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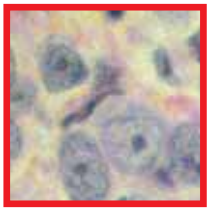

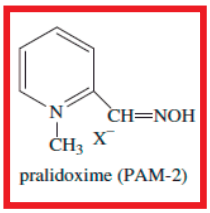

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Садржај / Contents

У овом броју

Др Ђузепе Д'Анкона (Dr. Giuseppe D'Ancona) и сарадници послали су нам чланак о новом типу операције на срцу и о искуству које је тим проф. др Миралема Пашића стекао након првих петсто таквих оперативних захвата изведених у *Deutsches Herzzentrum,* Berlin. Реч је о транскатетерској имплантацији аортног залиска TAVI (*transcatheter aortic valve implantation*), која постаје све чешћи хируршки захват код пацијената с аортном стенозом јер је у таквим случајевима конвенционални хируршки захват високо ризичан. У овом чланку се, сем осталог, описује како је формиран и обучаван тим који с великим успехом обавља овај деликатан хируршки захват.

Група истраживача Завода за патолошку анатомију из Бање Луке саопштила је резултате вишегодишњег истраживања које је имало за циљ да се одреди однос између експресије фактора раста васкуларног ендотела (VEGF-a, *vascular endothelial growth factor*) и морфолошких одлика меланоцитног невуса и меланома коже.

Scripta Medica доноси и три ревијска чланка: један је посвећен неуротоксичним поремећајима и лечењу особа отрованим органофосфорним једињењима; други приказује стварање еритроцита из ћелија плацентарне крви за потребе трансфузије; а трећи је посвећен значају фактора TGF-beta (*transforming* growth factor) у артеријској плућној хипертензији.

Четири приказа случаја су из следећих дисциплина: патолошка анатомија, офталмологија и анестезиологија, а поглавље „Слике из клиничке медицине" садржи два интересантна чланка. Први је из офталмологије (приказана је Purtscher ретинопатија која је настала након што је трактор прешао преко грудног коша одраслог мушкарца), а други из кардихирургије (описана је хируршка корекција митралне регургитације након руптуре папиларног мишића код запуштеног случаја, девет недеља након инфаркта миокарда). Овај последњи пример показује да се клиничка пракса некада мора ослонити на дијагностички поступак када није дао снимке који се срећу у уџбеницима, али је омогућио хирурзима да брзом интервенцијом спасе живот пацијента.

Један од најбољих познавалаца ксенотрансплантације, др Давид Купер (Dr. David Coopper, University of Pittsburgh, USA), послао је писмо уреднику поводом чланка о тој врсти трансплантације, који је објављен у нашем часопису (*Scrpta Medica*, Vol. 43, No, 1, 2012; www.scriptamedica.com). Да ли је близу дан револуције у медицини до које би довела ксенотрансплантација? Из писма др Давида Купера и одговора на то писмо аутора чланка др Зорана Ивановића (Aquitaine-Limousin Branch of French Blood Institute/UMR 5164 CNRS/ University Bordeaux, France) читаоци ће сазнати шта стоји на путу том изузетно значајном прогресу медицине – конкретно колико смо близу/далеко од тога да ткива и органи генетски модификоване свиње постану замена за истрошене делове људског организма.

Стручна рубрика коју већ одавно објављујемо под насловом „Питања и одговори" (*Questions and Answers*) има за циљ да се заинтересованим читаоцима олакша изучавање стручног енглеског језика. Она истовремено служи као подсетник на неке детаље медицинске струке. У овом броју тај прилог је у великој мери посвећен неуропсихијатрији.

Уводник је посвећен писању информације за масовне медије (press release). Повод за тај текст је чињеница да интересантни чланци из малих биомедицинских часописа са подручја бивше Југославије ретко бивају примећени или упућени широкој публици, а и то што понекад новинари, махом због неразумевања материје, дају сензационалистички тон налазима објављеним у часописима и тако штете и часопису и ауторима. Зато је најбоље када аутор, на захтев часописа, пише кратку и јасну информацију за медије коју ће новинарима и јавности упутити редакција часописа.

Редакциони одбор овог међународног часописа је у мају 2012. године одлучио да се сва оригинална истраживања, ревијски и специјални чланци, као и прикази случајева објављују само на енглеском језику. Историјски чланци, прикази књига, чланци у рубрици *in memoriam*, извештаји са значајнијих научних скупова и други информативни чланци биће објављивани на српском језику. Преводе наслова чланака који су објављени на енглеском и наслови чланака који се објављују на српском биће штампани у садржају само ћирилицом.

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EDITORIAL

Writing a Press Release

After publication of a particularly interesting or significant scientific work, some journals circulate a press release to the media. This is a short, readily understandable written statement, the purpose of which is to attract notice by the general public. Alternatively, journalists may ask authors of key articles for a statement that will help them spread the message to a wider audience beyond professional physicians and scientists. Neither the authors nor journals should distribute unpublished reports until the journal's media embargo has expired.[1]

Local media, seeking sensationalism, sometimes exaggerate the importance of certain scientific reports. This is not beneficial for either the authors or the journal. To avoid misunderstandings, it is best to have the author write a press release and allow the medical journal to release it.

BMJ recently published a retrospective cohort study on medical journal press releases and associated news stories.[2] Going backwards from January 2009, the authors of this study reviewed five major medical journals, *Annals of Internal Medicine, BMJ, Journal of the National Cancer Institute, JAMA, and New England Journal of Medicine* to identify the first 100 original research articles with quantifiable outcomes that had generated newspaper coverage. They identified 759 associated newspaper stories, and 68 journal press releases and concluded that high quality press releases issued by medical journals could make the quality of associated newspaper stories better, whereas low quality press releases would likely make them worse.

A good press release includes a brief and clear headline. The body of the text should be short and to the point. A text of between 150 and 250 words should be sufficient to convey the main message. The sentences should be short, and the terms used understandable to lay people. Obscure terminology should be avoided whenever possible, and technical words and abbreviations should be explained when first used. For lay readers and listeners, approximations are preferable to percentages when reporting data. For example, 8 percent becomes "nearly one in ten", and 57 percent becomes "more than half." The press release should contain the name, address, telephone, and e-address of the senior author, but if there are multiple authors, one could be selected to talk to the media.

The writer of a good press release should direct his attention to his audience, which consists of journalists and people looking for health-related information. A journalist's primary goal is to provide correct information to his readers. A precisely worded and easily understandable press release is the way to avoid sensationalism and wrong information.

Rajko Igić

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SPECIAL ARTICLE

Transapical Transcatheter Aortic Valve Implantation: The Berlin Experience

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ABSTRACT
Transcatheter aortic valve implantation (TAVI) has been recently introduced as a means to treat patients with severe aortic valve stenosis at high risk or rejected for conventional surgery. In the present manuscript we summarize the Deutsches Herzzentrum Berlin (DHZB) experience with transapical TAVI starting from the initial steps for adequate TAVI team building and training and focusing on the technical issues and results in the first 500 operated patients. We will also comment upon the future of TAVI in the light of the results from large recent multi-center trials.

KEY WORDS
TAVI, TAVI team, surgical complications, future of TAVI.

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Aortic valve stenosis (AVS) is the most common valvular cardiac pathology in Europe and North America. Its prevalence increases progressively with aging, and it presents as calcified aortic stenosis in 2-7% of the population aged >65 years.[1] Surgical indications for aortic valve replacement (AVR) are well defined in the international guidelines where it is specified that AVR should be performed only in symptomatic patients with severe AVS or in asymptomatic patients whenever there is an objective and subsequent left ventricular dysfunction.[1]

Although the results of surgical AVR are satisfactory in both the perioperative and long term follow-up phases, the perioperative risks increase for elderly patients and for those with complex comorbid profiles, which includes the vast majority of patients referred for AVR. Moreover, many patients with severe symptomatic AVS are not referred to surgical treatment because the examining physician considers surgery too traumatic and a prohibitive mortality risk.[2]

Conventional AVR requires full sternotomy, heart lung machine adoption, cardiac arrest (cardioplegia), and "open-heart" replacement of the native aortic valve. Despite excellent results achieved by conventional AVR in the past, it is mandatory to consider alternative interventions for a less invasive procedure. In this context, trans-catheter aortic valve implantation (TAVI) was recently introduced to treat patients with severe AVS that were rejected or considered too high risk for conventional surgery. Ten years after the first human implant,[3] TAVI is a well accepted technique for treatment of over 35.000 patients worldwide.

Multicenter prospective randomized trials recently demonstrated the superiority of TAVI over conventional medical therapy for management of those patients with severe AVS who were rejected for standard surgery.[4] In addition, TAVI is not inferior to conventional surgery for treatment of high risk surgical candidates (PARTNER trials).[5] The benefits of this innovative technique remain consistent even at mid-term follow-up.[6]

We will summarize the Deutsches Herzzentrum Berlin (DHZB) experience with transapical TAVI starting from the establishment of DHZB TAVI team building and training principles in 2008 and moving towards the acquisition of a standardized patient selection and operative protocol. In the second part, we present results obtained with the first 500 patients treated via this revolutionary surgical operation.

Building and training the TAVI team

Since the beginning of our experience with TAVI in 2008, the DHZB policy has been to structure two TAVI teams with complementary and synergic action: the core team and the virtual team. The core team consists of two anesthesiol-ogists with expertise in trans-esophageal echocardiography, five surgeons from four different surgical generations and two experienced cardiologists. The virtual team consists of about 50 persons involved in the chain of treatment of TAVI patients from the beginning to the end of hospitalization and in the out-patient follow-up.

A computer simulator, training by dry runs, and visiting centers that performed procedural life-case demonstrations provided procedural preparation. After these early theoretical stages, a first phase of proctoring was mandatory for the initial phases of the program building. External proctoring for the first cases was applied by pioneering experts, depending on the type of TAVI procedure.[3,7] This proctoring was continued by occasional and repeated visits by other experts in order to exchange experience, improve details of the procedure and identify possible systemic errors inherent within the TAVI process.

After external proctoring stages, internal proctoring was managed as a self-appraisal relationship with regard to surgical experience between the team members, where opinions were exchanged in an open discussion during evaluation of the TAVI processes. This internal proctoring involved the most experienced surgeon/cardiologist ("senior proctors"); it was then gradually taken over by other members of the team ("younger proctors"), according to their surgical experience. This allowed those who were taught to "be proctor and proctored." During the initial phase, senior proctors performed all procedures if possible complications were expected due to patients' clinical condition (e.g. poor LVEF, cardiogenic shock, age ≥ 90 years, special situations).

Dhzb clinical and procedural policies for TAVI patients selection and procedural planning

Inclusion Criteria. As previously emphasized, TAVI is proposed as a novel form of treatment for patients rejected from or at prohibitive risk for conventional aortic surgery to correct symptomatically severe AVS. Since the beginning of our experience at DHZB we have applied a "no exclusion" policy for referring patients to TAVI.

In fact, our idea is that every high-risk patient, including those with poor left ventricular ejection fraction (LVEF =10-20%) or cardiogenic shock or those requiring a rescue procedure, should be considered for TAVI; exceptions would be those with endocarditis or too large annuli. No patient should be refused because of co-morbidities.

Patients are evaluated and accepted for TAVI according to contemporary accepted criteria that emphasize the importance of a predicted high mortality score (Euro-SCORE >20%, STS-score>10%). We believe that an institutional evaluation should be performed on the basis of the single center experience with conventional AVR and TAVI. Furthermore, some patients who do not fulfill the high risk classification criteria may be accepted for TAVI for technical surgical reasons (e.g. porcelain aorta) or because of a high risk for conventional operation due to factors not covered by the risk scores (e.g. liver cirrhosis, malignancy, or in special situations such as in patients with assist devices).

The reason(s) must be widely acceptable and must be clearly formulated and documented. We consider only active or recent endocarditis to be an absolute contraindication for TAVI. Obviously, an annulus size exceeding the recommendations of the valve manufacturers would also preclude TAVI.

Valve Sizing and Prosthesis Selection. We have developed a standardized system for evaluating native AV annulus size that allows us to select the appropriate trans-catheter prosthesis. The aortic annulus is assessed by preoperative trans-thoracic echocardiography, multislice computed tomography and intra-operative trans-esophageal echocardiography. A two mm over-sized valve is generally used when adopting the Edwards SAPIEN valve (Edwards SAPIEN THV, Edwards Lifesciences, Irvine, California). This device is the most frequently used trans-catheter aortic valve prosthesis at DHZB.

The Edwards SAPIEN heart-valve system consists of a trileaflet bovine pericardial valve and a balloon-expandable, stainless steel support frame (Fig. 1). Generally, we choose a valve size of 23 mm for aortic valve annuli smaller than 21 mm, a 26-mm prosthesis for annuli between 21 mm and smaller than 24 mm, and a 29 mm valve for annuli between 24 mm and smaller than 27 mm. A native aortic annulus of 19 mm diameter is the lower limit orientation value for the 23-mm valve. In borderline cases, the decision is made on an individual basis, taking into account

Figure 1. Edwards SAPIEN valve (Edwards SAPIEN THV, Edwards Lifesciences, Irvine, California) consists of a trileaflet bovine pericardial valve and a balloon-expandable, stainless steel support frame.

additional factors. These include: the distances from the annulus to the coronary artery ostia, the shape of the annulus (oval versus circular), the amount of material in the leaflets, aortic diameters at the level of the sinuses of Valsalva, the sino-tubular junction, the ascending aorta, and the extent of calcification in structures, such as the left ventricular outflow tract, anterior mitral leaflet, and aortic valve leaflets themselves.[8]

Intraoperative phases for transapical TAVI. Since the beginning of our experience, TAVIs have been done in our special operating room (Fig. 2) that combines a catheter laboratory with all equipment and conditions necessary for surgery and sterile valve preparation before implantation; it includes requisite equipment for anesthesia, appropriate lighting, and a heart-lung machine.[8] All operations

Figure 2. Hybrid operating room at the DHZB. This is a special operating room that combines a catheter laboratory with the preconditions necessary to perform surgery and sterile valve preparation before implantation, anesthesiologic equipment, appropriate lighting, and the heart-lung machine. *(From Pasic M, Unbehaun A, Dreysse S, et al. Transapical aortic valve implantation in 175 consecutive patients: excellent outcome in very high-risk patients.* J Am Coll Cardiol 2010;56:813-20.*)*

are performed under general anesthesia with fluoroscopy and echocardiography guidance.

The patient is placed in a supine position, and a small anterior thoracotomy is performed 2 to 3 cm below the left mammary groove. The left ventricular apex is more easily reached, and the procedure more comfortably performed, when the chest is entered at the 6th or 7th intercostal space. In this way, the amount of chest retraction and dissection can be reduced. In any case, the correct position of the left ventricular apex is previously evaluated by chest computed tomography, and the actual position of the apex is checked digitally by the operator from a small opening in the intercostal space before extending the thoracotomy.[8]

Once the chest is entered, the pericardium is opened and the pericardial cradle is suspended to the thoracotomy

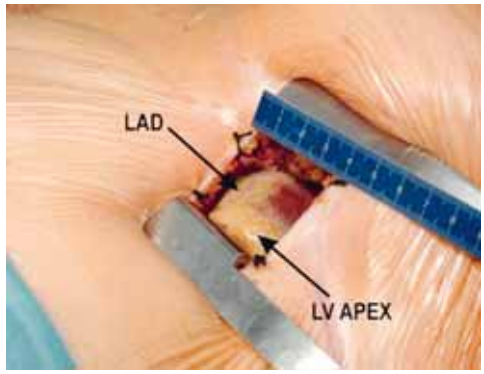

Figure 3. LV Apex exposure and preparation for TAVI through a small left anterior thoracocotmy in the 6th-7th intercostal space. LAD: Left anterior Descending coronary artery. *(From Pasic M, Unbehaun A, Dreysse S, et al. Transapical aortic valve implantation in 175 consecutive patients: excellent outcome in very high-risk patients.* J Am Coll Cardiol 2010;56:813-20.*)*

cutaneous edges. At this stage, the left ventricular apex is identified along with its anatomical boundaries (Fig. 3). Two polypropylene purse strings (either 3-0 or 2-0) are placed in an area of transaction between the left ventricular apex and the anterior wall of the left ventricle. The purse strings are reinforced with equine pericardium pledgets. At this stage a diagnostic pigtail is placed in the aortic root and pushed within the deepest aortic sinus to perform the first aortography. The ideal radiographic projection is to have the nadirs of two aortic sinuses (normally the left and right sinuses) lined up. When all three sinuses are visible in the same projection, their nadirs should line up. This condition is usually achieved with a radiographic projection of around 15 degrees left anterior oblique and 15 degrees cranial. Once the ideal projection is achieved, heparin is administered (100 IU/kg of body weight) and the left ventricular apex is punctured with a needle. A guide-wire is then passed through the aortic valve under fluoroscopy and echocardiography guidance. A small bore introducer (14 F) is passed over the wire and through the valve. An exchange of the initial standard guide-wire is made with a stiff guide-wire protected by an angiography catheter. The valvuloplasty balloon is passed over the wire and through the calcified valve. An aortic balloon valvuloplasty is then done under rapid ventricular pacing (160 BPM).

After balloon deflation, the external pacing is discontinued and the small bore introducer removed. At this stage, a large bore (24-26 F) introducer is inserted into the apex over the stiff wire. The prosthetic valve, previously mounted on a balloon, is inserted in the large bore introducer and positioned within the native aortic valve annulus under fluoroscopy guidance. [7, 8] Aortography at this stage allows the initial selection of the valve position. Once the proper

starting position has been identified, rapid ventricular pacing (160 BPM) is started. In this moment, while starting to slowly inflate the balloon, injection of 10 or 20 mL of contrast medium through the pigtail catheter (pulled back from the sinus of Valsalva 2 to 3 cm distally into the middle part of the ascending aorta just above the sinotubular junction) allows visualization (Fig 4). The balloon is initially expanded by about 30-40%. The valve usually opens at the left ventricular end first. The position of the valve is corrected if necessary, and the balloon is further inflated to about 60-70%. When the valve is 50- 60% opened, fine correction of the valve position is still possible. The optimum positioning of the valve is obtained by precise alignment relative to the coronary artery ostia, native valve, and native aortic valve annulus. These structures are perfectly visible by this technique.[9]

Finally, the balloon is completely inflated, and the valve is deployed in the desired position (Fig. 4). At this stage, the balloon is deflated, external pacing is discontinued, and the balloon is retracted into the transapical sheath. After controlling the correct position and function of the newly implanted valve with the last aortography (Fig. 4), the introducer together with the balloon-catheter and the super-stiff guide-wire are pulled back and the apical purse strings are tied up.

