M. Expression of nestin, mesothelin and epithelial membrane antigen (EMA) in developing and adult human meninges and meningiomas. Acta Histochem. 2010 Oct 11. [Epub ahead of print]**

*Department of Pathology, Cytology and Forensic Medicine, University Clinical Hospital Mostar, Kralja Tvrtka bb, Mostar, Bosnia and Herzegovina; Department of Histology and Embryology, School of Medicine, University of Mostar, Bijeli brijeg bb, Mostar, Bosnia and Herzegovina.*

The spatial and temporal pattern of appearance of nestin, epithelial membrane antigen (EMA) and mesothelin proteins was immunohistochemically determined in the cells of normal developing and adult human meninges and meningiomas. Human meninges developed as two mesenchymal condensations in the head region. The simple squamous epithelium on the surface of leptomeninges developed during mesenchymal to epithelial transformation. Nestin appeared for the first time in week 7, EMA in week 8, while mesothelin appeared in week 22 of development. In the late fetal period and after birth, nestin expression decreased, whereas expression of EMA and mesothelin increased. EMA appeared in all surface epithelial cells and nodules, while mesothelin was found only in some of them. In adult meninges, all three proteins were predominantly localized in the surface epithelium and meningeal nodules. In meningothelial meningiomas (WHO grade I), EMA was detected in all tumor cells except in the endothelial cells, mesothelin characterized nests of tumor cells, while nestin was found predominantly in the walls of blood vessels. The distribution pattern of those proteins in normal meningeal and tumor cells indicates that nestin might characterize immature cells, while EMA and mesothelin appeared in maturing epithelial cells. Neoplastic transformation of these specific cell lineages contributes to the cell population in meningiomas.

**15. Poljac E, Montagne B, de Haan EH. Reduced recognition of fear and sadness in post-traumatic stress disorder. Cortex. 2010 Oct 21. [Epub ahead of print]**

*Laboratory of Experimental Psychology, University of Leuven, Leuven, Belgium; Department of Psychology, International University of Sarajevo, Sarajevo, Bosnia and Herzegovina.*

Post-traumatic stress disorder (PTSD) is associated with impairments in emotional experience and expression. The current study examined the recognition of emotional facial expressions in PTSD patients and matched healthy controls, both in terms of accuracy and sensitivity. The task involved short video clips of a neutral face changing (morphing) into one of the six basic emotions (happiness, anger, fear, surprise, disgust and sadness). Clips differed in length, with short clips terminating at 20% of maximum emotional intensity, and the longest ones ending with a full-blown expression. We observed a specific impairment in the PTSD group for recognizing the emotions fear and sadness. This result was observed via a reduced accuracy and a decreased sensitivity for these emotions. We discuss the observed altered affective processing and its possible clinical implications.

**16. Prohić A, Kasumagić-Halilović E. Identification of Malassezia species from immunocompetent and immunocompromised patients with seborrheic dermatitis. Eur Rev Med Pharmacol Sci. 2010 Dec;14(12):1019-23.**

*Department of Dermatovenerology, University Clinical Center of Sarajevo, Sarajevo, Bosnia and Herzegovina.*

BACKGROUND AND OBJECTIVES: Differences in prevalence, clinical and histological manifestations between seborrheic dermatitis (SD) in immunocompetent and immunocompromised patients suggest that these two populations might also differ in a spectrum of isolated Malassezia species. The purpose of our study was to analyse the prevalence of Malassezia species in immunocompromised and non-immunocompromised patients with SD and to examine if the range of isolated yeasts varies between these two study groups. PATIENTS AND METHODS: Specimens were taken from 50 patients with SD: 30 without any underlying disease and 20 with confirmed immunosuppression. The samples were obtained by scraping the skin surface of the scalp and trunk lesions of all subjects and then incubated on modified Dixon agar. The yeasts isolated were identified by their morphological and physiological properties according to Guillot et al method. RESULTS: In both groups, the most commonly isolated species from the scalp lesions were Malassenzia restricta and Malassenzia globosa, the later being the most common species isolated from lesional trunk skin. No significant differences were found between immunocompromised and immunocompetent patients from both sampled sites. CONCLUSIONS: There is no difference in the distribution of Malassezia species isolated from SD lesions between immunocompetent and immunocompromised patients. However, the much higher percentage of positive cultures in immunocompromised patients confirms that impaired cellular immunity may facilitate fungal survival on the skin.

**17. Redžić S. Use of wild and semi-wild edible plants in nutrition and survival of people in 1430 days of siege of Sarajevo during the war in Bosnia and Herzegovina (1992-1995). Coll Antropol. 2010 Jun;34(2):551-70.**

*Academy of Sciences and Arts of Bosnia and Herzegovina, Sarajevo, Bosnia and Herzegovina.*

This study is a systematic overview of data on use of wild and semi-wild edible plants in nutrition of people in 1430 days of the siege of Sarajevo during aggression on Bosnia and Herzegovina (1992-1995). The author of this study spent all that time in Sarajevo. In 1993, the author prepared a survival program for people that included usage of edible wild plants. In addition, he conducted a detailed survey, including special interviews, on 630 people of average age 37.4 years (55% residential inhabitants, the rest were refuges), 310 males and the rest were females. According to survey, 91 species of mostly wild plants and three species of fungus were used: Küchneromyces mutabilis, Armillariella mellea and Coprinus comatus. Wild vegetables included 49 species, spices 24, wild fruits 16, and 2 species of bread-plants. They belong to 26 plants communities, and grew on 24 different habitats (urban surfaces, river coasts, low forest and scrubs, meadows and rocky grasslands). The 156 plant parts (leaves, young branches, fruit, flower, seed, root and rhizome) from 91 plant species were used. Vegetables were dominant category of use (soups, pottages, sauces) with 80 ways of preparation (30.53%), then salads 41 (15.65%), spices 39 (14.89%), different beverages 38 (14.50%), sweets 21 (8.02%), nutritive teas 15 (5.73%), and other preparations. In order to improve conventional food (war pasta, rice, lentils, old beans) people used spices made from different wild plants.

**18. Sesar K, Šimić N, Barišić M. Multi-type childhood abuse, strategies of coping, and psychological adaptations in young adults. Croat Med J. 2010 Oct 15;51(5):406-16.**

*Center for Mental Health, Health Center, Dr J Grubišića 11, Široki Brijeg, Bosnia and Herzegovina.*

AIM: To retrospectively analyze the rate of multi-type abuse in childhood and the effects of childhood abuse and type of coping strategies on the psychological adaptation of young adults in a sample form the student population of the University of Mostar. METHODS: The study was conducted on a convenience sample of 233 students from the University of Mostar (196 female and 37 male), with a median age of 20 (interquartile range, 2). Exposure to abuse was determined using the Child Maltreatment Scales for Adults, which assesses emotional, physical, and sexual abuse, neglect, and witnessing family violence. Psychological adaptation was explored by the Trauma Symptom Checklist, which assesses anxiety/depression, sexual problems, trauma symptoms, and somatic symptoms. Strategies of coping with stress were explored by the Coping Inventory for Stressful Situations. RESULTS: Multi-type abuse in childhood was experienced by 172 participants (74%) and all types of abuse by 11 (5%) participants. Emotional and physical maltreatment were the most frequent types of abuse and mostly occurred together with other types of abuse. Significant association was found between all types of abuse (r=0.436-0.778, P<0.050). Exposure to sexual abuse in childhood and coping strategies were significant predictors of anxiety/depression (R(2)=0.3553), traumatic symptoms (R(2)=0.2299), somatic symptoms (R(2)=0.2173), and sexual problems (R(2)=0.1550, P<0.001). CONCLUSION: Exposure to multi-type abuse in childhood is a traumatic experience with long-term negative effects. Problem-oriented coping strategies ensure a better psychosocial adaptation than emotion-oriented strategies.