Special conditions

Use of femoro-femoral cardiopulmonary bypass (CPB). Our DHZB policy states that elective normothermic femoro-femoral CPB should be considered in patients with low LVEF (10-20%), cardiogenic shock or rescue procedure, as well as in patients with significantly enlarged right ventricle (RV) with poor RVEF and severe pulmonary hypertension.[10-12]

Prophylactic use of intra-aortic balloon pump (IABP). IABP should be considered in patients with poor LVEF (10-20%) and in patients in cardiogenic shock.[10-12]

Simultaneous treatment of combined coronary artery disease (CAD). Simultaneous elective percutaneous coronary intervention (PCI), or coronary artery bypass grafting if PCI is not possible, is considered for patients with concomitant CAD. Only the most relevant coronary artery stenosis is treated.[13-16]

"Valve-in-valve" concept for degenerated biological valve prosthesis. This concept may be considered in high-risk patients as a therapeutic or a palliative approach.[17, 18]

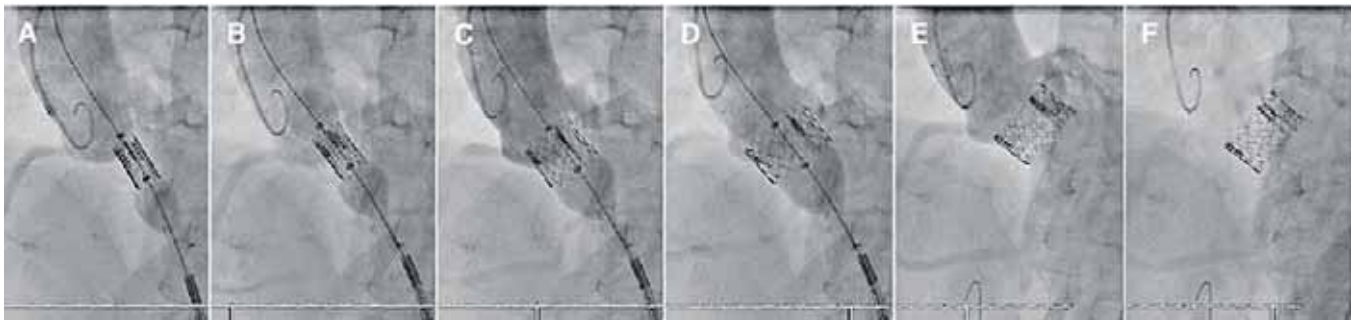

Figure 4. Valve deployment with angiographic monitoring. **The starting position of the valve is intentionally chosen to be lower than usual because of a short distance from the aortic annulus to the ostium of the dominant right coronary artery (8 mm as assessed by computed tomography). The balloon is only slightly inflated, the valve deployment begins, and angiography is performed (A). Then the valve position is corrected by pushing the valve slightly forwards into the aorta before full deployment (B). Concomitant angiography enables excellent visualization of the important anatomic factors in the proximal ascending aorta throughout the valve deployment and immediately after the valve is in its definitive position (C, D, E). In this particular case of high risk for occlusion of the right coronary artery, the valve is positioned very precisely and slowly and deployment requires a second injection of the contrast medium into the ascending aorta (D). Fluoroscopic view without contrast medium (F).** ***(From Pasic M, Dreysse S, Drews T, et al. Improved technique of transapical aortic valve implantation: "the Berlin addition".*** **Ann Thorac Surg 2010;89(6):2058-60.*)***

This slow and controlled release of the prosthesis is an original modification of the procedure by the DHZB TAVI team. It has been published in the medical literature as the "Berlin Addition."[9]

Combined atrioventricular valve pathology. Concomitant significant functional atrioventricular valve pathology is not treated in combination with TAVI, but later on by conventional surgery if necessary. The exception is grade IV tricuspid valve regurgitation.

Post-implant paravalvular leakage or transvalvular regurgitation. Aortic regurgitation after valve

implantation of grade 1 to 2 should be treated by additional balloon dilation of the valve and, if necessary, by implantation of a second valve. In the case of grade ≥2, if it is not correctable, conventional surgical aortic valve replacement should be considered.[19]

Intra-procedural bleeding of unknown origin. Any arterial bleeding with no identifiable cause should be considered as a suspected annulus rupture. It requires immediate institution of CPB, and a median sternotomy should be performed, even in patients who are considered formally "inoperable" or "not suitable for conventional surgery." Standard aortic valve replacement is required, and, in the case of myocardial rupture, the left ventricular outflow tract (LVOT) should be reconstructed with an over-sized pericardial patch. No attempt should be made to close the ruptured left ventricle from the outside.[20]

Dhzb results in the first 500 transapical TAVI

This section presents results from the first 500 patients treated by trans-apical TAVI at the DHZB. All patients have been treated according to the indications and institutional protocols as discussed above.

The study cohort consists of 311 (62%) female and 189 (38%) male patients. The mean age was 79.5 ± 8.1 years (median 80.6 years, range 28.9-98.9 years). The mean logistic EuroSCORE (European System for Cardiac Operative Risk Evaluation) of the study cohort was 36.3 ± 20.9% (median 30.4%, range 4.1 to 96.7) and the mean STS PROM was 16.7% ± 14.2% (median 12.2%, range 1.2 to 89.5). There were 28 (5.6%) patients with cardiogenic shock during the study period. The mean follow-up was 458.2 ± 368.1 days with a range from 0 (death during the procedural day) to 1363 days with a total of 628 patient years. At the time of the last data collection 374 (74.8%) patients were alive and 126 (25.2%) patients had died during the follow-up.

Surgical complications directly due to the procedure included surgical revision of apical pseudoaneurysm in two patients, revision for bleeding in eight patients, one iatrogenic aortic dissection (treated by transapical placement of an uncovered aortic endostent; patient survived), one valve migration. Annular rupture occurred in six patients throughout the study period.

The overall 30-day mortality for the entire study cohort of 500 patients was less than 5%, with 23 deaths among 500 patients. The 30-day mortality for patients without preoperative cardiogenic shock was 4.0% (19 deaths among 472 patients). The overall 6 month, 1 year and 2 year survival rates were 83.9 ± 1.7%, 80.1 ± 1.9% and 68.4 ± 2.7 for the whole group.

Epilogue

Since the first procedure in 2002, [3] TAVI has become standardized, with over 35,000 implants performed all over the world. Our own results with transapical TAVI along with those from multicenter prospective randomized trials using transfemoral and transapical TAVI [4-6] clearly indicate an encouraging midterm outcome along with acceptable perioperative mortality and rate of complications.

Results from the PARTNER trial reveal similarly high mortality at 2 years with TAVI and with surgical aortic valve replacement. In fact, follow-up mortality and complications rates presumably relate to the complex comorbid profile of elderly patients with symptomatic severe AVS.[6]

Perioperative results may impact follow-up outcome independently. In this context, residual aortic valve regurgitation after TAVI has been underestimated in the past. Paravalvular regurgitation is a design limitation of transcatheter aortic valves. This problem is most often secondary to incomplete and heterogeneous circumferential apposition of the prosthesis within the native annulus.

Recent scientific evidence [6] supports our DHZB policy of not accepting residual aortic insufficiency greater than two. In fact, even mild paravalvular aortic regurgitation seems to be independently associated with increased late mortality. [6] Avoiding valve under sizing and employing a patient's tailored post-TAVI re-dilation of the released transcatheter prosthesis can prevent this problem.

The future of TAVI. The successful application of TAVI in high risk patients ensures that it will, in the future, be used to treat even those individuals with low perioperative risk for conventional aortic valve surgery. The extension of TAVI indications will be aimed at minimizing the invasiveness of the procedure so as to further reduce the length and complexity of postoperative care. The SURTAVI Trial will start soon to evaluate the safety and efficacy of TAVI for treatment of symptomatic severe AS in subjects who are at intermediate risk for aortic valve surgery (STS mortality risk score ≥3% and ≤8%).

In conclusion, TAVI is a revolutionary technique that has already changed the standard treatment of severe AVS. It should be emphasized that TAVI in general, and transapical TAVI in particular, is a "temptingly easy procedure (...) with 1001 sequences." [8] Failure to respect one of the preoperative and/or perioperative steps may lead to abrupt catastrophe. Consistent results can be achieved and maintained in even the sickest patients, and the procedure can be eventually extended to a lower risk population through a systematic and structured TAVI training program.

Authorship statement

G D'Ancona has written the manuscript and is a DHZB TAVI team member; M Pasic has reviewed the manuscript and is the DHZB TAVI team and TAVI program Chief and senior surgeon; T Drews-S Buz- S Dreysse- A Unbehaun are DHZB TAVI team members; R Hetzer is a DHZB TAVI team member and Chief of DHZB .

Financial disclosure

Prof. Pasic and Drs. Unbehaun, Drews, Buz, and Dreysse have been proctors to Edwards Lifesciences since July 2009. There are no other disclosures.

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ORIGINAL ARTICLE

Expression of Vascular Endothelial Growth Factor (VEGF) in Melanocytic Skin Alterations

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ABSTRACT

Introduction. The study of growth factor expression allows further development of therapeutic modalities in the treatment of malignant diseases of the skin. This study aims to determine the relationship between the level of VEGF expression and morphological parameters (biological behavior of lesions, histological type, the defect on the surface, the density of inflammatory infiltrate, mitotic index, stage of growth and cell type) in melanocytic nevi and melanomas of the skin in different regions.
Methods. The study included skin biopsy material of 73 patients, divided in two groups (group I-melanomas, group II- nevi). The following parameters were determined: histological type, thickness of alteration (Breslow), Clark level, pTNM stage, the width of alteration, the density of lymphocytic infiltration of the tumor, mitotic index, stage of tumor growth, the presence of ulceration, tumor cell type, location and level of expression of VEGF.
Results. Most of benign melanocytic alterations in the skin shows low expression levels of VEGF in 91.18% of cases. In the group of melanomas, a high level of expression was seen in 61.54 % of cases. Nodular and acral lentiginous type of melanoma more often showed a high level of expression of VEGF, while superficial spreading melanoma often showed a low level of VEGF expression.
Conclusion. Benign melanocytic alterations have low, while malignant melanocytic alterations have high level of expression of VEGF.

KEY WORDS

Vascular endothelial growth factor; skin; melanocytic alterations; prognosis.

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Nevomelanocytic nevi are formed by nevomelanocytic clusters in the epidermis (junctional nevus), in the dermis (intradermal nevus), or on both places (compound nevus).[1] They are distinguished from other nevi by the ability of malignant alteration towards skin melanoma.[2] Melanoma is a heterogeneous disease of the skin and mucous membranes which shows a significant increase in worldwide incidence in the past decades (from 2.7 to 6.0 of 100 000 residents per year in men and from 4.6 to 8.5 of 100 000 residents in women).[3] Because of clinical and biological characteristics, the World Health Organization (WHO) has offered the classification of melanoma, where because of the frequency,are described as superficial spreading melanoma, nodular, and acral lentiginous melanoma.[4]

The skin retains the ability of rapid neovascularization, or secondary angiogenesis in response to numerous pathological stimuli, injury, inflammatory dermatoses, and neoplasia.[5]Processes that occur during the angiogenic cascade are regulated by various factors, stimulators and inhibitors, whose balance limits the process.[6] Stimulators of angiogenesis are: growth factor of endothelial cells lining the blood vessels (VEGF), basic and acidic fibroblast growth factors (b-FGF, aFGF, FGF-2, FGF-1), endothelial cell growth factor originating from platelets (PDECGF), angiopoetin-1, and others.[7]

The VEGF family consists of five isoforms: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor. VEGF-A, also known as vascular permeability factor, or simply VEGF, was described as a potent endothelial cell

mitogen which stimulates the proliferation and migration of endothelial cells. VEGF is overexpressed in almost all solid tumors and correlates with vascularity, grade, and prognosis. Several studies have examined the expression of members of the VEGF signaling pathway in melanoma. Secretion of VEGF occurs during progression of early cutaneous melanocytic lesions, with low VEGF expression in benign nevi increasing significantly in dysplastic nevi and more so in malignant melanoma. [8] The transition of melanomas from the radial to the aggressive vertical growth phase is also marked by increased VEGF production. [9,10] Tumor blood flow in melanomas thicker than 0.9 mm was detected using Doppler ultrasound, and endogenous VEGF expression and secretion in melanoma tumor cells were later established. [11]Statistical analysis showed that the expression rate of VEGF in choroidal melanoma was much higher than that in the control group, and was dependent on tumor size, which suggested that VEGF played a role in the progression of choroidal melanoma by stimulating angiogenesis required forpromotion of tumor growth. [12]

The study of growth factor expression allows further development of therapeutic modalities in the treatment of malignant diseases of the skin. Targeted vascular treatment decreases the possibility of creating new blood vessels in tumor, and thus indirectly affects tumor cells and slows tumor growth and development.[13]

The aim of this study was to determine the correlation between the level of VEGF expression and morphologic parameters (biologic behavior of lesion, histological type, surface defects, inflammatory infiltrate density, mitotic index, stage of growth, and cell type) in melanocytic nevi and skin melanoma fromdifferent anatomical regions.

Materials and methods

Patients

The research was done on bioptic skin samples of 73 patients with melanocytic skin alterations, taken at the Clinical Center, Banjaluka between 2004 to 2007. Based on histopathological analysis, patients were divided into two groups: group I - 39 patients with melanoma and group II - 34 patients with nevi.

Morphological analysis

In all specimens, the following was determined: the histological type- determined by the analysis of histological samples according to the WHO histological classification;[4] the thickness of alteration; Clark level- determined histologically and by the layers of tumor location (level I to level V); pTNM stage- on the basis of histological analysis and insight into the history of the disease according to the 7th pTNM classification;[14] tumor infiltration by lymphocytes; mitotic index: the number of mitoses was determined in 10 visual fields at high magnification; the estimation of growth phase: radial or vertical growth phase[4] ;the presence of surface defect: presence or absence of ulceration; cell type of alteration: epitheloid cells, spindle cells, mixed type (epitheloid + spindle cells);localization: alterations have been classified according to the localization into the following subgroups: head and neck, trunk, extremities.

Immunostaining

To detectthe primary antigen VEGF,commercial mouse monoclonal anti-human VEGF antibody (Daco M7273) was usedat a dilution of 1:25. For visualization, we used the LSAB + (Daco K0690) system and chromogen DAB Liquid (K3466).

The presence or absenceand the intensity of vascular endothelial growth factor was assessed by semi-quantitative ranking using a scale from 0 to 3, taking the level of immunostaining of keratinocytes as an internal control. The quantification was as follows: score of 0, no difference in immunostaining for VEGF between melanocytes and ke-

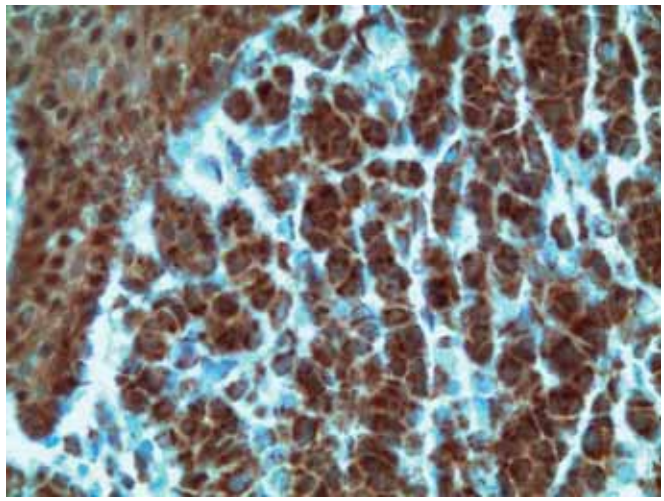

Figure 1. Medium level of VEGF expression in melanocytic nevus, score 2 (anti-VEGF x 400)

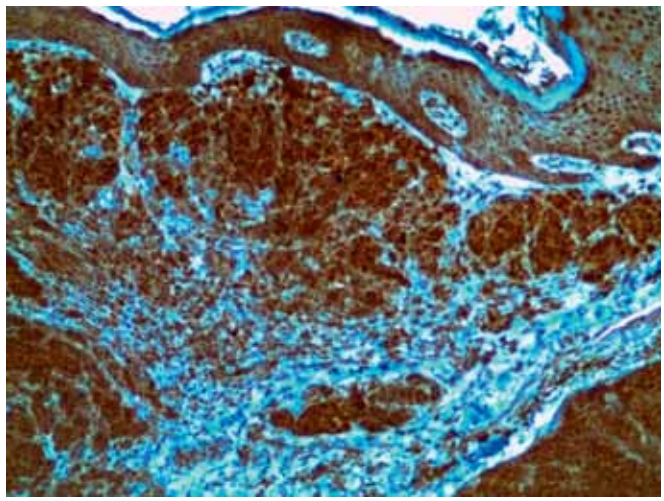

Figure 2. High level of VEGF expression in melanoma, score 3 (anti-VEGF x 200)

ratinocytes; score of 1 - less than 25% tumor cells show an expression of higher intensity compared to the level of staining of keratinocytes; score of 2 - 25 - 75% of tumor cells show an expression of higher intensity compared to the level of staining of keratinocytes (Figure 1); score of 3 - more than 75% tumor cells show an expression of higher intensity compared to the level of staining of keratinocytes (Figure 2).

Statistical analysis

The results were analyzed by methods of descriptive and correlative statistics. Statistical analysis was performed using the SPSS software version 15.0, and the following tests were applied: $\chi 2$ and the related methods of analysis of categorical variables (Fisher's exact test, Kendall tau rank correlation coefficient) and Mann Whitney U- test.

Results

The average age of examinees was 45 years. The gender distribution is 1,92:1 in favor of women.

Most of benign melanocytic alterations in the skin showed low expression level of VEGF (score of 0 and 1) in 91,18% of cases. In the group of melanomas, high expression levels were found in 61.54% of cases (level 2 and 3). A statistically significant difference exists in the expression of VEGF in groups. In group I, expression was often high (score of 2 and 3), and in group II, more often the expression was low (score of 0 and 1) ($\chi 2=21,658$; df= 1; $p<0.001$)[1]

Histological type. A statistically significant difference was not found in level of VEGF expression when different histological types of nevi were compared ($\chi 2=2.062$; $p=0.724$). Nodular and acral lentiginous melanomas more often showed a high level of expression of VEGF, while superficial spreading melanomas often showed a low level of VEGF expression ($\chi 2 = 6.858$, $p = 0.032$) [*].

The presence of surface defects. A statistically significant difference was not present regarding the level of expression of VEGF and the presence of a defect in nevi (Fischer's test, p = 0.101). Unlike nevi, in the melanoma group there was a statistically significant difference in the level of VEGF expression and the presence of ulceration was found ($\chi 2= 4.545$ p = 0.033)[*].

The thickness of alteration. Based on statistical analysis, we can conclude that there was no statistically significant correlation between the expression of VEGF and the thickness of nevi ($\chi 2= 1.009$, p = 0.604). A higher level of expression was present in melanomas that were thicker (higher stage according to Breslow) ($\chi 2=11.211$, p = 0.011, $p<0,05$). Based on the analysis of the Mann-Whitney test, we can conclude that there was no statistically significant difference in the level of expression of VEGF and the width of benign melanocytic lesions (U = 38.000 for the significance of 0.605) and in the melanoma group (U = 142.000 for the significance of 0.273).

Tumor infiltration by lymphocytes. Analysis showed no statistically significant differences between nevi with different densities of lymphocytic infiltration in relation to the expression of VEGF (Chi-square of 1.019, p = 0.601). On the basis of statistical analysis, in the group of melanomas, we can conclude that there was a statistically significant difference in the level of expression of VEGF and the density of lymphocytic infiltration (Chi-square of 8.555, p = 0.014, $p<0,05$). The low level of expression of VEGF is more common in melanomas with dense lymphocytic infiltration, while a high level of expression was found in melanomas with rare lymphocytic infiltrate[*].

Mitotic index. In the studied material, mitotic activity in benign melanocytic alterations was verified in only one case (2.94%), and level of VEGF expression in this case was 1. In the group of melanomas, based on Kendall tau rank correlation coefficient, there was no statistically significant difference in the level of VEGF expression in relation to mitotic activity (t= 0.256, p = 0.060)[*].

Estimation of growth phase. Analysis revealed that there was no statistically significant difference in the level of VEGF expression and the growth phase of nevi (Chi-square of 5, p = 0.07). Melanomas presented with vertical growth phase had showed a higher level of expression of VEGF (Chi-square of 4.840, p = 0.028, $p<0.05$)[*].

Cell type of alteration. All examined nevi had an epitheloid cell type. Analysis of Chi square analysisshowed a statistically significant difference in the level of VEGF expression and cell type of melanoma (Chi-square of 8.871 p = 0.031, $p<0.05$). A high level VEGF expression was more often verified in melanomas with epitheloid cells[*].

Localization. Analysis using Chi square test showed there was no statistically significant difference in expression of VEGF, with respect to the localization of nevus (Chi-square of 2.765, p = 0.251). In the melanoma group based on Chi square test, we could conclude there was a statistically significant difference in the level of expression of VEGF and localization of melanoma (Chi-square of 7.831, p = 0.05, $p<0.05$). Melanomas localized on the extremities showed a higher level of expression of VEGF (score 2 and 3), while melanomas localized on the head, neck and trunk showed a low level of expression of VEGF[*].

Based on analysis of Kendall tau rank correlation coefficient, no significant difference in the level of expression of VEGF and the level of invasion according to Clark was found (t= 0.244, p = 0.063)[*].

[*] Contact the corresponding author for the detailed data.

Statistical analysis using Kendall tau rank correlation coefficient showed a statistically significant difference in the level of VEGF expression and pT stage melanoma (t= 0.259, p = 0.050). Melanomas in higher pT stage of the disease showed higher expression of VEGF (score 2 and 3). **

Discussion

Early diagnosis and differentiation between benign and malignant tumors of the skin is of utmost importance. So far there is an insufficient number of studies that would indicate that routine screening for skin may be important in the prevention of malignant skin tumors and contribute to better treatment of patients suffering from these diseases.[15]

In this study, we have found that melanocytic nevi showed expression of VEGF in the most cases (79.41%). The expression is usually at low grade (grade 1 immunostaining). In the group of melanomas, a low expression of VEGF is present in 38.46% of the cases (score 0 and 1), while a high level of expression was present in 61.54% of the cases (score 2 and 3). Carazo and Peyri[16] reported that the majority of melanoma had showed lower levels of expression (score 0 and 1), which is different from our results. A logical explanation for thisis that the authors examined the selected group of melanomas ("thin melanomas" ie. Breslow less than 1 mm), while we presented results from anunselected group (Breslow thicknesseven greater than 1 mm). The results of Einspahr and associates suggest that the level of VEGF expression may be a significant parameter which indicates the malignant transformation of melanocytic skin alterations. The study demonstrated that the level of VEGF expression in benign melanocytic alterations is low or absent, while in dysplastic nevi it is significantly higher, and the expression is much higher in malignant melanocytic alterations (melanoma). Thus, increased expression of VEGF may be a good indicator of preneoplastic changes in melanocytic alterations.[17] Brychtova and collaborators determined the presence of VEGF expression in benign and malignant melanocytic alterations. More often, the high level of VEGF expression can statistically be verified in melanomas in relation to nevi.[18]

The difference in the level of VEGF expression and morphological parameters (histological type, the defect on the surface, the density of inflammatory infiltrate, mitotic index, stage of growth and cell type) has not been demonstrated in the examined nevi. In the melanoma group, a statistically significant difference exists in the level of VEGF expression and the presence of ulceration and thickness according to Breslow. Boone and associates failed to demonstrate a positive correlation between the expression of VEGF-C and the presence of ulceration, tumor thickness according to Breslow, and the level of invasion according to Clark.[19] In our study, nodular and acral lentiginous types of melanoma were more likely to exhibit high VEGF expression level, while superficial spreading melanoma often showed a low level of expression of VEGF. These results are in line with literature data.[19]

In the examined material, we did not find malignant changes in Clark level I. A number of melanoma cases showed a high expression of VEGF (score 2 and 3) in 24 (61.54%) cases. The cases with higher expression are generally at higher Clark level. Based on analysis of Kendall's tau b test, we found no significant difference in the level of expression of VEGF and the level of invasion according to Clark. Salven and associates also did not find any differences in the manifestation of VEGF (measured by immunohistochemical methods) between small and large primary melanomas.[20] However, this contrary to other reports. Redondo and associates believe that the more Clark's or Breslow's level increases, the percentage of positive immunostaining for VEGF increases, thus linking it with the development of primary tumors, although a prognostic study has not been performed.[16,21]

Melanomas presented with vertical growth phase showed a higher level of VEGF expression. Looking at the value of immunostaining for VEGF according to the Breslow level, we found very important information which we consider fundamental: melanomas in radial growth phase, and those are the ones thathave not undergone change to malignant eclipse, show less VEGF, which is significantly different as measured by precise Fisher's test (p = 0.002) compared to those melanomas who have already penetrated.[22]

In our series, benign melanocytic lesions were located on the trunk in 26 (76.47%) cases, followed by the extremities in 5 (14.71%) casesand head and neck in 3 (8.82%) cases. Melanomas in our material were located on extremities in 15 (38.46%) cases, followed by head and neck in 13 (33.33%) cases and on the trunk in 11 (28.21%) cases. Melanomas located on the extremities showed a higher level of expression of VEGF (score 2 and 3), while melanomas located on the head, neck and trunk show a low level of expression of VEGF.

Melanocytic alterations show the expression of VEGF, regardless of their clinical behavior. Benign melanocytic alterations often indicate low, while malignant melanocytic alterations often show high level of expression of VEGF. Presence and level of expression of VEGF show no difference regarding to histological type, surface defect, density of inflammatory infiltrate, mitotic index, growth phase, cell type, and location of nevi. A high level expression of VEGF is present in the nodular and acral lentiginous types

** Individual morphological parameters and VEGF expression in the nevi and melanomas, with statistical analysis, may be obtained from the corresponding author.

of melanoma, in melanomas with ulceration and rare inflammatory infiltrate in the stroma, high mitotic index, in higher stage disease (Breslow, Clark, pT), and in melanomas located on extremities.

Authorship statement

RG had full access to all data in the study and as corresponding author takes full responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: RG, VG, ZK, IS, TB. Acquisition of data: VG, ZK, IS, TB. Analysis and interpretation of data: RG, VG, ZK. Drafting of the manuscript: RG, IS, TB. Critical revision of the manuscript: VG, ZK. Statistical expertise: IS, TB. Administrative, technical, or material support: RG, VG, IS. Study supervision: ZK, TB.

Financial disclosure

No potential conflicts of interest was reported.

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Vaskularni endotelni faktor rasta (VEGF) u melanocitnim kožnim promjenama

APSTRAKT

Uvod. Istraživanje faktora rasta je značajno za dalji razvoj terapijskih modaliteta u liječenju malignih bolesti kože. Cilj ove studije je da odredi odnos između nivoa ekspresije VEGF-a i morfoloških parametara (biološko ponašanje lezije, histološki tip, defekt površine, gustina inflamatornog infiltrata, mitotski indeks, stadijum rasta i ćelijski tip) u melanocitnim nevusima i melanomima kože različitih regija.

Materijal i metode. Ispitivanja su urađena na biopsijskim materijalima kože 73 pacijenta, koji su podijeljeni u dvije grupe (grupa I- melanomi, grupa II- nevusi). Određivani su sljedeći parametri: histološki tip, debljina promjene (prema Breslow-), Clark-ov nivo, pTNM stadijum, širina promjene, gustina limfocitnog infiltrata u tumoru, mitotski indeks, stadijum tumorskog rasta, prisustvo ulceracije, ćelijski tip tumora, lokalizacija i nivo ekspresije VEGF-a.

Rezultati. Većina benignih melanocitnih promjena kože pokazuje nizak nivo ekspresije VEGF-a u 91.18% slučajeva. U grupi melanoma, visok nivo ekspresije je uočen u 61.54 % slučajeva. Nodularni i akralni lentiginozni tip melanoma češće pokazuju visok nivo ekspresije VEGF-a, dok površinski šireći melanom obično pokazuje nizak nivo ekspresije VEGF-a.

Zaključak. Benigne melanocitne promjene imaju nizak, a maligne visok nivo ekspresije VEGF-a.

KLJUČNE RIJEČI

Vaskularni endotelni faktor rasta; koža; melanocitne promjene; prognoza.

REVIEW ARTICLE

Neurotoxic disorders and medical management of patients poisoned with organophosphorus pesticides

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ABSTRACT
In this article the neurotoxic disorders appearing in patients poisoned with organophosphorus pesticides (OPs) are reviewed. OPs cause four important neurotoxic effects in humans: the cholinergic syndrome, the intermediate syndrome, organophosphate-induced delayed polyneuropathy and chronic organophosphate-induced neuropsychiatric disorder. Compared to the cholinergic syndrome, that causes millions of cases of poisoning with fatality of more than 15% each year, other disorders involve much smaller number of patients. This article is focused on neurotoxic disorders appearing after acute and chronic exposure to OPs with emphasis on clinical presentation, molecular mechanisms and possibilities of medical treatment.

KEY WORDS
Organophosphorus pesticides; cholinergic syndrome; intermediate syndrome; chronic organophosphate-induced neuropsychiatric disorder; pyridinium oximes; atropine; acetylcholinesterase.

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Organophosphates (OPs) have been used as pesticides and developed as warfare nerve agents such as soman, sarin, tabun, VX and others. Some OP insecticides have found its application in human and veterinary medicine. Pesticide poisoning results from occupational, accidental, and intentional exposure. The epidemiological pattern of poisoning shows significant variation in number of deaths and form of poisoning between developing and industrial countries[1-3]. According to the World Health Organization, about 1 million accidental and 2 million suicidal poisonings with organophosphorus insecticides are reported per year, with more than 300000 fatalities[1]. Medical management is difficult, with case fatality generally more than 15% [4,5].

OP esters cause four neurotoxic disorders in humans: the cholinergic syndrome, the intermediate syndrome, organophosphate-induced delayed polyneuropathy (OPIDP) and chronic organophosphate-induced neuropsychiatric disorder (COPIND). These syndromes, arising from severe exposures, may be caused by OP pesticides or and some of them by warfare nerve agents[6]. Most of the cases of poisoning can be prevented by better administrative control, restricted access to OP pesticides, effective measures of personal protection and education of OP pesticide applicators and medical personnel.

The cholinergic syndrome

Signs and symptoms of cholinergic syndrome occurring in acute poisoning with OP pesticides are predictable from their biochemical mechanism of action and are directly related to the levels of acetylcholinesterase (AChE) activity in the central nervous system. In cases of human poisoning, general acute symptoms of peripheral nicotinic and muscarinic intoxication are clearly apparent[7]. These symptoms include miosis (unreactive to light); sweating, rhinorrhea, lacrimation, and salivation; abdominal cramps and other gastrointestinal symptoms; respiratory difficulties and cough; dyspnea, constriction sensation in the chest, wheezing; twitching of facial muscles and tongue, tremors, and fasciculations; bradycardia and ECG changes, pallor, and cyanosis; anorexia, nausea, vomiting, diarrhea, and involuntary urination and defecation. These signs and symptoms are accompanied by central effects such as dizziness, tremulousness, and confusion; ataxia; headache, fatigability, and paresthesia. Finally, seizures, convulsions, twitching, coma, and respiratory failure may occur. If the poisoned patient survives the first day of poisoning,

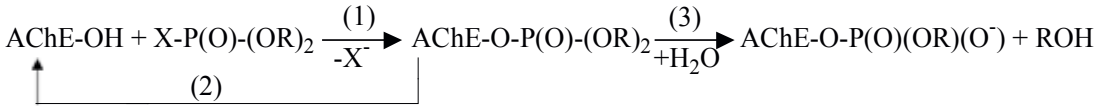

Figure 1. Interaction of acetylcholinesterase (AChE-OH) with organophosphorus compounds. Reaction 1: interaction of organophosphate molecule with the serine hydroxyl group at the active site of AChE. Inhibited AChE cannot further serve its physiological function, which causes the accumulation of acetylcholine at the nerve endings. Reaction 2: spontaneous reactivation of inhibited AChE which occurs relatively quickly for dimethylphosphates but slowly for other OP compounds. Reaction 3 ("aging"): non-enzymatic, time-dependent loss of one alkyl group (R) bound to the phosphorus. X = acyl radical (i.e. Cl^-, F^-, CN^-, *p*-nitrophenol etc.).

there are personality changes, mood swings, aggressive events and psychotic episodes including schizoid reactions, paranoid delusions, and exacerbations of preexisting psychiatric problems. Sleep is poor from nightmares and hallucinations; disturbances or deficits in memory and attention, and additional delayed effects also occur. Death usually occurs due to respiratory failure resulting from a combination of central and peripheral effects, paralysis of the respiratory muscles, and depression of the brain respiratory center[7-11]. The first four to six hours are the most critical in acute poisoning with OP pesticides. If there is improvement in symptoms after initial treatment then the patient is very likely to survive if adequate treatment is continued [9]. The data presented in Table 1 summarize the muscarinic, nicotinic and CNS effects in patients poisoned with OP pesticides observed at the National Poison Control Center in Belgrade during 1998-2007 period[5]. These findings are consistent with the results of other studies[12].

Clinical diagnosis of acute poisoning with OP compounds is relatively simple and is based on medical history, circumstances of exposure, clinical presentation, and laboratory tests. Confirmation of diagnosis can be made by measurement of erythrocyte AChE or plasma cholinesterase (ChE). Activities of these enzymes have been accepted as biomarkers of exposure and/or toxicity of OP. Erythrocyte AChE is identical to the enzyme present in target synapses and its levels are assumed to reflect the effects in target organs. For that reason, erythrocyte AChE is regarded as biomarker of toxicity of these compounds. On the other hand, ChE level in plasma frequently does not correlate well with clinical presentation of OP poisoned patients.

The mechanism of OP poisoning involves inhibition of AChE at synapses and neuromuscular junctions in cholinergic pathways leading to accumulation of acetylcholine and overstimulation of postsynaptic muscarinic and nicotinic receptors (Figure 1). Inhibition of AChE occurs after phosphorylation of hydroxyl group at serine at the active site of the enzyme. Following inhibition, AChE can be spontaneously reactivated at the rate that depends on chemical structure of OP. For OP having dimethyl radicals the AChE reactivation is relatively rapid with a half-time of about 1-2 hours, while that for OP having diethyl functional groups is 31-57 hours[13,14].

Table 1. Muscarinic, nicotinic, and CNS effects in patients poisoned with OP pesticides. From the National Poison Control Center in Belgrade (1998-2007).[5]

Muscarinic	No. (%) of Patients*	Nicotinic	No. (%) of Patients*	CNS	No. (%) of Patients*
Miosis	196 (61.8)	Fasciculations	46 (14.5)	Coma	80 (25.2)
Bronchorrhoea	164 (51.7)	Hypertension	35 (11.0)	Somnolence	23 (7.3)
GIT**	161 (50.8)	Fibrillation	32 (10.1)	Convulsions	14 (4.4)
Hypotension	88 (27.8)	Tachycardia	30 (9.5)	Sopor	13 (4.1)
ARI**	83 (26.2)	Tremor	4 (1.3)	Disorientation	4 (1.3)
Bradycardia	29 (9.1)	Arrhythmia	1 (0.3)	Agitation	1 (0.3)
ACF**	15 (4.7)				
Cardiac arrest	8 (2.5)				

*Based on total number of patients poisoned with OPs during 1998-2007 (n=317).

**Abbreviations: GIT = gastrointestinal symptoms; ARI = acute respiratory insufficiency, ACF = acute circulatory failure.

Medical management of patients with cholinergic syndrome

Medical management of patients showing symptoms of cholinergic syndrome include general and supportive measures and specific treatment. General measures in treatment of acute OP poisoning include decomination of exposed tissues, representing a vital step in reduction of the dose of pesticide absorbed, and resuscitation when needed. The patients should be observed carefully for several days (or even weeks) because respiratory arrest may occur[15,16].

Supportive measures should be directed towards the cardiorespiratory system with particular emphasis on maintenance of ventilation, cardiac rhythm and blood pressure; the removal by suction of respiratory and oral secretions which may cause respiratory distress; and the oxygenation of the patient. Severely poisoned patients disconnected from the ventilator when the general condition improves, must be carefully watched for rapid deterioration and development of the intermediate syndrome during the following few days in the Intensive Care Unit[9,15]. In addition, the patients should be warned to report to hospital if signs of organophosphate-induced delayed polyneuropathy appear 2-3 weeks after exposure.

Ingested organophosphates should be removed by early gastric aspiration and then lavage, with protection of the airway - this may be the best remedy in unconscious patients. Gastric lavage is most effective within 30 minutes of ingestion, but might be still effective up to 4 hours post ingestion, as organophosphates are rapidly absorbed from the gastrointestinal tract[7]. Administration of oral activated charcoal, in conventional doses, may be considered for reducing further absorption of some organophosphorus pesticides[7, 17].

Specific treatment of acute poisoning with OP pesticides include administration of atropine (as direct antidote), diazepam (as anticonvulsant) and a pyridinium oxime (as specific reactivator of inhibited AChE).

Atropine acts by blocking the effects of excess concentrations of acetylcholine (ACh) at muscarinic cholinergic synapses following OP inhibition of AChE. Atropine is the initial drug of choice in acute OP poisoning. Atropine sulphate in combination with an oxime has been used in traditional therapy for OP intoxications including pesticides. Atropine can relieve the following symptoms of OP poisoning: sweating, salivation, rhinorrhoea, lacrimation, nausea, vomiting and diarrhea, and can help control of bradycardia and circulatory depressions, dilating the bronchi and abolishing bronchorrhoea. Atropine does not bind to nicotinic receptors and cannot relieve nicotinic effects in OP poisoning[7]. In addition, there is evidence on anticonvulsant properties of atropine in OP poisoning[18,19].

The standard dosing of atropine depends on the severity of OPC poisoning. The initial dose is usually 2 mg in an adult (0.02 mg/kg in a child) given every 5-10 min until hyperatropinization (flushing, dryness of the mouth, nose, lungs and the skin, heart rate 80-100/min, normal blood pressure, mydriasis) is reached, which should be maintained during further treatment. The dose may be increased as required. Patients poisoned with OP appear to be resistant to toxic effects of atropine and may require relatively large doses of atropine administered during prolonged periods of time. In severe OP poisoning total dose of atropine given during 5 weeks of treatment can be as high as 30000 mg[15].

Diazepam is a well-known benzodiazepine most frequently used for the treatment of convulsions that appear in OP poisonings. Diazepam enhanced the clinical efficacy of low doses of atropine. In the cholinergic nervous system, diazepam appears to decrease the synaptic release of ACh. The main consequence of the action of benzodiazepines in CNS is hyperpolarization of neurons making them significantly less susceptible to cholinergically-induced depolarization. The ultimate result is cessation of propagation of convulsions [16,20,21].

In patients poisoned with OP, benzodiazepines may have a beneficial effect in reducing anxiety and restlessness, reducing muscle fasciculation, arresting seizures, convulsions, controlling apprehension and agitation and possibly reducing morbidity and mortality when used in conjunction with atropine and an oxime. Diazepam should be given to patients poisoned with OP whenever convulsions or pronounced muscle fasciculations are present. The recommended dose of diazepam in cases of OP poisoning is 5-10 mg intravenously over three minutes in the absence of convulsions and 10-20 mg intravenously in cases with convulsions, which may be repeated as required [9,16,22].

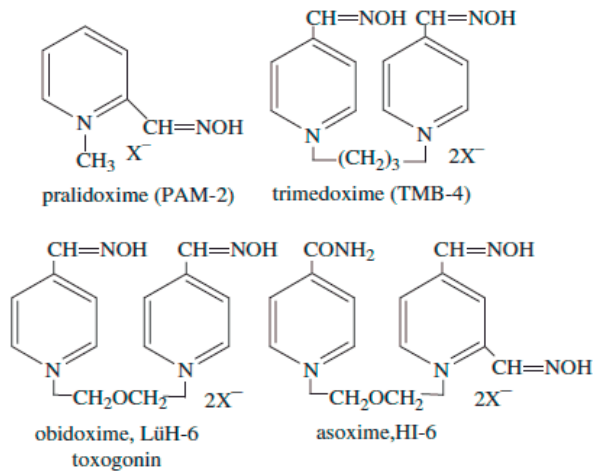

Figure 2. Chemical structures of pyridinium oximes used to treat human OP poisoning. X indicates an anion.

Pyridinium oximes acceleratine the rate spontaneous reactivation of AChE inhibited by OP by displacing the phosphoryl moiety from the enzyme. The oximes can only be of benefit as long as inhibited AChE is not completely converted to the aged form which is resistant to both spontaneous and oxime-induced reactivation. Pyridinium oximes are effective against OP-inhibited AChE in the peripheral nervous system, but have a limited penetration across the blood-brain barrier (about 10% of the given dose) due to their pharmacokinetic profile and the presence of quaternary nitrogen atom(s) in their structure[23].