**19. Spasojević GD, Malobabić S, Suščević D, Stijak L, Nikolić V, Gojković I. Morphological variability of the subcallosal area of man. Surg Radiol Anat. 2010 Aug 21. [Epub ahead of print]**

*Department of Anatomy, Faculty of Medicine, University of Banja Luka, Banja Luka, Bosnia and Herzegovina.*

BACKGROUND: Knowledge of morphology of human anterior cingulate and medial frontal cortex related to the knee of corpus callosum is important in the diagnostics and neurosurgical treatment. Neuroimaging studies did not provide a clear picture of this region, what is also caused by terminological inconsistencies. It is not always clear what is actually defined under the terms subcallosal area, subcallosal cingulate gyrus, subcallosal gyrus, subcallosal region, or subgenual prefrontal gyrus. Our study of subcallosal area provides morphological data useful for recent imaging studies. METHODS: Digital photographs (42 formaline fixed brains: 26 males, 16 females) were used in morphometry (surfaces and lengths) of subcallosal area. We defined all its boundaries: superoanterior, anterior, posterior, and inferior one. If anterior subcallosal sulcus, as anterior delineation of subcallosal area was absent, we used intercommissural line system. RESULTS: Anterior subcallosal sulcus we found in 86.9% of cases. Its variations were classified into four types: Type 1-vertical or slightly oblique sulcus (most often type on right hemispheres-30.1%), Type 2-in form of letter C or inverted letter C (most often type on left hemispheres-42.9%), Type 3-sulcus in form of letter S or inverted letter S, and Type 4-sulcus not belonging to any previous type. Mean surface of subcallosal area was in males 2.65 cm(2) (right hemispheres), and on left 2.70 cm(2). In females on right it was 2.56 cm(2), and on left 2.55 cm(2). All measured values were not significantly different (left/right; males/females). CONCLUSIONS: Accurate neuroanatomical localization requires exact determination of boundaries of subcallosal area. Therefore, standardized criteria were proposed for definition of subcallosal area.

**20. Vidić A, Ilić Z, Deljkić D, Adrović F. Exposure of workers in Tusnica coal mine. Radiat Prot Dosimetry. 2011;144(1-4):672-4. Epub 2010 Oct 19.**

*Institute for Public Health of FBiH, M. Tita 9, Sarajevo, Bosnia and Herzegovina.*

The aim of this paper is to identify potential exposure of the workers in the coal mine Tusnica. The results of the investigation showed increased activity of brown coal up to 1060±88 Bq kg(-1) for (238)U, 976±30 Bq kg(-1) for (226)Ra and 118±31 Bq kg(-1) for (232)Th. Dose rate measurements ranged from 0.07 to 0.25 μSv h(-1). The annual effective dose, taking into account external exposure to ambient gamma radiation and internal exposure due to inhalation of the resuspended dust, would be 1.6 mSv a(-1). The results presented lead to the conclusion that Tusnica coal mine contains brown coal with significant radioactivity, indicating that the working hours in the area should be regulated and the use of respiratory protective equipment is obligatory.

**21. Vidić D, Ćavar S, Šolić ME, Maksimović M. Volatile constituents of two rare subspecies of Thymus praecox. Nat Prod Commun. 2010 Jul;5(7):1123-6.**

*University of Sarajevo, Faculty of Science, Department of Chemistry, Sarajevo, Bosnia and Herzegovina.*

Hydrodistilled essential oil and the corresponding headspace volatiles of Bosnian wild growing Thymus praecox ssp. polytrichus (A. Kern. Ex Borbàs) Jalas and Thymus praecox ssp. skorpilii (Velen.) Jalas were subjected to capillary GC-MS analysis. This work presents a detailed essential oil analysis of these two rare Thymus subspecies from Bosnia, as well as the very first report on their headspace composition. Eighty-seven volatile constituents were identified in four samples. Two alcohols were the major constituents in the essential oil of T. praecox ssp. polytrichus, a monoterpene, linalool (13.9%), and a sesquiterpene, (E)-nerolidol (10.4%), while linalyl acetate (36.7%) and linalool (22.7%) were the most abundant volatiles in the corresponding headspace sample. Oxygenated monoterpenes (57.5%) predominate in the essential oil of T. praecox ssp. skorpilii with linalyl acetate (28.7%) and linalool (14.4%) as the main representatives. Headspace sample of this subspecies also showed richness in linalyl acetate (52.4%), while the second most abundant compound was alpha-pinene (14.5%), a monoterpene hydrocarbon.