Among the many classes of oximes investigated so far, those with clinical application can be divided in two groups - the monopyridinium and bispyridinium oximes. Currently, the only used monopyridinium oxime is pralidoxime, while the most significant bispyridinium oximes comprise: trimedoxime (TMB-4), obidoxime (LüH-6, Toxogonin) and asoxime (HI-6), and their chemical structure is presented in Figure 2. Bispyridinium oximes are less frequently used in treatment of OP poisoned patients, due to their limited commercial availability, and may have some advantages over pralidoxime in special circumstances (i.e. poisoning with warfare nerve agents).

Pralidoxime is currently most important pyridinium oxime being used in clinical practice for about a half of a century. In poisoning with OP pesticides pralidoxime chloride should be administered to adults in a dose of 500 mg/h, continuously maintained until clinical improvement is obtained, or 30 mg/kg body weight bolus intravenously over 4 to 6 hours or 8 to 10 mg/kg/h intravenously until full recovery occurs. In children, pralidoxime chloride should be administered in a dose of 25 mg/kg intravenously for 15 to 30 minutes, followed by a continuous infusion of 10 to 20 mg/kg/h. The therapy can continue for 18 hours or longer (even several days), depending on the clinical status[9,15] and the presence of OP or its metabolites in blood/urine.

Detailed protocols on medical treatment of cholinergic crysis are presented in several excellent reviews[5,7,10,14,16,22,24,25].

Intermediate syndrome

The term Intermediate Syndrome (IMS) was first described by Senanayake and Karalliedde (26) because it appeared in the interval between the end of the cholinergic crisis and the onset of OPIDP. Following exposure to various OP pesticides, clinical manifestations of IMS typically occur within 24 to 96 hours, and affect patients without fasciculation or other cholinergic signs. The reported incidence of IMS ranges from 7.7% to as high as 84% [27]. Although IMS is well recognized as a disorder of neuromuscular junctions, its exact etiology, incidence, and risk factors are not clearly understood. IMS generally occurred among patients with prolonged and severe inhibition of AChE, however not every patient with severe AChE inhibition developes IMS. Other risk factors of IMS include delayed metabolism of OP pesticides due to toxicokinetic factors or impaired organ function, severity of poisoning, elevated muscle enzymes, and inadequate or late oxime therapy. IMS has been linked with exposure to specific OP pesticides having dimethyl phosphate structure (e.g. fenthion, dimethoate, monocrotophos, dichlorvos, methylparathion) but also developed after exposure to parathion (ethyl phosphate) and methamidophos (phosphoramidate)[28,29]. Two typical cases of IMS caused by fenthion and diazinon were recently described by Jokanović and coworkers[5].

Marked weakness of neck flexion and varying degree of proximal limb muscle weakness, manifesting as weakness of shoulder abduction and hip flexion, are the regular clinical features. Respiratory insufficiency is also common and frequently draws medical attention to the onset of the syndrome. Other possible manifestations are involvement of muscles innervated by motor cranial nerves and decreased deep tendon reflexes. Studies conducted in nineties have shown that intermediate syndrome goes along with excretion of cholinesterase inhibitor metabolites in the urine and by severe depression in cholinesterase levels. It was suggested that the condition might reflect the recirculation of lipid soluble cholinesterase inhibitors from body fat compartments or gastric fluids[30]. IMS could be explained by the reduction in number of functioning cholinergic receptors at the postjunctional membrane, or a failure of acetylcholine release. All these abnormal findings on electromyography suggested a combined presynaptic and postsynaptic defect, without sensory impairment[31].

With appropriate therapy, recovery from IMS occurs 5-18 days after the onset of weakness. The recovery among patients who survived IMS follows a distinct pattern, starting first with muscle power recovery in cranial nerve-innervated muscles, followed by respiratory muscles, proximal muscles, and neck flexors. Since IMS carries a high risk of death among patients with respiratory failure, prompt recognition of the syndrome is the basis of IMS treatment. IMS management is mainly supportive since there are no specific antidotes available for this life threatening syndrome. As IMS generally takes place at the same time with severe OP toxicity and persistent inhibition of AChE, early gastrointestinal decontamination, followed by appropriate therapy involving atropine and pralidoxime, and prompt institution of respiratory support, should be helpful in ameliorating the magnitude and/or the incidence of IMS. The prognosis of IMS appears to be favorable if respiratory failure can be promptly recognized and treated accordingly[28-30].

Table 2. Organophosphorus pesticides as a cause OPIDP in man.[6,36,37]

OP insecticide	No of cases	Location	Year
Chlorpyrifos	2	Italy, India	1986
Dichlorvos	5	Romania, Turkey, Brazil, Korea, India	1980, 2002-2006
Ethyl parathion	1	Germany	1993
Fenthion	3	USA	1985
Isofenphos	1	Israel	1987
Isofenphos/phoxim	1	Italy	1995
Leptophos	80	USA	1974
Malathion	2	Japan, Turkey	1991, 2009
Merphos	1	USA	1977
Methamidophos	> 45	Sri Lanka, Italy, China, Turkey, USA	1981, 1998
Mevinphos	1	Serbia	2010
Mipafox	3	UK	1952
Omethoate	1	France	1972
Phosphamidon/ Mevinphos	1	China	2002
Trichlorfon	22	Romania, Iran, Japan, Hungary	1983 -1986
Trichloronat	1	Poland	1975

Organophosphate induced delayed polyneuropathy

Organophosphate induced delayed polyneuropathy (OPIDP) is unique toxicological phenomenon in that it is caused by a single exposure to certain OP with effects usually appearing after 10 to 20 days or later. OPIDP is toxicologically different from the cholinergic syndrome in that it is based on different mechanisms which do not involve AChE and appear a few weeks after OP poisoning has been medically solved with standard therapeutic measures and patient dismissed from hospital. OPIDP is also a different syndrome from IMS.

The interest in OPIDP appeared after thousands cases of poisoning with triorthocresyl phosphate (TOCP) that occurred mainly due to beverage and food contamination in USA in 1930 and Morocco in 1959 [32-35]. By the end of twentieth century, there were many cases of OPIDP due to TOCP poisoning in Romania, Sri Lanka, former Yugoslavia and China. In addition to TOCP, several other OP pesticides have been reported to cause OPIDP in man (Table 2) [6,11,36-38].

OPIDP is relatively rare neurodegenerative disorder in humans that is characterized by loss of function and ataxia of distal parts of sensory and motor axons in peripheral nerves and ascending and descending tracts of spinal cord. The early neurological symptoms usually are sharp, cramp-like pains in the calves, tingling in the feet and hands followed by distal numbness and paresthesia. Pain and weakness in muscles spread rapidly and patients become unsteady and unable to keep their balance. Progressive leg weakness occurs, together with depression of tendon reflexes. Symptoms may also appear in the arms and forearms. Sensory loss may be mild. Muscle tonus of the limbs gradually increase and spasticity appears in the lower limbs. Physical examination reveals distal symmetrical mainly motor polyneuropathy, with wasting and flaccid weakness of distal limb muscles, especially in the lower limbs. In severe OPIDP quadriplegia with foot and wrist drop were observed as well as mild pyramidal signs[34]. There may be some functional recovery in less severe cases with more distal involvement and sparing of spinal cord axons, but pyramidal and other signs of central neurological involvement may become more evident with time. The recovery affects only sensory nerves, while motor neurons may permanently lose its function as indicated by Morgan[33] who described the lack of improvement during 47 years in 11 patients poisoned with TOCP. The prognosis for functional recovery depends on the degree of pyramidal involvement with ataxia and paralysis representing a permanent outcome of severe OPIDP. It appears that clinical signs of OPIDP in children are considerably milder than in adults [11,35,37,38].

OPIDP is initiated by phosphorylation and subsequent aging of >70% neuropathy target esterase (NTE) in peripheral nerves. Physiological role and importance of NTE were recently discussed by Jokanović et al. [11].

Medical treatment of OPIDP in humans is symptomatic. Standard treatment of OP poisoned patients comprising atropine, oxime and diazepam was not effective in treatment of OPIDP. However, there were several reports in the literature describing attempts of treatment of OPIDP in animals and these studies were reviewed by Lotti [34] and Jokanović et al. [11,35], but none of these treatments have been tested in patients so far.

Chronic organophosphate-induced neuropsychiatric disorder

Chronic exposure to OPs has been associated with impaired neurobehavioral performance in some, but not all, epidemiological studies[39]. Chronic organophosphate-induced neuropsychiatric disorders (COPIND) occur without cholinergic symptoms and apparently are not dependent on AChE inhibition[39,40]. COPIND usually appears with a delay and persists for a long period possibly suggesting the permanent damage of the central nervous. The most common symptoms of COPIND include cognitive deficit (impairment in memory, concentration and learning, problems with attention, information processing, eye-hand coordination and reaction time), mood change (anxiety, depression, psychotic symptoms, emotional lability), chronic fatigue, autonomic dysfunction, peripheral neuropathy and extrapyramidal symptoms such as dystonia, resting tremor, bradikynesia, postural instability and rigidity of face muscles[11,39-46]. Suicidality and alcohol intolerance have also been reported[42]. Similar clinical features have also been reported by soldiers suffering from the Gulf-War Syndrome, which led to the, so far unproven, hypothesis that the illness was caused by chronic exposure to chemical agents with similar effects to OPs[47].

Diagnostic criteria for COPIND include: 1) Repeated exposure to organophosphates; 2) At least four of the following: a) personality change and destabilization of mood, b) impairment of concentration, c) impaired exercise tolerance, d) reduced tolerance to alcohol, e) heightened sensitivity to organophosphates; 3) At least three of the following: a) exacerbation of "dippers flu", b) impulsive suicidal thinking, c) language disorder, d) heightened sense of smell, e) deterioration of handwriting[48].

In several epidemiological studies conducted among farm workers and pesticide applicators, neuropsychological damage accompanied with damage of peripheral nervous system, anxiety and depression were predominant among the poisoned group[49-51]. Agricultural workers tested about 2 years after a pesticide poisoning episode showed significantly lower performance in verbal and visual attention, visual memory, sequencing and problem solving[52]. Levin et al.[53] found a high level of anxiety in commercial sprayers of insecticides. Savage et al.[54] showed abnormalities in psychometric testing and motor reflexes. Mild intoxication can also induce COPIND, farm workers with mild OP pesticides intoxication requiring no hospitalization performed worse on tests of cognitive and psychomotor function than nonexposed workers did tested 2 years later. Epidemiological study from Spain revealed a link between exposure to organophosphates and increased suicidal rate[55]. A literature review of mortality and morbidity studies related to suicide among pesticide-exposed populations, revealed high suicide rates in farming populations. Epidemiological studies conclude that acute and chronic OP exposure is associated with affective disorders[50,56].

The underlying mechanism of COPIND has not been established. Tan et al.[46] hypothesized that COPIND could be derived from withdrawal of OP pesticide after chronic low-level exposure or acute exposure. In addition, other scientists have suggested that mechanisms other than the inhibition of AChE might also be involved. Studies in animals suggested that cognitive enhancing action and changes in behavior of low doses of certain OPs, such as dichlorvos, in rats were unrelated to AChE inhibition[14,57,58]. Finally, London et al.[50] reported that exposure to OP may cause serotonin disturbances in the central nervous system, which are implicated in depression and suicide in humans.

Authorship statement
Both authors contributed equally.

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We declare that we have no conflicts of interest.

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Neurotoksični poremećaji i lečenje otrovanih organofosfornim pesticidima

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APSTRAKT

U ovom radu opisani su neurotoksični poremećaji koji se javljaju kod pacijenata otrovanim organofosfornim (OF) pesticidima. OF pesticidi izazivaju četiri značajna neurotoksična efekta kod ljudi i to holinergički sindrom, intermedijarni sindrom, organofosfatima izazvanu naknadnu polineuropatiju i hronični organofosfatima izazvani neuropsihijatrijski poremećaj. U poređenju sa holinergičkim sindromom, koji se javlja kod više miliona slučajeva trovanja svake godine u svetu sa mortalitetom većim od 15%, ostali poremećaji su opisani kod znatno manjeg broja slučajeva. Rad je fokusiran na neurotoksične poremećaje koji se javljaju posle akutne i hronične ekspozicije OF pesticidima sa naglaskom na kliničku sliku, objašnjenje molekularnih mehanizama i mogućnosti lečenja zatrovanih pacijenata.

KLJUČNE RIJEČI

Organofosforni pesticidi, holinergički sindrom, piridinski oksimi, atropin, acetilholinesteraza.

REVIEW ARTICLE

Production of Hematopoietic Cells From Umbilical Cord Blood Stem Cells for Transfusion Purposes: Focus on *Ex Vivo* Generation of Red Blood Cells

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ABSTRACT
Cord blood contains a heterogeneous population of stem cells and progenitors and, in addition to transplantation these cells could be used also for ex vivo production of mature cells, as red blood cells (RBC). The experimental ex vivo production of RBC, first demonstrated with cord blood CD34+ cells as a "raw material", is today a reality. The proof of principle for transfusion of ex-vivo-generated erythrocytes was provided recently. In this purpose, the CD34+ cells of other sources could be used: bone marrow, peripheral blood in steady state and after "mobilization" treatment, but also embryonic stem cells and adult Induced Pluripotent Stem Cells – iPSC. It is imperious today to decide which cell population will be used to produce ex-vivo erythrocytes for clinical applications. In addition, the culture system should be optimized with respect to physical parameters (for example oxygenation), up-scaling to the clinical grade and transferred to industrial level.

KEY WORDS
Umbilical cord, stem cells, red blood cells, transfusion of generated red blood cells.

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Cell engineering for cell therapy and transfusion

Traditional transfusion is based on injection in circulation of « labile hemoproducts » which, apart plasma, represent concentrates of mature blood cells. Thus, injection of the cells (mainly Red Blood Cells (RBC) and platelets) is aimed to replace temporarily their lack in circulation. The point is that transfusion is operating with the cells having definitive life span i.e. the cells which are not going to replicate and reconstitute in vivo a cell lineage or a tissue. On the contrary, cell therapy is based on injection of stem and progenitor cells i.e. the cells which should produce for short- or long-term, a cell offspring, hence, ensure a sustained production of mature cells belonging to one or more cell lineages.

The future of cell therapy is closely related to development of ex vivo technology for transformation and amplification of cells. Cell engineering should allow improving the grafts based on stem cells and progenitors that already became reality in field of hematology, cardiology, endocrinology....[1]

But cell engineering could also enable ex vivo production of mature cells for transfusion purposes, starting from CD34+ cells isolated from bone marrow, cord blood, or mobilized to peripheral blood; it is possible to produce megakaryocyte, granulocyte, as well as RBC. In this review, we are going to discuss some points related to ex vivo red blood cells generation from cord blood CD34+ cells.

CD34+ cells as a source of stem cells

Bone marrow, cord and peripheral blood cells expressing CD34 antigen are extremely heterogenous cell population from a functional point of view. The idea that the expression of CD34 antigen is related to hematopoietic stem cells

has been a permanent source of misunderstanding and confusion (reviewed in [2]). It should therefore be stressed that the fact the majority of hematopoietic stem and progenitors cells express CD34, does not mean that all CD34+ cells are stem cells or progenitors. In CD34+ population of cord blood, 30 to 50 % of cells are progenitors (CFU-GM, BFU-E, CFU mix and CFU Mk) and only a small percentage is a subpopulation of primitive stem cells. Approximately one half of CD34+ cell population does not exhibit either progenitor or stem cells functional properties. Furthermore, the situation is even more complicated: some stem cells do not express CD34 in steady state,[3] and expression of this molecule could be reversible,[4] and is not related to functional capacities of stem cells.

However, from a practical viewpoint, due to well developed and easily available technology for isolation of CD34+ cells, this cell population, relatively enriched in progenitor and stem cells represents a good starting point to ex vivo production of mature cells.

Cord blood CD34+ cells

CD34+ population from cord blood is composed of progenitors and stem cells exhibiting a higher proliferative capacity than those in CD34 cell population isolated from adult bone marrow and peripheral blood.[5] In fact, cord blood is nothing else than neonatal blood, i.e. its part remaining in placenta after the umbilical cord is cut. These high proliferative capacities of stem and progenitor cells from cord blood seem to be attractive for ex vivo production of mature cells, since enabling a good yield. In addition, the sampling of cord blood is neither harmful for the mother nor for the baby nor related to serious ethical issues as some other sources of stem cells (embryonic stem cells). On the other hand, the need for cord blood cells in transplantation purposes could appear as a limiting factor for their use for ex vivo generation of mature cells. In fact, this problem could be easily solved by choosing only cord blood units rejected from the bank due to a cell number/volume lower than the bank standards. For all these reasons, CD34+ cells isolated from cord blood were and are regularly used in experiments aimed to enable a production of mature cells ex vivo.

Ex vivo production of RBC from stems cells: a need in transfusion medicine

Fulfilling the worldwide need for 96 million red cells transfusion per year meets several limitations.[21] First problem comes from demographic evolution which predicts important increase of world population aging in the coming decades (e.g. percentage of persons over 60, will reach 32% in 2050 in France). This will increase considerably the number of malignant haematological conditions which have important requirement for RBC transfusion.[22] On the other hand, the numbers of blood donors will raise much more slowly. Secondly, even in developed transfusion system, the risk of transfusion transmitted infection (TTI) can be really high due to elevated prevalence of the infectious disease spreading world widely and not adequate blood testing infrastructure.[21] Therefore, finding the alternatives to conventional transfusion practice as it is transfusion of ex vivo generated RBC, may become unavoidable future. In addition, development of strategies for ex vivo production of RBC offers the possibility to generate RBC expressing patient's specific blood group antigens or even "universal" RBC lacking membrane expression of two principal blood group systems, ABO and RHD. This might resolve the problem of the alloimunisation in the frequently transfused patients (patients with thalassemia major and sickle cell disease) or transfusion of patients with rare blood phenotype. Finally, ex vivo produced RBC could be used as control samples for the immunehematologic testing and screening of RBC alloantibodies in patients who received multiple transfusions.

RBC from CD34+ cells

Appearance of colonies composed of hemoglobinized cells in semi-solid medium indicates in vitro erythroid differentiation from progenitor to precursor state.[6] These cultures in semi-solid medium are still in use to enumerate erythroid progenitors in a cell population. Their efficiency is highly improved with combination of cytokines and growth factors allowing/stimulating differentiation of erythroid progenitors. The "red" colonies (CFU-E, BFU-E) are composed mainly of erythroid precursors (starting from pro-erythroblasts), but in these cultures, the final erythroid maturation does not occur. This methodology didn't enable the production of homogene population of erythroid cell types produced which can be easily isolated and analyzed. This problem was technically accomplished by development of two phase liquid culture ex vivo models of erythropoiesis, proposed by Fibach et al (24) and modified after by several groups (24-28). Briefly, In the first, erythropoietin-independant phase peripheral blood mononuclear cells or CD34+ derived from different origin (bone marrow, cord blood, peripheral blood) were cultivated in presence of different combination of the growth factors (originally, in presence of the conditioned medium from human bladder carcinoma). This phase allows generation of primitive erythroid progenitors BFU-E which proliferate and differentiate in more mature CFU-E. In second, EPO-dependant phase, presence of EPO in combination with other synergistic cytokines, allows generation of erythoid precursors with high yields of orthochromatic normoblasts and minority of enucleated erythrocytes. Just

for the illustration, we can mention that using this model Freyssinier et al obtained CD36 + population of erythroid progenitors by cultivated CD34+ derived cord blood and mobilised peripheral blood in serum-free condition with SCF, IL-3, IL-6, in the first phase culture. These cells developed in erythroid precursors in the secondary culture in presence of EPO. Production overview was that from 10^6 CD34+ cells input this model enabled generation of 10^7 CD36+ cells at Day 7 and of 1.5x 10^8 precursors after 3 days of secondary culture (100-fold expansion).[26]

It should be mentioned that the others managed to reach terminal erythroid maturation using mono-phase culture system. For example Malik et al, showed that in presence of IL-3, GM-CSF, and high concentration of EPO, ex vivo production of erythroid precursors from CD34+ from bone marrow, cord blood, and peripheral blood, was supplemented with ten to forty percentage of the enucleated erythrocytes showing the characteristics of reticulocytes and expressing γ-globin and β-globin depending of the source.[29]

More efficient and sophisticated era of the ex vivo RBC production started when professor Douay's group published its first paper explaining how to get a massive amplification of a pure erythroid precursor population by applying a sequential specific combination of growth factors during the culture, designed first to amplify hematopoietic stem cells, to differentiate them to erythroid progenitors, and then to induce their terminal differentiation.[7] This approach used a three-phase culture. In the first phase, aimed to differentiate committed progenitors till precursor stage from CD34+ cord blood cells, was a classical liquid culture supplemented by FLT-3 ligand (FLT-3L) stem cell factor (SCF) and thrombopoietin (TPO). After this week long phase, the cells were resuspended in medium containing SCF insulin growth factor (IGF) and erythropoietin (EPO) for further 7 days. Finally, the cells were washed and resuspended in medium with EPO only.

Although not ideal, this procedure showed that a massive and exclusive production of erythroid precursors (up to 99%) containing fetal hemoglobin (amplification up to 200000 times) is possible ex vivo. Since in cord blood, RBC fetal hemoglobin is a major component, the proportion fetal/adult hemoglobin decreased after culture but not reaching the proportion in adult RBC, where adult hemoglobin is predominant. Even if reticulocytes were only occasionally present in culture endpoint cell population (all stages from pro-erythroblasts to ortho-chromatic erythroblasts were present), these ex vivo generated erythroid cells injected in non-obese diabetic/severe combined immunodeficient (NOD/Scid) mice continued with terminal maturation and gave enucleated RBCs in vivo producing adult hemoglobin.[7] The fact that the complete terminal differentiation is accomplished only in vivo, pointed to a crucial role of erythropoietic microenvironement for the full maturation of RBC. That's why, few years later, the same group improved the system by combining a liquid culture with a co-culture with stromal cells.[8] Starting from cord blood, bone marrow or peripheral blood CD34+ cells the first culture phase (8 days) was stimulated by interleukin-3 (IL-3), SCF, EPO in presence of hydrocortisone. The cells were then transferred to a stromal layer (murine stromal cell line MS5 or adult human bone morrow obtained from healthy donors) in presence of EPO only for next 3 days and then washed and reseeded on adherent layer in medium without growth factors.[8]

These modifications resulted in virtually pure population (90-100%) of enucleated cells, mainly composed of reticulocytes. These ex vivo generated erythrocytes display characteristics close to those of native ones (volume and hemoglobin content). Interestingly, in contrast to bone marrow or peripheral blood CD34+, ex vivo generated RBC from cord blood CD34+ cells contained mostly fetal hemoglobin. In these conditions, the expansion reached amazing 10^6-10^7 fold with respect to the starting number of CD34+ cord blood cells.[8]

After being injected into circulation of NOD/SCID mice, these ex vivo produced reticulocytes mature up to final RBC. These cells show normal enzyme expression, membrane deformability and oxygen dissociation characteristic similar to those of native erythrocytes.

Ex vivo RBC production yield

If one would like to utilize ex vivo generated RBC for purpose of transfusion, the number of produced cells should correspond to one presented in the unit of conventional RBC "concentrate" which contains approximately 2000 billions of cells (2000 x 10^9). To accomplish this challenge, the best choice of the starting material, according to our and others' experience,[22] is cord blood CD34+ cells. Their proliferative capacity is 5 to 10 times higher than those of peripheral blood CD34+ cells.

Ex vivo systems enabled to produce 4 to 22 millions of RBC from one cord blood CD34+ cell. Knowing that average cord blood donation contains from 2 to 5 million of CD34+ cells, we can easily calculate that from only one cord blood unit equivalent of the 10 to 50 RBC units can be produced.

Improving the system

These results have been confirmed by other groups,[9,10] including ours.[11] In attempt to improve the system according to our "Oxygen Stem Cell Paradigm",[12] we hypothesized that the yield of RBC could be further improved

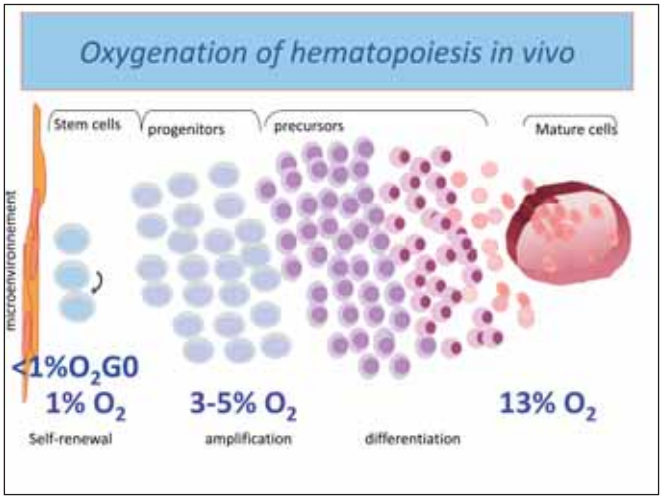

Figure 1. Oxygen gradient in bone marrow.

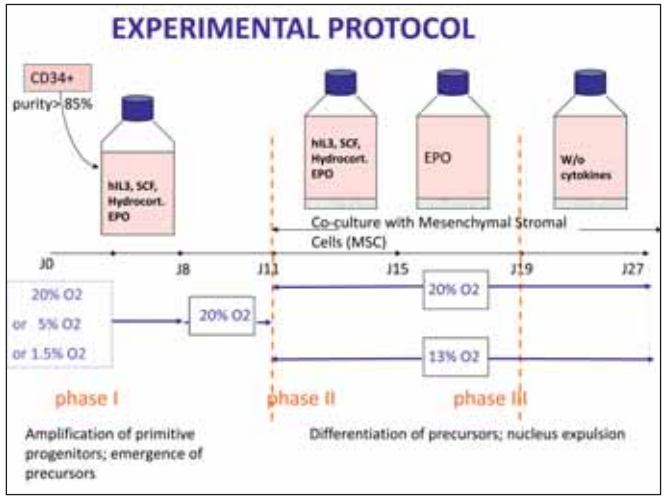

Figure 2. Culture designed to mimic the bone marrow oxygen gradient.

by adapting the oxygenation of each stage of culture to physiologic one. It is known that erythropoiesis takes on in micro-environment of bone marrow niches exposed to relatively low O_2 concentrations (from almost 0 near the endosteum to 8 % at the edge of blood vessels).[13-16] In the course of their proliferation, differentiation and maturation, erythroid cells migrate towards the longitudinal access of bone marrow, reaching more oxygenated areas as they approach blood circulation (Figure 1). Also, some literature data obtained with cord blood cells strictly suggested that at 1% O_2, the production and amplification of BFU-E (EPO-independent cells) seemed to be enhanced, while their commitment toward CFU-E and further is accomplished at higher O_2 concentrations.[17]

Thus, we extended our "Oxygen Stem Cell Paradigm" to erythroid progenitors,[12] and hypothesized that the sequential establishment of an optimal O_2 concentration for each development stage of hematopoiesis/erythropoiesis enhanced the amplification of primitive stem cells and erythroid progenitors as well as the proliferation of the latter, and consequently, boosted RBC production. So, adapting the early phase of erythropoiesis to physiologically low oxygenation (1.5 to 5%) (Figure 2), we increased by several fold the amplification of primitive erythroid progenitors before inducing latter steps of erythropoiesis. An additional advantage was obtained during the phase 2 and 3 of culture (erythroid maturation and nucleus expulsion) if the culture was exposed to 13% O_2 concentration with respect to that of air (20-21%) (Figure 2). Thus, just by approaching culture oxygenation conditions to the physiological one, we realized 2 to 3 fold higher yield of mature enucleated RBCs (morphology at each culture point, Figure 3).[11]

The other interesting proposition came from Fujimi et al, who cultivated CD34+ cord blood cells on a telomerase gene

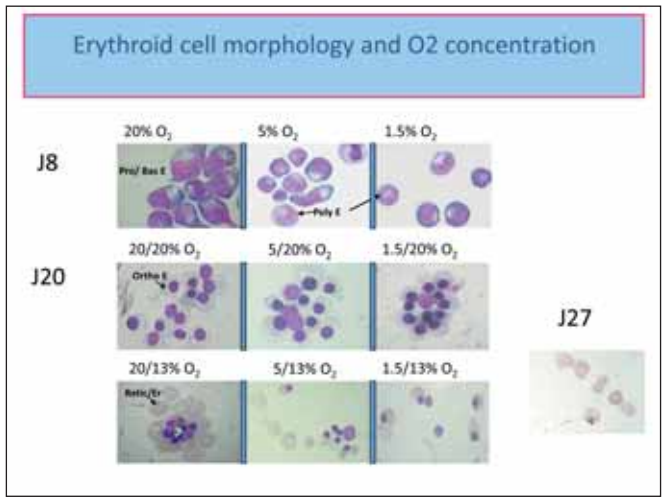

Figure 3. Morphology of erythroid cells at different culture time-points and with respect to oxygenation level.

transduced (hTERT) human stromal cell line, followed by the simple culture phase, succeeded by the co-culture with macrophages derived from parallel co-culture of CD34+ cells on hTERT stromal cells in presence of macrophage colony stimulating factor (M-CSF) and granulocyte colony stimulating factor (G-CSF**).** But this costly system didn't overcome the degree of enucleation in the final phase and the expansion rate obtained in previous protocols.[8,23]

Simplification of the procedure

In order to simplify the procedure for the ex vivo RBC production interesting proposal came form Miharada et al, who developed a stroma-free protocol. Using cord blood CD34+ cells in liquid culture in presence of VEGF,

IGF-2, mifepristone, antagonist of glucocorticoid function and human serum, they were capable of generating $4X10^{12}$ erythroid cells from a single donation of around $5x10^6$ CD34+ cells (700,000 fold expansion).[30] They demonstrated that contact with stromal cells is not absolutely necessary for the enucleation since it was not completely abrogated by elimination of contact between MS-5 cells and erythroid cells. According to the obtained results, authors concluded that: .... the interaction of erythroblasts with other cells is not necessary and that signals mediated by humoral factors seem to be sufficient for efficient autonomous completion of erythroblast enucleation.

But, this system was not as efficient as previous ones:[8] it enabled terminal differentiation where the percentage of enucleated cells was 77.5% compared with 98% reported by Giarratana et al.[8]

Proof of principle for transfusion of in vitro generated RBCs

All these data were obtained in experimental conditions in small culture systems allowing production of RBC volumes sufficient only to confirm the principle. Professor Douay's group produced milliliter-range quantities of RBCs from peripheral blood CD34+ cells).[18] Peripheral blood CD34+ obtained by leukapherese after mobilization with G-CSF were expanded by using three-step protocol previously described by the same group; but in this work stromal cells were replaced by detergent virus-inactivated human plasma, which was sufficient to accomplish erythroid differentiation and enucleation (68%) Immunophenotypic characterization of enucleated cells confirmed their reticulocytes profile. These cells synthesized mostly adult Hb and showed enzyme content, deformability and Hb functionally corresponding to those of native RBC. Blood group antigens expression from ex vivo generated RBC and native RBC from the same donor didn't show notable difference.

Homogeneous sample of 10^{10} Day 18 reticolocytes generated under good manufacturing practice conditions and labeled with 51 Cr were re-injected to the same donor (autologous context).[18] The level of these cells in the circulation 26 days after injection was between 40-60% which is comparable with reported half-life of approximately 28 days for the native RBC.

This work demonstrated a normal behavior and functional properties of ex vivo generated RBCs. This is the first proof of principle for transfusion of in vitro generated RBCs. Although similar assay was not done with RBCs ex vivo generated from cord blood CD34 cells, we could suppose with reasonable probability that it would yield similar results.

Conclusion and perspectives

Experimental RBCs ex vivo production from cord blood CD34+ cells turned out to be feasible. Ex vivo generated RBCs exhibit similar properties as "natural" RBCs. Although not done with RBCs generated from cord blood CD34+ cells, a proof of principle for transfusion of ex vivo generated RBCs is provided. The challenge now is to develop a large-scale procedure allowing to get clinically relevant RBCs quantities that should be compatible with industrial production.[19] Of course, red cell concentrates produced this way should be tested to demonstrate their efficiency that will require clinical trial with very large number of patients.

However, there is a conceptual dilemma raising a question which should be addressed before launching such an expansive enterprise: from where to start,[20] i.e. which cell population use as raw material for RBC production. It seems that the traditional source of CD34+ cells as bone marrow, blood or umbilical cord are less attractive today than the pluripotent embryonic stem cells or adult Induced Pluripotent Stem Cells (iPSC).[20,21]

Very complicated matter is the blood type phenotype. With that respect also, iPSC seem to provide an advantage. It is estimated that 3 different cell lines, could enable the RBC offspring "covering" 99% of erythrocyte phenotype needed for Caucasian population.[20]

Whatever choice of starting cell population, RBC production will surely be costly, at least at the beginning, before establishing a large-series industrial process. While initially, when adult and CD34+ cells were primarily considered, it was supposed that ex vivo production of RBCs could be particularly useful to produce RBCs of rare blood type phenotypes, these new possibilities with iPSC bring a hope to "cover" all patients. The industrial-scale RBC production, which depends on the amount of investment, probably would not start in 5 to 10 years.

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Both authors contributed equally.

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Perspektiva korištenja hematopoetskih ćelija iz krvi pupčane vrpce (placentarne krvi) u transfuziji: fokus na stvaranje eritrocita *ex vivo*

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APSTRAKT

Krv iz pupčane vrpce (placentarna krv) sadrži populaciju matičnih ćelija i progenitora opredeljenih za ćelije pojedinih krvni loza. Te ćelije mogu se koristiti u svrhu transplatacije, ali i za proizvodnju zrelih ćelija - poput eritrocita. Eksperimentalna proizvodnja eritrocita ex vivo pomoću CS34+ ćelija je ostvarena pre jedne decenije. Odnedavno postoji dokaz da se tako stvoreni eritrociti mogu koristiti u transfuziji. Matične ćelije i progenitori iz drugih izvora (kostna srž, periferna krv) kao i embrionalne matične ćelije i pluripotentne matične ćelije indukovane iz zrelih ćelija mogu se takođe koristiti za proizvodnju eritrocita za transfuziju.

KLJUČNE RIJEČI

Placentarna krv, matične ćelije, eritrociti, transfuzija proizvedenih eritrocita.

REVIEW ARTICLE

Transforming Growth Factor-Beta Superfamily Members in the Pathogenesis of Pulmonary Arterial Hypertension

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ABSTRACT
Pulmonary arterial hypertension (PAH) is a devastating and rapidly progressing disease that induces substantial pulmonary vascular remodeling. The pathologic changes especially in pulmonary microvasculature result in progressive increases in the mean pulmonary artery pressure and pulmonary vascular resistance, which, if untreated leads to right-ventricular failure and death. Although it is clear that PAH has a multifactorial pathobiology, recent discoveries pointed out crucial role of Transforming Growth Factor (TGF)-beta family members in the pathophysiology of PAH. The TGF-beta superfamily is composed of multifunctional mediators, including the TGF-beta isoforms and the Bone Morphogenetic Proteins (BMPs). Germline mutations in the gene coding for BMP receptor 2 (*BMPR2*) have been identified in 60% of familial and 10-30% of idiopathic PAH. Mutations in the TGF-beta receptors, ALK-1 and endoglin, have been found in PAH patients with a personal or family history of hereditary hemorrhagic telangiectasia. Non-canonical TGF-beta pathways as well as TGF-beta receptor ligands (i.e. BMP9) are also involved in PAH development. Our improved understanding of TGF-beta pathway regulation will have important implications for the development of novel therapeutic strategies for this complex and serious disease. Animal models will undoubtedly have an important role in this process; however human studies will give the final answer about the efficacy and safety of the novel treatments for PAH. This review provides an overview of the TGF-beta and BMPs potential role in PAH.

KEY WORDS
Transforming growth factor-beta, pulmonary arterial hypertension, bone morphogenetic proteins, endoglin, ALK1.

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Pulmonary arterial hypertension (PAH) is either due to cardiac, pulmonary or extrathoracic disorders (secondary PAH) or without any detectable cause (primary or idiopathic PAH; IPAH).[1] In some cases, primary PAH occurs in young patients with a mitochondrial disorder,[2] but whether there is a causal relation between them, remains to be elucidated. Regardless of the etiology, unrelieved pulmonary hypertension advance to right-sided heart failure, progressive debilitation and death, often within 2 to 3 years after its initial diagnosis. Lung specimens from patients with PAH and from experimental models underline the importance of vascular cell proliferation and obliteration of small pulmonary arteries by smooth muscle cells and myofibroblasts in the pathogenesis of this disease. In addition, plexiform lesions comprising endothelial cells and myofibroblasts are found in about 50% of cases.

Over the last decade, some major advances have led to substantial improvements in the management of PAH. Much of this progress was pioneered by work in animal models. Although none of the current animal models of PAH completely recapitulate the human disease, they do provide

insight into the cellular pathways contributing to its development and progression. Genetic studies have revealed heterozygous mutations in the *BMPR2* gene encoding the type II bone morphogenetic protein receptor (Bmpr2), a member of the TGF-beta superfamily of receptors, underlying the familial form of the disease.[3] Subsequently, *BMPR2* mutations were found in about 25% of apparently sporadic cases of IPAH, many of which are examples of familial transmission with low disease gene penetrance. These studies pointed toward a critical role for the TGF-beta superfamily in the pathogenesis of PAH through the molecular mechanisms that include regulation of pulmonary vascular cell growth and differentiation. [4]

TGF-beta signalling pathways

TGF-beta superfamily has over 35 structurally related pleiotropic cytokines that also includes TGF-beta1, TGF-beta3, Activins, Nodals and bone morphogenetic proteins (BMPs). Signalling by TGF-beta family members occurs through type II serine/threonine kinase receptors and type I receptors, also termed Activin-receptor-like kinases (ALKs).[5] Each member of the TGF-beta superfamily binds to a characteristic combination of type I and type II receptors. Ligand binding induces the assembly of type I and type II receptors into complexes, within which type II phosphorylates type I receptor and this phosphorylation is both essential and sufficient for TGF-beta signalling (Figure 1). Endoglin, an accessory receptor, modulates TGF-beta signaling by regulating surface TGF-beta receptors and their activation.[6] Once activated, the type I receptor can induce several signaling outcomes which include phosphorylation of cytoplasmic proteins called regulatory Smads (Smad2/3 or Smad1/5/8) that trimerize with Smad4 leading to nuclear shuttling and gene transcription regulation. In addition, non-Smad signaling pathways can also be induced (Figure 1) including members of the mitogen activated protein kinase (MAPK) pathway, JNK, p38, p42/44 (ERK), PI3-kinase and Rho pathways.[7] Involvement of non-Smad pathways in pathogenesis of PAH is well known, especially the ras homolog family member A (RhoA)-Rho kinase (ROCK) axis which specific inhibition is currently the most promising therapeutic approach for PAH.[8] Also,