**22. Vranić S, Frković-Grazio S, Lamovec J, Serdarević F, Gurjeva O, Palazzo J, Bilalović N, Lee LM, Gatalica Z. Adenoid cystic carcinomas of the breast have low Topo IIα expression but frequently overexpress EGFR protein without EGFR gene amplification. Hum Pathol. 2010 Nov;41(11):1617-23. Epub 2010 Aug 4.**

*Department of Pathology, Clinical Center of the University of Sarajevo, Sarajevo, Bosnia and Herzegovina.*

Adenoid cystic carcinoma of the breast is a rare subtype of breast cancer with basal-like features. Published studies on breast adenoid cystic carcinoma are limited, resulting in relatively scarce information on the value of predictive tumor markers. We studied

20 primary cases of adenoid cystic carcinoma of the breast for expression of estrogen receptor, progesterone receptor, androgen receptor, epidermal growth factor receptor, HER-2/neu, and topoisomerase IIα using immunohistochemistry and fluorescent in situ hybridization methods. Estrogen and progesterone receptor expression were detected in 1 case each. All tumors were uniformly negative for Her-2/neu expression. Androgen receptor and topoisomerase IIα expression were weakly positive in three cases and 7 cases, respectively. Epidermal growth factor receptor overexpression was detected in 13 cases (65% of all cases). Amplification of TOP2A or HER-2/neu gene was not detected in any of the cases. Our study shows that the majority of adenoid cystic carcinomas of the breast do not overexpress Her-2/neu, topoisomerase IIα, or estrogen receptor, and thus, they are unlikely to respond to therapies targeting these proteins. However, these tumors frequently over-express epidermal growth factor receptor, indicating a potential benefit from anti-epidermal growth factor receptor therapy for patients with advanced adenoid cystic carcinomas of the breast.

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**23. Zerem E. Reply to: draining sterile fluid collections in acute pancreatitis? Primum non nocere! Surg Endosc. 2011 Mar;25(3):979-80. Epub 2010 Jul 7.**

No abstract available.

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**24. Zerem E, Imamović G, Jusufović R. Is total splenectomy unavoidable in the treatment of splenic benign cyst? Dig Surg. 2010;27(4):336; author reply 337. Epub 2010 Aug 3.**

No abstract available.

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**25. Zerem E, Imamović G, Omerović S, Ljuca F, Haračić B. Percutaneous treatment for symptomatic pancreatic pseudocysts: Long-term results in a single center. Eur J Intern Med. 2010 Oct;21(5):393-7. Epub 2010 Jul 24.**

*University Clinical Center Tuzla, Trnovac bb, Tuzla, Bosnia and Herzegovina.*

PURPOSE: The aim of the study was to present and evaluate the long-term results of percutaneous catheter drainage (PCD) in the treatment of symptomatic pancreatic pseudocysts (PPC). METHODS: We performed a retrospective analysis of 128 patients with 140 PPC treated by PCD from 01/01/1989 to 12/31/2008. All procedures were performed under ultrasound control. Surgical treatment was planned only in patients with failed PCD. The patients were followed up monthly with sonography for 12 months. The primary outcome was conversion rate to surgery. Secondary outcomes were disappearance of PPC, requirement for additional treatment, length of hospital stay, and catheter dwell time. RESULTS: During the follow-up, 42 of the 140 cysts (30%) recurred. 19/42 cysts were small and they were followed up without intervention and 23/42 cysts required further intervention. These patients were offered a second attempt but 5 patients declined it and they chose to undergo surgery. The remaining 18 patients underwent second PCD and 10 of them developed recurrence. All of them underwent third PCD and 6 of the 10 patients developed recurrences. Four and 2 of them necessitated surgery and follow-up, respectively. In total, 9 of the 128 patients (7%) underwent surgery during the study period. Medians (interquartile ranges) of hospital stay and catheter dwell time were 19 (14-23) and 23 (15-43) days, respectively. There were no complications related to the procedure. CONCLUSION: PCD is a safe and effective management for PPC, with low recurrence rates and complication rate and it can eliminate the need for surgery in majority of patients with PPC.

by Nerma Tanović

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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 and medical 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. On the grounds of poor English manuscripts may be sent back to an author for rewriting or language correction.