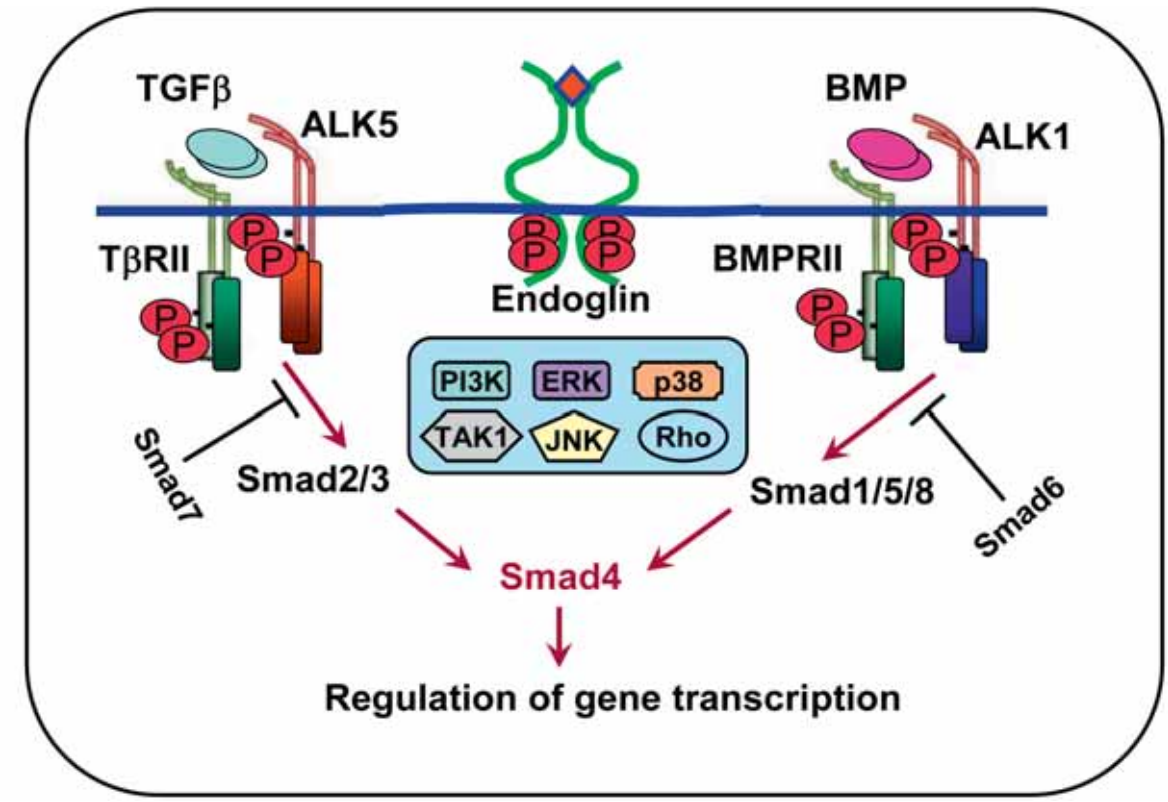

Figure 1. TGF-beta family signalling pathways. Ligands (TGF-beta and BMPs) bind to the receptors and induce heteromeric complex formation between specific type II and type I receptors. Endoglin, an auxilliary receptor, is able to modulate and regulate both TGF-beta and BMPs effects. The type II receptors phosphorylate the type I receptors, leading to their activation. Subsequently, phosphorylation of receptor-regulated (R) Smads occur, which form complexes with Smad4 (common Smad) and translocate into the nucleus where interact with transcription factors and regulate gene responses (canonical Smad signaling pathway). Inhibitory Smads 6 and 7 inhibit receptor activation of R-Smads. In addition, the activated type I receptors can signal through non-Smad pathways (PI3 kinase, mitogen activated protein kinases (MAPK) ERK, p38, TAK1, and Jun kinase, and GTP-ase Rho).

it was recently reported that *BMPR2* deficiency promotes pro-proliferative and anti-apoptotic responses in pulmonary arterial smooth muscle cells through the activation of TGFâ-TAK1-MAPK pathways in PAH.[9]

TGF-beta receptor mutations in PAH

Although mutations in the *BMPR2* gene are the most frequent germline mutations identified in familial (~60%) and idiopathic PAH (10-30%), they are absent in some families and in the majority of sporadic and associated cases of PAH. This fact led to the investigation of other gene involvement from TGF-beta family in PAH. Indeed, mutations in the TGF-beta receptors, ALK-1 and endoglin, have been identified in PAH patients with a personal or family history of hereditary hemorrhagic telangiectasia (HHT).[10]

It is important to stress out that TGF-beta signalling is essential for regulation of vasculogenesis and angiogenesis. Mutations in at least five genes result in HHT (Rendu Osler-Weber-syndrome), an autosomal dominant disorder with a prevalence of about 1 in 5-8,000 individuals, but mutations in two genes (*Endoglin, ENG* causing HHT1 and *ALK1* causing HHT2) account for approximately 85% of cases. HHT is characterized by epistaxis, mucocutaneous and gastrointestinal telangiectases and arteriovenous malformations (AVMs) in pulmonary, hepatic and cerebral circulation.[11] Deficiency in endoglin or Alk1 causes cardiovascular defects leading to embryonic lethality. Mouse heterozygous models for HHT1 and HHT2 has been developed allowing further insight in the role of TGF-beta family in blood vessel and heart development and vascular homeostasis.[12, 13] Our and other studies have revealed a marked deficiency in nitric oxide (NO) mediated vasodilation in endoglin-haploinsufficient mice ($Eng^{+/-}$), as well as that endoglin has an important role in endothelial NO synthase (eNOS) activation.[14, 15] Moreover, endoglin and Alk1 associate with and stabilize the eNOS activation complex leading to NO production. In heterozygous conditions for either endoglin or Alk1, eNOS becomes uncoupled and produces more superoxide than NO which leads to tissue damage and impaired vascular tone control. Interestingly, our recent work also revealed that adult *Eng* and *Alk1* heterozygous mice have signs of pulmonary arterial hypertension including increased right ventricular (RV) systolic pressure, RV hypertrophy, degeneration of the distal pulmonary vasculature, and muscularization of small arteries. PAH that heterozygous mice develop is attributable to uncoupled eNOS activity and increased superoxide ($\bullet O_2$) production, which can be prevented by antioxidant treatment.[16, 17]

On the other hand, *Bmpr2* heterozygous mice had no or mildly elevated pulmonary pressure.[18, 19] However, the infusion of serotonin caused several signs of PAH in $Bmpr2^{+/-}$ mice, further increased by hypoxic conditions. Authors showed that *BMPR2* haploinsufficiency increased susceptibility to PAH and pulmonary vascular remodeling *in vivo* providing a link between two key systems widely implicated in the pathogenesis of PAH.[19] Also, a transgenic dominant-negative form of Bmpr2 specifically expressed and activated after birth in smooth muscle cells led to increased pulmonary arterial pressure suggesting that the mutation need to be expressed in smooth muscle to produce the phenotype.[20] More recently, Hong et al. have used conditional knockout mice in which *BMPR2* gene was deleted in pulmonary endothelial cells. They showed that endothelial *BMPR2* deletion is in itself not sufficient to cause PAH but can increase the susceptibility to PAH.[21]

It is therefore clear that PAH is very complex disease with initial stages likely involving the interaction between genetic predisposition (i.e. *BMPR2*, *ALK1*, and *ENG* mutations) and environmental risk factors. Genetic mutations of TGF-beta family members are not sufficient to induce PAH, but are serious predisposing factors. To add even more complexity to the topic, mutations of *SMAD4* (common Smad; Figure 1) do not predispose to PAH, but are seen in patients with juvenile polyposis (JP), colorectal cancer, and with the combined syndrome of JP and HHT (JP-HHT).[22] Finally, one report showed patient with *BMPR2* mutation exibiting PAH with HHT features, particularly pulmonary AVMs.[23] That pointed out the possibility that PAH and HHT have a common molecular pathogenesis.

Not only the TGF-beta family receptors but also their ligands could have important role in PAH pathobiology. The recent finding of circulating BMP9 as the true physiological ligand for ALK1, acting in combination with Bmpr2 (Figure 1), supports a role for BMP9 in PAH. Endoglin potentiates the effects of BMP9 and can therefore affect these pathways. Moreover, BMP9 can stimulate endothelin 1 (ET-1) release from pulmonary endothelial cells. [24] ET-1 involvements in the pathogenesis of PAH is well known and endothelin receptor antagonists have been a therapeutic mainstay in PAH, providing benefit to many patients.[25]

Epilogue

TGF-beta superfamily has a critical role in the development and maintenance of the pulmonary vasculature. Genetic defects and imbalance of TGF-beta and BMP pathways contribute significantly to the development of pulmonary arterial hypertension. Pharmacological approach and identification of small inhibitory molecules to these pathways will allow modulation of the TGF-beta superfamily signaling and assist in development of future therapeutics for PAH.

However, the complex regulation of these pathways and their involvement in many fundamental biological processes such as cell growth, differentiation, embryonic de-

velopment and tissue homeostasis, may raise many safety issues and slow the pace of progress. Nevertheless, our growing knowledge of the TGF-beta pathway regulation should teach us how to restore the balance that is lost in patients with PAH. It should assure the progress in this field and open new avenues and possibilities of gene replacement therapy and personalized medicine in the treatment of this life threatening disease.

Financial disclosure
I declare that I have no conflicts of interest.

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Uloga transformišućeg faktora rasta-beta i srodnih molekula u patogenezi plućne arterijske hipertenzije

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APSTRAKT

Plućna arterijska hipertenzija (PAH) je veoma teška i progresivna bolest koja za kratko vrijeme dovodi do anatomskih promjena na krvnim sudovima pluća. Patološke promjene, koje su naročito izražene u plućnoj mikrocirkulaciji, dovode do progresivnog porasta srednjeg plućnog arterijskog pritiska i vaskularnog otpora. Netretirane, ove promjene dovode do insuficijencije desnog srca i često imaju fatalan ishod. Široka lepeza faktora je uključena u patofiziologiju nastanka PAH. Međutim, najnovija naučna istraživanja ističu ključnu ulogu transformišućeg faktora rasta-beta (TGF-beta) i njemu srodnih molekula u nastanku PAH. Familija ovih proteina obuhvata različite forme liganda i receptora TGF-beta, kao i koštane morfogene proteine (BMP), koji imaju višestruke i složene funkcije. Mutacije gena za BMP receptor 2 (BMPR2) identifikovane su u 60% slučajeva porodične PAH i u oko 10-30% slučajeva idiopatske PAH. Mutacije receptora TGF-beta, kao što su ALK1 i endoglin, nađene su kod pacijenata sa PAH i ličnom ili porodičnom istorijom hereditarne hemoragične teleangijektazije. U patogenezu PAH uključeni su i brojni ligandi receptora TGF-beta (npr. BMP9), kao i faktori koji aktiviraju ne samo glavne nego i sporedne signalne puteve. Brojni napori, usmjereni ka boljem razumijevanju funkcije članova porodice TGF-beta imaće direktan uticaj na razvoj novih terapeutskih strategija za liječenje ove teške bolesti. Početnu fazu takvih napora pretstavljaju eksperimentalna istraživanja na životinjama. Međutim, predklinička i klinička istraživanja će dati konačan odgovor o primjenljivosti i efikasnosti novih terapeutskih strategija u liječenju PAH. Ovaj reviski članak sažeto prikazuje naša sadašnja znanja o ulozi članova porodice TGF-beta u PAH.

KLJUČNE RIJEČI

Transformišući faktor rasta-beta, plućna arterijska hipertenzija, koštani morfogeni proteini, endoglin, ALK1.

CASE REPORT

Treatment of Bilateral Postoperative Aphakia in a Young Patient With Still-Chauffard Syndrome

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ABSTRACT
An 18-year-old female patient was referred to our clinic with low vision in both eyes. She had been treated for Still-Chauffard syndrome since birth, had choric uveitis and underwent cataract surgery in young childhood; she was since left aphakic in both eyes. After bilateral implantation of Verisyse Aphakia phakic intraocular lense was done, her vision and quality of life improved dramatically over the course of one year.

KEY WORDS
Still-Chauffard syndrome, aphakia, Verisyse aphakia.

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Case presentation

Still-Chauffard syndrome is a systemic form of juvenile idiopathic arteritis (JIA).[1] It is present in 20% of all cases of JIA and equally represented in male and female patients. JIA is characterized by remittent high fevers, maculopapular rash, generalized lymphadenopathy, hepatosplenomegaly and serositis.[2] Ocular manifestations include a triad of symptoms: band keratopathy, anterior uveitis and cataract.

An 18-year-old female patient was referred to our clinic due to low vision in both eyes. She underwent bilateral cataract surgery when she was 8 years old and was left aphakic in both eyes. Prior to cataract surgery, she had chronic recurrent anterior uveitis in both eyes and was treated with high dose of corticosteroids, both topically and systemically. Her uncorrected visual acuity (UCVA) was 20/800 and visual acuity corrected with+12.00 spherical diopters was 20/60 for the right eye and 20/80 for the left eye. Slit lamp examination showed band keratopathy in both eyes, mild chronic uveitis without Tyndall light and iridodonesis. The fundus was unremarkable. Oculus Pentacam II corneal topography showed low astigmatic patterns and deep anterior chambers in both eyes.

Considering the exam results together with the patient's age and current general health condition, we decided to implant Verisyse Aphakia (Abbott Medical Optics, Inc., Abbott Park, Illinois, USA) phakic intraocular lenses (pIOL) in both eyes. Prior to the surgery, the patient received sub Tenon anesthesia in both eyes. The standard surgical procedure included a clear corneal incision (5.0 mm), viscoelastic instillation, lens implantation and fixation to the anterior iris; we used an extended 10/0 nylon suture with intra cameral antibiotic administration. There were no postsurgical complications during the follow up period. One week after surgery, UCVA on the right eye was 20/25 and 20/40 on the left eye. One year after surgery UCVA on the right eye was the same, and the left eye had improved to 20/25.

Discussion

Still-Chauffard syndrome occurs in 20% of all cases of JIA. Due to treatment of the specific ocular symptoms, these patients have a long-term exposure to the topical and systemic corticosteroids that can result in subsequent cataract formation.

This case was the first case of pIOL implantation for aphakia correction in a young patient in our clinic and in our region. Nonetheless, studies[3] with the same type of pIOL show similarly beneficial results, without intra-operative or postoperative complications. Even though our patient was young and had a chronic systemic illness, her lenses remained stabile in the anterior chambers, there was no endothelial cell loss and she did not experience any increase in intraocular pressure.

In summary, our findings show that implatantion of phaking itraocular lense is a safe procedure not only for elderly aphakic patients, but also for young ones as well.

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Lečenje bilateralne postoperativne afakije mladog pacijenta obolelog od Still-Chauffard sindroma

Bojan Kozomara

APSTRAKT
Osamnaestogodišnja djevojka javila se u našu kliniku radi lošeg vida na oba oka. Od djetinjstva se liječi od Still Chauffard-ovog sindroma, sa hroničnim uveitisom i kataraktama koje su operisane u ranoj mladosti i nakon kojih je bila afakna na oba oka. U našoj klinici urađena je bilateralna implantacija fakičnih intraokularnih sočiva (Verisyse Aphakia), nakon čega su se vidna oštrina i kvalitet života drastično popravili u periodu od jedne godine.

KLJUČNE RIJEČI
Still-Chauffard syndrome, afakija, Verisyse Aphakia.

CASE REPORT

Dexmedetomidine as Adjunctive Therapy for Delirium Tremens

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ABSTRACT
This case report describes a patient with severe alcohol withdrawal complicated by a potentially difficult airway management that was treated by adjunctive dexmedetomidine infusion. Benzodiazepines are the mainstay of conventional therapy, but they may not be optimal choices in some complicated situations. Our patient underwent excision of the cancer on the floor of the mouth which involved radical neck dissection and flap rotation. During the course of intensive care he developed airway obstruction, in part, due to somnolence. While we need a better understanding of how dexmedetomidine works in delirium tremens, its use as an adjunctive agent appears warranted in complex clinical situations like this one.

KEY WORDS
Dexmedetomidine, delirium tremens, airway obstruction.

DOI: 10.7251/SMD1202113S *(Scr Med 2012;43:113)*

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A 62-year-old male with a past medical history of hypertension and hepatitis C, had a squamous cell carcinoma on the floor of the mouth. He underwent excision of the lesion which involved radical neck dissection and flap rotation. The patient was taken to the SICU one hour after the end of surgery because he did not meet criteria for extubation in the operating theater. He was transferred to the general wards on POD#2 in a stable condition. On POD#3, he was transferred back to continued intensive care due to altered mental status and partial airway obstruction. A fiberoptic airway examination revealed supraglottic edema. While in the SICU, the patient had received glycopyrrolate, racemic epinephrine by nebulizer, and dexamethasone. In addition, prior to his airway examination, he had received haloperidol, morphine, and lorazepam for "agitation." When it was determined that the airway obstruction likely resulted from somnolence, flumazenil was given to reverse the sedative affect of benzodiazepine. The patient woke up and was able to maintain his airway with no further assistance. However, then he began to exhibit the signs and symptoms of severe alcohol withdrawal. At that time, dexmedetomidine drip was introduced with judicious supplementation of lorazepam. After two days patient was transferred back to the medical floor.

Discussion

Alcohol withdrawal is a common problem in the peri-operative period for certain adult patients. The extreme form of alcohol withdrawal is delirium tremens, which can result in significant morbidity and mortality if not competently managed. Benzodiazepines are the mainstay of conventional therapy, but they may not be optimal choices in some complicated situations. We present a patient with severe alcohol withdrawal complicated by a potentially difficult airway that was managed by adjunctive dexmedetomidine infusion.

The influence of noradrenergic neurotransmission in alcohol withdrawal is well established (1-3). The sympathetic system overdrive (anxiety, agitation, increased blood pressure, tachycardia, and tremor) during alcohol withdrawal may facilitate progression of withdrawal symptoms. By decreasing norepinephine release alpha-2 adrenergic agonists may aid in reducing alcohol withdrawal symptoms.[3] In contrast to benzodiazepines, this class of drugs offers no benefit for prevention or treatment of alcohol withdrawal seizures or delirium tremens. In our patient's case, benzodiazepines given in sufficient doses to control all symptoms would have resulted in over sedation with attendant airway obstruction. While we need a better understanding of how dexmedetomidine works in delirium tremens, its use as an adjunctive agent appears warranted in complex clinical situations like this one.

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CASE REPORT

Severe Non-Opioid-Induced Pruritus Following Spinal Block

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ABSTRACT
This case report describes an occurrence of intractable localized pruritus after spinal anesthesia in the absence of neuraxial opioid. Pruritus was confined to soles of booth feet. Symptoms were attributed to possible subclinical diabetic distal sensory neuropathy. Pruritus was refractory to IV diphenhydramine, IV lidocaine but responded quickly to subhypnotic dose of IV propofol. The close relationship between pathways for pain and pruritus may result in severe and unusual symptoms. Diagnostic workup should include tests for distal sensory neuropathy. Further understanding of neuropathic pruritus is needed.

KEY WORDS
Spinal block, non-opioid-induced, propofol.

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A 57-year-old, 122 kg, 6'1" male, had a past medical history of hypertension, glucose intolerance, benign prostatic hypertrophy and right-sided Bell's palsy. This patient was admitted for cystolithotrypexy under spinal anesthesia. Subarrachnoid block produced a prompt onset of anesthesia with satisfactory sensory blockade to the level of the tenth thoracic dermatome. Propofol infusion was maintained for sedation, and a Ramsay scale of 5 was obtained. The patient tolerated the procedure with no untoward events and was transferred to the Post Anesthesia Care unit. Approximately 60 minutes after arrival in the recovery room, the patient complained of intense itching confined to soles of both feet. He reported the onset of this discomfort soon after he awoke in the OR. Upon evaluation, the patient had no motor block and sensory recovery to the level of L3 dermatome. Treatment of the present severe pruritis was initiated with intravenous lidocaine. Ten minutes later the patient reported no relief (verbal analog scales 10/10). His discomfort was so great that physical restraint was required to keep him from getting of the bed and excoriating his feet. Subsequently, IV diphenhydramine controlled his agitation but had no effect on the severe itching. Subhypnotic propofol infusion was started with a total infusion time of thirty minutes. The itching subsided dramatically after five minutes and after 20 min was no longer present. At that time, there were a complete resolution of sensory and motor block and the patient was discharged from the recovery room and advised to pursue a work up for diabetic neuropathy and to seek follow up in the pain clinic if symptoms recurred. In the mean time patient had another cystoscopy procedure under spinal anesthesia and had the exactly the same course in the Post Anesthesia Care unit. The only difference was that prior to the placement of subarrachnoid block he received IV fentanyl as a premedication.