**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 Arial. All pages must be numbered. Prepare texts with double spacing (except those of tables, which are made with table tools in Word or in Excel). 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** (2000 characters with spaces per page), the limits are as follows:

- **Review** - up to 24 pages (maximum count 48000 characters with spaces),
- **Original research** or **clinical report** - up to 20 pages (maximum count 40000 characters with spaces),

- **Statistical and methodological compilations** – up to 16 pages (maximum count 32000 characters with spaces),
- **Case reports** and **letters** – up to 3 pages (maximum count 6000 characters with spaces),
- **Images in clinical medicine** – maximum 4 figures, the text of the legend up to 250 words (maximum count 1900 characters with spaces), up to 3 authors and no more than two references.

**Electronic copy**. Please observe the following instructions when preparing the electronic copy: (1) label the disk with the name of the author and the title of the article; (2) ensure that the written text is identical to the electronic copy; (3) arrange the main text as a single file; do not split it into smaller files; (4) only when necessary, use italic, bold, subscript, and superscript formats; do not use other electronic formatting facilities; (5) do not use the hyphen function at the end of lines; (6) avoid the use of footnotes; (7) distinguish the numbers 0 and 1 from the letters O and l; (8) avoid repetition of data in the tables, figures and text. Please indicate the software programs used to generate the files. Acceptable program files include MS Word, Excel, JPEG, GIF, TIFF. (Please do not send PDF files).

**Organization of the text**. The text of original articles is usually divided into sections with the following headings: Introduction, Materials (Patients) and methods, Results, Discussion and Conclusion. This 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 more flexible structure of the text. If possible, use standard abbreviations. Non-standard abbreviations should be defined when first used in the text.

## Title page (the first page)

The title page should carry the following information:

1. Type of the article.
2. Title of the article, which should be as short and concise as possible. Authors should include all information in the title that will make electronic retrieval of the article both sensitive and specific.
3. A short title (up to 50 characters with spaces), which will appear in the heading of an article in the journal.
4. 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).
5. The name of the department(s) and institution(s) to which the work should be attributed.
6. 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.
7. Specify sources of support in the form of grants, equipment, drugs, or others, if any and a statement about existence or non-existence of the conflict of interests.
8. Total number of pages, words and characters with spaces (Microsoft Word enables the simple acquisition of these data), number of figures and tables. A word count for the text only (excluding abstract, acknowledgments, figure legends, and references) allows editors and reviewers to assess whether the information contained in the paper warrants the amount of space devoted to it, and whether the submitted manuscript fits within the journal's word limits. A separate word count for the Abstract is also useful for the same reason.

## Second page

Abstract and Key Words are written on the 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 (up to 250 words), without authors' names and institutional affiliations. Its structure should be similar to that of the text. For original articles, the abstract needs to have the structure with the following subtitles: Objective, Materials and methods, Results and Conclusion. Abstract for review articles and case reports also needs to have up to 250 words and does not have to be structured, but it has to have a Conclusion. 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. The key words should not repeat the title of the manuscript. Terms from the Medical Subject Headings (MeSH) list of Index Medicus should be used; MeSH terms are available from: www.nlm.nih.gov/mesh/

## Third page

Should carry the manuscript of article. Text should be under the following headings:

**Introduction**. Needs to be short and to specify to the reader, clearly and with arguments, reasons for the research presentation, and the novelties that the article brings. In Introduction maximum 3 to 4 pertinent and directly related works need to be cited. At the end of Introduction, an author needs to clearly specify the set aim of the research.

**Methods**. This part needs to provide the following information: selection and description of participants, precise technical information about all methods *(*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 other specificities related to the presented research). Upon reporting about humane experiments, an author needs to indicate if the used procedures were in accordance with the Declaration of Helsinki from 1975 and its amendments from 1983. In addition, there needs to be stated if and which ethical committee gave consent for carrying out the research. A separate subtitle is *Statistical Analysis*. Authors need to indicate all statistical tests that were used. In addition, there needs to be stated the level of significance selected beforehand (P), that is which value P the authors considered to be statistically important (ex. 0.05 or 0.01, or some other). The results should be stated with pertaining confidence intervals (CI).

The editorship recommends to the authors to follow STARD instructions published in 2003 in the researches of diagnostic accuracy. At the end of the paragraph authors need to state which computer statistical program they have been using, as well as indicate the manufacturer and version of the program.

**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 and for the manuscript preparation in the acknowledgements section.

**References**. Need to be on a 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. If the paper has been published in electronic form on PubMed the confirmation of acceptance is not needed. 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). Examples of references please see on the following pages.

**Tables**. Need to be submitted separate from the main text. The preferred software for tables is Microsoft Excel (save each table in a file with single worksheet). Only tables made with table tools in Microsoft Word are acceptable. For the paper version, type or print each table on a separate sheet of paper. Number tables consecutively in the order of their first citation in the text. Use Arabic numerals. Each table needs to have an explanatory title. Place the title above the table. Give each column a short or abbreviated heading. Also, visibly indicate the position of each table in the text, using its assigned numeral at the end of the sentence which is relevant to the table(s). Tables should be positioned in the text where the author feels is appropriate but the Editor reserves the right to re-organize the layout to suit the printing process. Authors need to place explanatory matter in footnotes, not in the heading. Explain in footnotes of the table all nonstandard abbreviations. For footnotes use the following symbols, in sequence: *, †, ‡, §, ||, ¶, **, ††, ‡‡. Identify statistical measures of variations, such as standard deviation and standard error of the arithmetic mean. *Be sure that each table is cited in the text.* If you use data from another published or unpublished source, obtain permission and acknowledge them fully.

**Figures** (illustrations: diagram, photograph, photomicrograph, radiograph, drawing, sketch, picture, outline, design, plan, map, chart, etc.). Need to be submitted separate from the main text. They need to be submitted as photographic quality digital prints or, exceptionally, as professionally drawn and photographed original illustrations. Figures should be in a digital format that will produce high quality images. Formats recommended include: JPEG, GIF, TIFF, Microsoft Word, Excel. Sending original photographs and slides is permissible when they cannot be digitized without professional help. In this case, send an explanation in the cover letter. Using Arabic numerals, number figures consecutively in the order of their first citation in the text. Also, visibly indicate the position of each figure in the text, using its assigned numeral in parentheses. Figures should be positioned in the text where the author feels is appropriate but the Editor reserves the right to re-organize the layout to suit the printing process.

Supply a legend for each figure. Titles and detailed explanations belong in the legends, however, not on the figures themselves. Figures should be made as self-explanatory as possible. Letters, numbers, and symbols on figures should therefore be clear and even throughout, and of sufficient size that when reduced for publication each item will still be legible. Photomicrographs should have internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background. If photographs of people are used, either the subjects must not be identifiable or their pictures must be accompanied by written permission to use the photograph.

*Legends for Figures* need to be included in the main manuscript text file, on a separate page - immediately following the references. Type or print out legends using double spacing.

For each figure, the following information should be provided: figure number (in sequence, using Arabic numerals - i.e. Figure 1 or Fig. 1); title of the figure; all necessary explanations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, identify and explain each one clearly in the legend.

## Units of measurement

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

## Abbreviation, Acronyms and Symbols

If possible for metric units use standard abbreviations. Non-standard abbreviations should be defined when first used in the text.

## 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.

Issue with supplement:

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

Issue with no volume:

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

Letters or abstracts:

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.

Article republished with corrections:

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.

Article with published erratum:

Malinowski JM, Bolesta S. Rosiglitazone in the treatment of type 2 diabetes mellitus: a critical review. Clin Ther. 2000;22(10):1151-68; discussion 1149-50. Erratum in: Clin Ther 2001;23(2):309.

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 monographs

Personal 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.

Chapter in a book:

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.

Conference paper:

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Dissertation:

Borkowski MM. Infant sleep and feeding: a telephone survey of Hispanic Americans [dissertation]. Mount Pleasant (MI): Central Michigan University; 2002.

## Other published material

Newspaper article:

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

Dictionary and similar references:

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

## Electronic material

CD-ROM:

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

Audiovisual material:

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

Journal article on the Internet:

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

Monograph on the Internet:

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

Homepage/Web site:

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

Part of a homepage/Web site:

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

Database on the Internet:

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

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