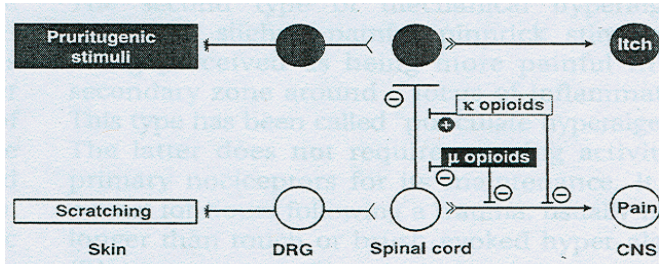

FIG. 1. Simplified schematic view of central interaction between pain and itch under physiological conditions. While having a similar inhibitory effect on the pain processing, μ and kappa opioids differentially modify the spinal itch processing. DRG: dorsal root ganglion; CNS: central nervous system.

Discussion

The close relationship between pathways for pain and pruritus may result in severe and unusual symptoms.[1-3] We report an episode of neuropathic symptoms following neuraxial anesthesia during the period prior to restoration of full sensation. The severe pruritus in the absence of

neuraxial opioid during spinal anesthesia could be an early manifestation of latent systemic disease, such as diabetic neuropathy.

Although the itch sensation seems to be transmitted via the C-fibers, which are different from those that transmit pain, increasing evidence supports an interrelation between these two distinct sensations. Painful stimuli, such as thermal, mechanical, or chemical, can inhibit itching, and inhibition of pain processing may enhance itching. Opioid receptors appear to be involved in the interactions between itching and pain (Figure 1).[1] Mild paresthesias during the return of full sensation are common. Possibly, our patient perceived paresthesia as an extremely irritating pruritus due to the presence of subclinical neuropathy.

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CASE REPORT

Metastatic Prostate Cancer Presenting as Interstitial Lung Disease

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ABSTRACT
Prostate cancer can metastasize to the lungs, but mostly in the form of pulmonary nodules and rarely as lymphangitic carcinomatosis. Also it is rare to have metastatic prostate cancer with no prostatic symptoms at diagnosis. We describe a rare case where a middle aged man presented with isolated pulmonary symptoms and CT scan findings which were consistent with interstitial lung disease, however on subsequent biopsy turned out to be adenocarcinoma arising from the prostate. The symptoms improved with chemotherapy.

KEY WORDS
Prostate, cancer, metastatic, interstitial lung disease, respiratory failure.

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Accepted: September 20, 2012

A 56-years-old man presented to us with a history of pneumonia and COPD diagnosed May 2010, treated with antibiotics but did not improve to baseline. In November 2010 he was seen at an outside hospital with shortness of breath (SOB), cough productive of yellow phlegm for 10 days. There was no history of sick contacts, recent travel, exposure to respiratory irritants, weight loss, fever, hemoptysis, incarceration or positive PPD. He had a 30-pack year history of smoking cigarettes. Vital signs were normal;Oxygen saturation was 92% on room air. Systemic exam was remarkable for rhonchi in the chest bilaterally. A CT scan was done which showed bilateral symmetrical extensive interlobular thickening with patchy ground glass opacities of unclear etiology. The pulmonary service was consulted. A presumptive diagnosis of interstitial lung disease was made. He improved with nebulizers, steroids and antibiotics.

The patient was readmitted 3 weeks later at our hospital with worsening shortness of breath so severe that he was unable to walk to the washroom or speak in full sentences. Review of systems was negative. He had no urinary or bowel complaints. He was tachypneic breathing over 22 breaths/minute and oxygen saturation was consistently <90% on room air. Systemic exam was remarkable for decreased air entry in the right lung base and dry crackles bilaterally. He was pale and there was bilateral 2+ pedal edema. The cardiovascular, abdominal and neurologic examinations were unremarkable.

CT scan of the chest (Fig. 1) showed increased interstitial markings and thickening bilaterally, in addition to bilateral small pleural effusions. He hadWBC count of 12,300/mm^3 with 79% neutrophils; hemoglobin was 8.1 g/dL and creatinine 1.1 mg/dL.

His condition worsened requiring admission to the Medical Intensive Care Unit because of concern for impending

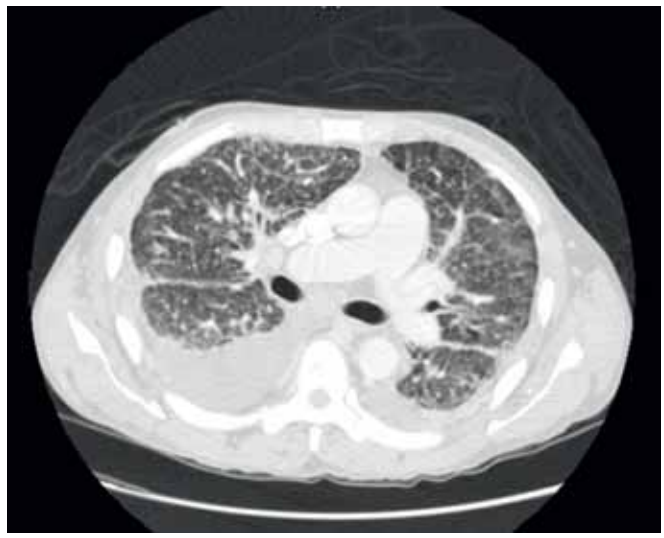

Figure 1. CT scan of the chest at presentation showing increased interstitial markings

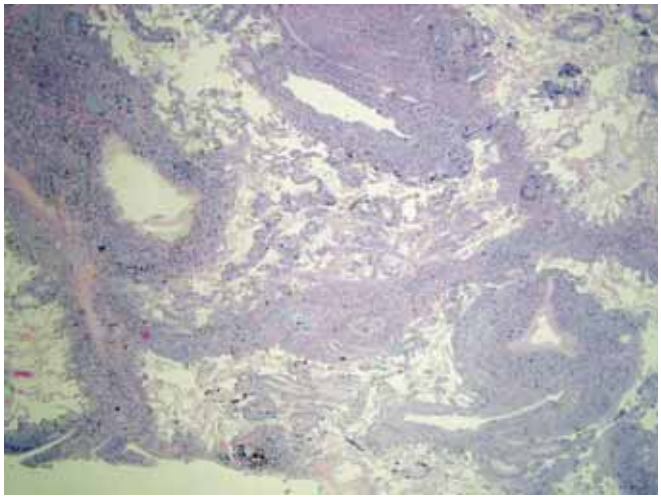

Figure 2. Adenocarcinoma involving bronchilovascular bundles, lymphatics, acinar septae and adjacent alveolar walls (6.5X)

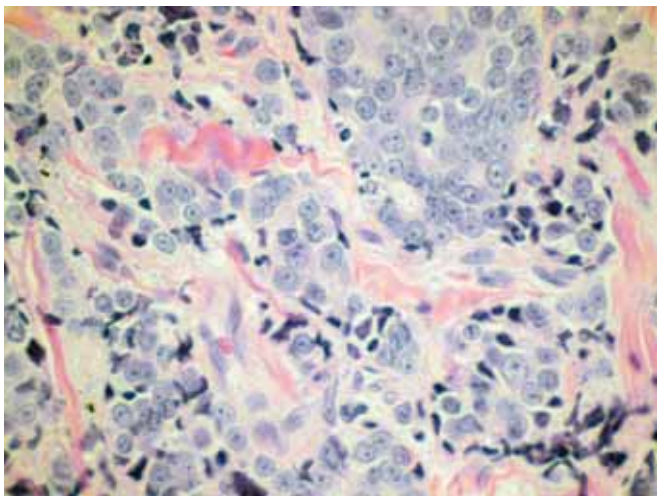

Figure 3. Adenocarcinoma cells with prominent nucleoli (90X)

respiratory failure. Due to an elevated white count and worsening respiratory status, he was empirically started on broad-spectrum antibiotics, consisting of Vancomycin and Piperacillin-Tazobactam, as well as respiratory adjuvants. With his clinical condition deteriorating and no definitive etiology for these parenchymal changes, which were not unlike the more commonly diagnosed pneumonitis or organizing pneumonia, a lung biopsy was requested.

He was taken to the operating room where he underwent left video-assisted thoracoscopic wedge biopsy of the left upper lobe. Grossly the lung tissue was granular, fibrotic and severely thickened such that the staple line had to be reinforced with running chromic suture because of obvious disruption. The parietal pleura was normal and there was 250ml of straw-colored pleural effusion evacuated from the pleural space. He left the operating room unable to be extubated after the procedure.

Pathology showed adenocarcinoma involving lymphatics, acinar septae, pleura, and bronchiolovascular bundles. In some areas the tumor was also invading into the alveolar walls located adjacent to bronchiolovascular bundles and acinar septae (Fig 2). Tumor cells showed mild variation in size and prominent nucleoli (Fig 3). Tumor cells by immunohistochemistry were strongly positive for prostate specific antigen (PSA), and therefore consistent with prostatic primary. The patient had a serum PSA level of 2864ng/ml. Subsequent CT scan of the abdomen showed a normal sized prostate with normal abdominal viscera but some suspicious bone lesions.

Clinically, he was unable to be weaned from the ventilator more than one week after surgical biopsy. Medical oncology was consulted and he was given a dose of docetaxel chemotherapy. He subsequently improved quite dramatically such that he was extubated and later discharged on home oxygen. The chemotherapy was continued as an outpatient. He initially refused androgen deprivation therapy but consented to it after cycle 4. His PSA dropped to 116 ng/ml. After 12 cycles of chemotherapy, his CT scan of the chest showed marked improvement. He continues to do well at this time and does not require home oxygen anymore.

Discussion

The interstitial spread of predominantly adenocarcinomas through the pulmonary lymphatics is commonly referred as pulmonary lymphangitic carcinomatosis (PLC). It constitutes 7% of pulmonary metastases[1]. The predominant age of presentation reported in the literature varies between 40-49 years. It is commonly seen in neoplasms originating from the breast (33%), stomach (29%) and lungs (17%).[2] The clinical manifestations of pulmonary lymphangitic carcinomatosis include coughing, panting, dyspnea. A normal or restrictive respiratory pattern could be seen on pulmonary function tests. Lung biopsy is the gold standard for the diagnosis but HRCT has been shown to be equally beneficial in this regard. The common HRCT findings of PLC include thickening of interlobular septa, fissures, and bronchovascular bundles.[3] The progression once the patient develops PLC is fairly rapid as noted in the literature with mean survival varying between 2-7 months except for prostatic primary carcinoma, which may have good remission with adjuvant hormonal therapy.[4]

It has been described more than two decades ago that pulmonary metastases from prostate adenocarcinoma are found at autopsy in 25% to 38% of patients but are evident on chest films in only 5.5% to 6.7%[5].

Intra-thoracic involvement from prostate adenocarcinoma could be in the form of lung nodules (84%), mediastinal lymphadenopathy (12%) or lymphatic spread (4%)[5]. There

are few case reports of prostate cancer presenting with pleural effusions[6].Most cases of metastatic carcinoma of the prostate to the lungs have symptomatic prostate primary or bone metastasis as in this case where a left posterior iliac crest biopsy was positive.

Pulmonary involvement with metastatic prostate cancer without bone metastasis has been described in a few case reports[2,4,5]. Interstitial thickening can be a part of the spectrum of radiological findings seen in lymphangitic carcinomatosis.[7] However, to our knowledge, metastatic prostate cancer presenting as purely interstitial lung disease has not been described.

We have described one of the rare presentations of prostate cancer, which is pulmonary involvement with no prostatic symptoms at diagnosis. Another rarity is the pattern of pulmonary involvement, in this case an interstitial process rather than cannonball lesions. This led to the delay in diagnosis until the patient's respiratory status declined requiring admission to the medical intensive care unit. Our case emphasizes that metastatic prostate cancer to the lungs can take many forms and this differential diagnosis must be considered if an old male patient presents with unexplained new pulmonary findings.

Authorship statement

OU performed the lung biosy. MS examined the tissue biopsy and made the diagnosis. SG and AS were involed in the oncologic treatment of the patient. CMPH was consulted for his expertise in the field. SG, OU, SG, CMPH and MS contributed to the conception and writing of this article.

Financial disclosure

The authors declare no conflict of interest.

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IMAGES IN CLINICAL MEDICINE

Purtscher's Retinopathy

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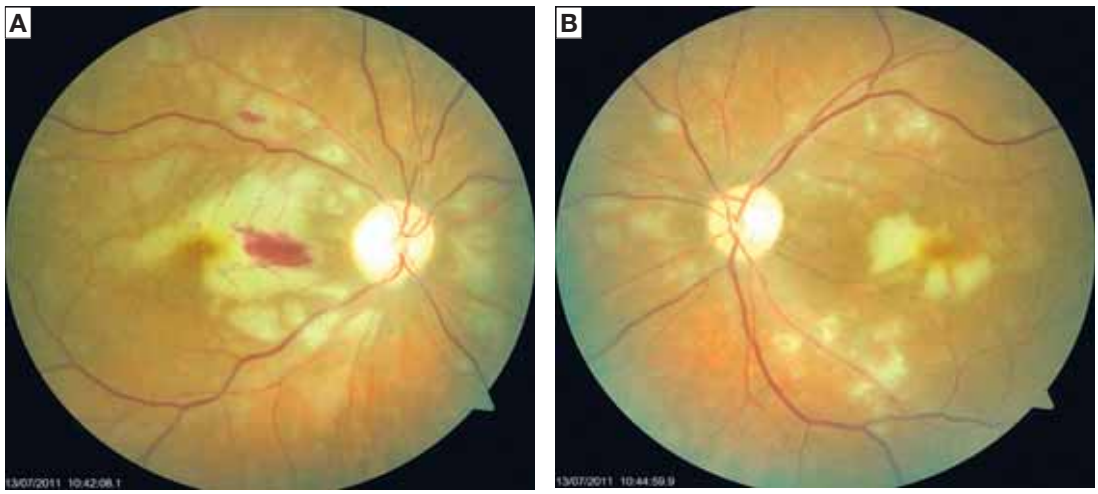

Figure 1. Putrscher's retinopathy – right eye A, left eye B.

A 62-year-old man with sudden loss of vision in both eyes was admitted as an emergency case. He gave a history of a recent trauma from an accident with a tractor that overturned on him and compressed his chest. At admission, his visual acuity in both eyes was limited to counting fingers at a half-meter distance. There were no other abnormal findings in the anterior segment apart from semi-mydriatic pupils with poor reaction to light.

We found numerous "cotton-wool" exudates in both ocular fundi as a sentinels of neuronal damage[1]; these were mostly within peri-papillary and inter-papillomacular areas. Our patient had a multiple retinal pale patches as a sign of ischaemia (Figures 1, A and B) and retinal hemorrhages (Figure 1 A).

Following acute compression injures to the thorax or head, a patient may experience visual loss due to Purtscher's angiopathic retinopathy in one or both eyes.[2,3] The mechanism remains unclear and somewhat controversial. Embolization of leukocytes can cause arterial occlusion and infarction of the microvascular bed. Because of its known association with trauma, acute pancreatitis and vascular diseases, leukocyte aggregation by complement C5a is believed to underly microvascular embolization in the eye.

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IMAGES IN CLINICAL MEDICINE

Surgical Correction of Mitral Regurgitation After Papillary Muscle Rupture

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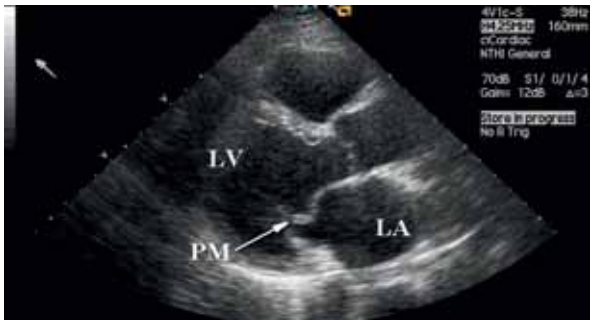

Figure 1. Preoperative transthoracic echocardiogram. It shows a mobile mass moving freely in the left ventricle. LA-left atrium; LV-left ventricle; PM-ruptured papillary muscle.

Figure 2. Specimen of the anterior mitral leaflet with ruptured anterior head of the posterior papillary muscle.

A 55-year-old Caucasian woman was transferred from an outside hospital with chest pain and dyspnea. The admitting diagnosis was pulmonary edema due to mitral regurgitation after an acute myocardial infarction 9 weeks earlier.

The patient survived previous time without any medical support until now, when dopamine and diuretic agents were administered immediately after hospital admission. Transthoracic echocardiography revealed severe mitral valve regurgitation due to rupture of the anterior head of the posterior papillary muscle [Figure 1]. Coronary angiography indicated single vessel disease of the right coronary artery. The mitral valve [Figure 2] was replaced with St Jude mechanical prosthesis #29; with preservation of the posterior mitral cusp, followed by right coronary artery bypass grafting. The patient was discharged 7 days after surgery without complications.

LETTER TO THE EDITOR

Xenotransplantation – Will It Be the Next Medical Revolution?

DOI: 10.7251/SMD1202121C *(Scr Med 2012;43:121)*

A recent issue of *Scripta Medica* included a brief review of the current status of xenotransplantation (cross-species transplantation) (1), which I and many others working in this field believe offers the prospect of a limitless supply of organs, tissues, corneas, cells, and even blood for clinical transplantation within the foreseeable future. Already a clinical trial of encapsulated pig islet transplantation is being undertaken in New Zealand. This trial is officially overseen and regulated by that country's Department of Health. More clinical trials will likely be initiated within the next few years.

There is no argument that the supply of organs and cells from deceased human donors is insufficient to meet the need for clinical transplantation and is likely to remain so in the future. In the USA alone, more than 110,000 patients are on the waiting list for an organ of one sort or another, and yet only approximately 30,000 organs will become available during the current year. Living donation improves the kidney transplantation situation, but makes little or no impact on the need for organs such as the heart, liver, and lungs. One million patients in the USA suffer from Type 1 diabetes and loss of islet function. Because Type 1 diabetes now constitutes a global epidemic, deceased human donors can *never* provide enough islets to make any real impact on this major health problem,

We still have further to go with clinical trials of whole organ xenotransplantation, although the increasing availability of genetically engineered pigs has helped to overcome the remaining immunological barriers (2). Clinical trials of pig islet transplantation now seem justified, since no fewer than five different groups found that islet transplantation resulted in insulin-independent normoglycemia in diabetic monkeys for periods of >6 months (3). In our own laboratory, we have controlled glycemia in two diabetic monkeys for periods of >1 year.

Progress in overcoming inter-species immunological barriers, although slower than we would like, is being made. What about the other aspects of xenotransplantation raised by Dr Ivanovic's review? With pigs housed under ideal biosecure conditions, the risk of transfer of a porcine microorganism to the recipient along with the transplanted organ will be very low. It would be less than that for transfer of a human pathogenic microorganism along with an organ from a deceased human donor, where the time for testing for pathogens is always limited. The potential risk associated with the presence of porcine endogenous retroviruses (PERV) is now considered low and could be controlled, if necessary, by siRNA technology. Ethical concerns about using pigs for the purpose of transplantation pale into insignificance when compared with the health problems associated with end-organ failure, diabetes, neurodegenerative disease, and the many other human conditions that might be cured by xenotransplantation. Nearly 100 million pigs in the USA are slaughtered for food; surely the use of pigs to provide life to terminally ill patients should be acceptable. The logistics of distributing *unlimited* organs *whenever required* will be much simpler than the present system of emergency retrieval of organs from deceased human donors.

Could alternative approaches provide the organs and cells that are needed? Regenerative medicine and tissue engineering may provide cells and potentially even islets, but it will be a long time before whole organs can be "grown" in the laboratory. Although cardiac assist devices have improved immensely in recent years, mechanical devices are a long way from replacing livers. Although the medical "revolution" of xenotransplantation has not yet occurred, I believe we can be optimistic that it is on the horizon, and that in many ways it will change the way we practice medicine.

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Financial disclosure
I declare that I have no conflicts of interest.

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IN REPLY

Only Time Will Tell

DOI: 10.7251/SMD1202122I *(Scr Med 2012;43:122)*

As can be appreciated from two recent reports,[1,2] there is a high level of agreement as to these facts: the supply of organs and cells from deceased human donors is insufficient to meet the need for clinical transplantation, and alternative solutions should be explored; there is significant progress in overcoming inter-species immunological barriers, but many problems still remain; whole organs xenotransplantation has not yet reached the level for clinical trials.

Knowing how long and tedious the way becomes, from initial phase clinical trials to routine therapeutic use, my enthusiasm for employing whole organ transplantation in routine clinical medicine is tempered. My view of the situation stems not only from biological and medical perspectives, but also the bureaucratic point of view. Furthermore, no clinical trials have started yet. Phases 1, 2 or 3 of clinical trials could reveal different problems and obstacles that could slow or impede employment of such a therapeutic procedure. For this reason, I do not believe that a medical "revolution" concerning xenotransplantation of whole organs will occur within the next decade. Even if our perception of the facts is similar, our estimations for success diverge. When we start to use the term "believe" (a delimitation mark between science and faith) the objective arguments become secondary to subjective feelings. Thus, "*seul l'avenir nous dira*."

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Financial disclosure
The author declares that he has no conflicts of interest.

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CONTINUING MEDICAL EDUCATION

Questions and Answers

Ова рубрика (Q & A) садржи незнатно измењене сегменте из наведене литературе или за ову прилику написан текст. Циљ нам је да ови прилози послуже читаоцу као вежба за унапређење стручног енглеског језика.

[This section includes short segments of texts from the published literature or original texts. The main purpose is to provide questions and answers that readers can use to improve their English.]

Scripta Medica

DOI: 10.7251/SMD1202123B *(Scr Med 2012;43:123)*

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Questions

1. How does contraction of airway smooth muscle differ from skeletal muscle?

2. During deep inspiration, the airways dilate. What neural mechanism contributes to this response?

3. Describe the cough reflex.

4. A 9-year-old girl has severe asthma that required the hospitalizations in the last year. She is now receiving therapy that has greatly reduced the frequency of these severe attacks. Which of the following therapies is most likely responsible for this benefit?
A. Albuterol by aerosol
B. Cromolyn by inhaler
C. Fluticasone by aerosol
D. Theophylline orally
E. Zafirlukast orally

5. The smaller upper four thoracic vertebrae share characteristics in common with the:
A. Cervical vertebrae
B. Thoracic vertebrae
C. Lumbar vertebrae
D. Sacrum
E. None of the above

6. The larger lower four thoracic vertebrae share characteristics in common with the:
A. Cervical vertebrae
B. Thoracic vertebrae
C. Lumbar vertebrae
D. Sacrum
E. None of the above

7. What is the radiofrequency catheter ablation?

8. Name the two major symptoms associated with neck diseases.

9. A 24-year–old female is referred for an evaluation to rule out bipolar disorder. She complains of rapid mood swings with uncontrollable anger and irritability. During assessment, it is noted that she has not had any sustained relationships for many years and feels empty. She fears abandonment by her friends and blames others for making her feel angry. What is the most important diagnosis in this patient?
A. Major depressive disorder
B. Borderline personality disorder
C. Histrionic personality
D. Depressive personality
E. Generalized anxiety disorder

10. A 40-year-old teacher presents with tremor in her hands that is most obvious when she awake and trying to perform an action. She had first noticed it several years ago, but is concerned that it may be very slowly worsening. A tremor of this type is most likely caused by disease in which of the following structures?
A. Thalamus.
B. Cerebellum
C. Substantia nigra
D. Spinal cord
E. Internal capsule

11. A 63-year-old man was forced to retire from iron working because of neurological condition which progressed over the past several years. It is characterized by tremor, rigidity and bradykinesia worse on the left side. The symptoms are somewhat alleviated by treatment with l-dopa/

carbidopa. This patients resting tremor is most likely to do which of the following upon falling asleep?
A. It becomes more rapid
B. Its amplitude increases
C. It generates to limbs that were uninvolved when the patient was awake
D. It disappears
E. It transforms into choreiform movements

12. Describe following degenerative lesions: osteoarthrosis, prolapsed intervertebral disc and spinal stenosis.

13. What are the most common causes of acute back pain?

14. Why does the back hurt?

15. What is the role of epidural steroid injections in low back pain?

16. What are the indications for laminectomy and discectomy?

17. What is the role of acupuncture in low back pain?

18. What is the role of exercise in low back pain?

19. What is the role of prolonged bed rest in low back pain?

20. What is the best method for treating acute and chronic low back pain?

21. Which of the following statements is true about the human heart?
A. It develops from two tubes that later divide into four chambers
B. It is first recognizable at 15 days gestation
C. It starts to pump at 8 weeks of gestation
D. Tetralogy of Fallot is the most common congenital anomaly
E. Congenital heart defects are present in 6 per 100.000 live births

22. A 30-year-old man is brought to the emergency room by his brother after he loses consciousness. He had similar episodes in the past, but this is the first one his brother has seen. During these episodes, he has been diaphoretic and pale. His brother says that the patient complained of graying of his vision, lightheadedness, and sensation of feeling warm. He also had repetitive jerks of his body. This lasted for 45 seconds, and then he became oriented to his surroundings in less than a minute. The patient is thin and underweight, has dry skin, teeth erosions and brittle nail. He complains of some muscle pain. His prolactin level is normal.

What is the most likely diagnosis?
A. Generalized seizure
B. Transient ischemic attack (TIA)
C. Pseudoseizure
D. Syncope
E. Hypothyroidism

23. How does nearsightedness develop in children?

24. What aspects of the mental state may be observed by the clinician?

25. A 41-year-old man presents to the emergency department complaining of a severe frontal headache that began suddenly and awakened him from sleep. The headache is associated with nausea, vomiting, and subjective fevers. He also complains of new-onset diplopia and photophobia, but denies any decrease in visual acuity. He denies experiencing any associated seizures, focal weaknesses, previous similar episodes, frequent headaches, or previous visual disturbances. He does not have any prior significant medical problems. He drinks socially, does not smoke, and denies recreational drug use.

What is the diagnosis?
A. Subarachnoid hemorrhage
B. Cerebellar infarction
C. Pituitary tumor apoplexy
D. Cavernous sinus thrombosis

26. A 17-year-old boy referred to his primary care physician by his parents for concerns related to deteriorating academic performance, increasingly withdrawn behavior, and persistent irritability. His parents have become increasingly frustrated with what they describe as a “lack of motivation” and growing conflict between them. He reports that the academic demands during his junior year seem to be much greater than in previous years and indicates that he has difficulty managing complex projects that require planning across a period of weeks or months. He is therefore often left to complete work at the last minute (or not at all), which results in poor grades. He reports a general lack of motivation for his academic work. He reports that he would rather spend time with his friends and playing video games and complains that his parents place “too much pressure” on him to complete his work.

What is the diagnosis?
A. Adolescent ADHD
B. Disruptive Behavior Disorders
C. Learning Disorders
D. Mood Disorders

27. Patient is a 56-year-old woman with chronic MDD, well-controlled type 2 diabetes, and well-controlled hypertension. Which of the following statements would most likely apply to her treatment response?
A. Her general medical condition will result in additional adverse effects and tolerability burden.

B. Her general medical conditions will results in reduced efficacy of antidepressant treatment.
C. Combination treatment for MDD is likely to be associated with an improved treatment response beyond that of monotherapy.
D. Traditional monotherapy, such as with SSRI, is a reasonable treatment option

Answers

1. Airway smooth muscle is not striated, has no Z-bands, and consists of discrete sarcomeres. Airway smooth muscle does not generate action potentials. Compared with skeletal muscle, airway smooth muscle contracts and relaxes very slowly and peak tensions may not be reached until 10 to 12 seconds after a maximal stimulus. Airway smooth muscle can also generate force at resting length substantially below its optimal point on the length-tension curve. Thus, airway smooth muscle can shorten much more than skeletal muscle.

2. The alveolar stretch receptors are activated. This inhibits vagal tone at the central nervous system level. This reduces parasympathetic constrictor tone on the airways, and they dilate.

3. The cough reflex links an afferent visceral sensory stimulus to activation of an efferent somatic motor response. The afferent receptor is a component of the autonomic nervous system. The efferent component of the cough reflex is the forceful movement of skeletal muscle to effect cough. Although the mechanism is not understood completely, the cough threshold is lowered by stimuli that cause bronchoconstriction. Patients with asthma have increased cough, and this is diminished by treatment that causes bronchodilatation.

4. Answer: C

Administration of corticosteroid directly to the lung significantly reduces the frequency of severe asthma attacks. This benefit is accomplished with minimal risk of the severe systemic adverse effects of corticosteroid therapy. Albuterol is only used to treat acute asthmatic episodes. The other agents may reduce the severity of attacks but not to the same degree or consistency as fluticasone (or other corticosteroid).

5. Answer: A

6. Answer: C

7. Catheter-mediated ablation of tachyarrhythmias was first reported in 1982, when direct current was used to ablate the atrioventricular (AV) junction. Following this rather primitive beginning, the technique developed rapidly, so that radiofrequency catheter ablation is now the procedure of choice for patients with a variety of tachyarrhythmias. With radiofrequency, the energy is applied as an alternating current at 500.000cycles per second, resulting in heating of the adjacent cardiac tissue without stimulating the underlying myocardium or causing the extensive and painful damage induced by direct current shock. Since it causes little discomfort, it does not require general anesthesia. Repetitive radiofrequency ablations can be performed safely and comfortably until the desired results is achieved.

In subjects with accessory AV pathways, as, for example, the Wolf-Parkinson-White syndrome, radiofrequency ablation has supplanted surgical resection as the therapeutic procedure of choice. The accessory pathway is ablated with delectable catheters. Or pathways located in the right AV groove or septum, the catheters are introduced via a femoral vein. For those located in the left AV groove, the catheters are introduced via a femoral artery, from which they are advanced to the left ventricle and/or atrium. Alternatively, the catheters may be advanced to the left atrium via a femoral vein across the interatrial septum.

Radiofrequency ablation is an effective procedure in subjects with AV nodal reentry. The ablation has been used with success in subjects with primary atrial arrhythmias. With intracardial mapping, these arrhythmias are localized to the right or left atrium. Immediate success is reported in more than 80 percent of patients, with the chance of recurrence being about 20 percent. As the technique is refined further, the procedure will become more effective and safer.

8. The two major symptoms associated with neck diseases are pain and limited motion.

The pain may be only in the neck or radiate to the shoulder and down the arm. The most common cause, considering all age groups as a whole, is myofacial syndrome, a condition of localized muscle spasm. Degenerative arthritis in the cervical vertebrae is the other common cause of neck pain, particularly in the older patient, and is known as cervical spondylosis. Trauma, particularly motor vehicle accidents, can lead to whiplash and other neck injuries. Sedentary occupations (e.g., deck work and driving motor vehicles) are often associated with pain, but the precise anatomic cause of pain is in many causes uncertain.

Most acute neck pain is caused by structural problems such as myofacial syndrome, muscle spasm, and traumatic ligament sprains. Diagnosis of these common conditions is clinical, relying entirely on the history and physical examination. Subacute and chronic neck pain is also most commonly structural, with osteoarthritis becoming more likely with increased age. Stress and biomechanics (e.g.,

positioning at work or in bed) are often important contributing factors to both acute and chronic neck pain. Less common but serious conditions must also be considered in developing differential diagnosis (structural, myofacial syndromes, ostheoarthritis, muscle spasm, disc prolapse, etc; nonstructural: ankylosing spondylitis, rheumatoid arthritis, bone infection, neoplasia-primary metastasis, lymphadenitis; other: stress, occupaation).

Issues of management. If nonstructural disease has been excluded , acute or chronic neck pain can be managed by simple measures such as ice or heat, analgesics, and rest, as well as more specific therapies (relaxation techniques, massage, cervical collar, injection therapy, mild traction, spinal manipulation).

Stretching and strengthening exercises are helpful, particularly in the late recovery phase. To prevent recurrence, exercise should be included in most neck rehabilitation programs.

9. Answer: B

Borderline personality disorder is characterized by pervasive instability in moods, interpersonal relationships, self-image and behavior. This instability, often disrupts family and work life, long-term planning, and the individual's sense of self-identity. It affects about 2% of adults, mostly young women. There is a high rate of self-injury without suicide intent, as well as a significant rate of suicidal attempts and completed suicide in severe cases.

10. Answer: B

Intention or kinetic tremors are most characteristic of damage to the cerebellum. Kinetic tremors of the hand and arms are most common with disease of the cerebellar hemispheres, but they may also develop with damage to the spinocerebellar tracts of the spinal cord. Damage to the substantia nigra, such as that occurring in Parkinson disease, produces a resting tremor that abates when the patient moves the involved limb intentionally. Damage to the thalamus is more likely to produce a sensory disturbance. Tremors may develop with spinal cord damage, but they do not follow a typical pattern and do not suggest a spinal cord origin. Internal capsule lesions typically cause weakness.

11. Answer: D

Patients with Parkinson disease often have a characteristic pill-rolling tremor of the hand while they are awake. The tremors associated with Parkinson disease are worse when the patient is at rest and not moving the affected limb. Paradoxically, this resting tremor ceases when relaxation progress to sleep. In fact most tremors and other types of movement disorders caused by disease of the caudate, putamen, and globus pallidus (ie, the basal ganglia) and the substantia nigra remit during sleep. Choreiform movements are jumping or dancelike movements and occur with Wilson disease (hepatolenticular degeneration) and Huntington disease, a hereditary degenerative disease of the basal ganglia.

12. **Osteoarthrosi**s indicates 'wear and tear" whereas 'osteoarthritis' infers inflammation. Joints so affected are stiff and difficult to move after disuse, but move more freely with use. Thus pain and stiffness after rest, relieved by activity but recurring as the patient tires, are characteristic of osteoarthrosis. This pain is often not immediately relieved by rest and the patient may have difficulty in settling comfortably.

Prolapsed intervertebral disc may start with pain spred over years. Before the acute episode there is frequently a period of vague aches and pains, often accepted by the patient as a normal reaction to activity. An acute episode occurs when bending or lifting, or on the day following such activities, or with no discernible cause. The pain subsides with rest, but may take several weeks to disappear. Thereafter periods of comfort are interspersed with majore and minor episodes of pain. Gradually this characteristically episodic pattern merges into the pattern of osteoarthrosis.

Spinal stenosis is a consequence of the spinal canal or neural foramina narrowing, usually caused by degenerative changes. It can results in back pain with radiation to the legs. In the latter site the pattern is similar to that of intermittent claudication in its relation to activity.

The pain of intermittent claudication is however localized to the muscle group involved in the leg, is cramp-like and not accompanied by numbness or tingling. It is relieved by standing still. The pain of spinal stenosis is often diffuse, accompanied by tingling and numbness and by malfunction of the muscles, all of which the patient finds difficult to describe. The symptoms increase with standing and walking and are relieved only by stooping, sitting or lying down.

13. In most cases of acute back pain, no clear pathophysiologic mechanism is defined. Episodes are usually preceded by minor trauma, heavy lifting, or a "near fall". Direct trauma is rarely a cause.

The first urgent crossroad in the diagnosis of low back pain is to decide whether the patient has a medically emergent condition (tumor, infection, or trauma) or not. More than 90% of cases of so-called benign acute low back pain resolve spontaneously.

14. Erect posture forces the spine into a position in which it is constantly exposed to minor trauma and to stress on pain-sensitive structures. These pain-sensitive structures

are the supporting bones, articulations, meninges, muscles, and aponeurosis.

15. There are not enough well-controlled clinical trials in select patient populations to define accurately the indications for epidural steroid injection. However, clinical experience shows that many patients have dramatic responses. A small amount of a corticosteroid is mixed with a small amount of lidocaine, and the mixture is instilled into the epidural space. In good hands, epidural steroid injections are a relatively ; pw-risk procedure. No more that three injections should be performed in any 6-moth period, because epidural steroid may lead to ligamentous laxity.

16. The indications are open to great dispute. Some surgeons believe that the only cure for lumbar radiculopathy is surgical removal of the causative dick. However, in many cases of low back pain, the herniated disk may not be causative factor.

17. Firm conclusions could not be drawn regarding the efficacy of acupuncture in acute low back pain. For chronic low back pain, it did seem to show some advantages for pain relief and functional improvement in the short term. Acupuncture should probably be viewed as a useful adjunct to other therapies in chronic low back pain.

18. Individually tailored exercise programs, aimed at stretching, strengthening, and general conditioning, may improve pain and function in chronic low back pain, when the exercises are supervised by a trained individual.

19. There is no evidence to suggest that the bed rest of more than 2 days is beneficial. In fact, for people with acute low back pain, bed rest may be less effective than staying active. There is little or no difference in outcome in patients with sciatica.

20. Further studies with adequate controls are required for definitive statement.

21. Answer: B

The heart develops from a single tube and is first recognizable at 15 days gestation. Congenital heart diseases have an incidence of 6 per 1.000 live births. Chamber septal defects are the most common congenital heart anomaly.

22. Answer: D

This is a young man with what may be an eating disorder and episode of syncope. This is not likely to be a seizure because seizures can be characterized by an aura, automatisms, tonic-clonic movements, tongue biting and incontinence. Patients with seizures often have a period after the seizure marked by amnesia, aphasia, Todd's paralysis, muscle pain, and disorientation. This postictal state usually lasts from several minutes to several hours. This patient became normal in a matter of seconds after the event. Prolactin levels measured within 20 minutes of the event may be elevated after a genuine seizure. The pattern of activity can also be helpful to distinguish seizures from pseudoseizures. Pseudoseizures are characterized by pelvic thrusting, nonconvulsive limb movements, voluntary eye closure, and normal prolactin level. There is often a history of prior sexual abuse. It is unlikely that he had a transient ischemic attack (TIA) because he is very young, and there is no reason to suspect a markedly increased risk of vascular disease. He also had no focal neurological abnormalities. Hypothyroidism can be excluded with laboratory tests. It is also vey unlikely that hypothyroidism would cause a seizure. Syncope is characterized by pallor, nausea, diaphoresis, and specific, provoking events.

23. Myopia or nearsightedness—difficulty seeing objects at a distance—develops in about 30% of children. Vision professionals typically think of myopia as a problem occurring when the eyeball becomes too long (anterior to posterior axis) for the optical power of the cornea and lens.

However, it has been unclear how this imbalance develops in children who previously had normal vision. To answer this question, Mutti and colleagues (2012) compared changes in eye growing for children who developed myopia at different ages versus those whose vision remained normal.

They found that, in children without myopia, the lens grew thinner and flatter to maintain normal vision as the eye grew. This adaptation maintained a normal balance between the optical power of the lens and the increasing length of the eyeball. From age nine months to nine years, eyeball length increased by an average of three millimeters.

However, in children who developed myopia, the lens stopped changing in response to eye growth. Nearsightedness developed not just because of increases in the length of the eyeball, but rather because the optical power of the lens no longer changed as the eye grew.

The imbalance occurred rather suddenly: about one year before children became nearsighted. For at least five years after the development of myopia, the eye kept becoming longer but the lens stopped flattening and thinning.

In contrast to the lens, changes in corneal growth showed little or no relation to the development of myopia. The cornea is responsible for about two-thirds of the optical power of the eye, and the lens for the remaining one-third.

The study provides vision professionals with an important new piece of information on why some children develop myopia. However, what's still unclear is why the lens sud-

denly stops adapting to continued growth of the eye. The mechanism responsible for this abrupt loss of compensatory changes in the crystalline lens might include restricted equatorial growth from internal mechanical factors.

24. Many of the clinical signs characterizing the mental state of the patient will have become apparent during history taking. The clinician now sets out to study systematically the different aspects of the mental state that can now be given special further attention: general appearance and behavior, thought processes, mood, delusions, hallucinations, obsessions, attention and concentration, evidence of intellectual defect (orientation, memory, attention and concentration, general information, intelligence), and insight and judgment. General information is tested by asking questions about current affairs, e.g. national politics and international problems), intelligence is assessed from the detail and subtlety of the patients' accounts of themselves, their capacity to reason, the extent of their knowledge, and the level of their occupational attainment; accurate measurement is made by use of standard intelligence tests to determine Intelligence Quotient (I.Q.)-the normal range of which is 80-120), while insight and judgment is the final sector in the examination (it deals with the extent of the patients' recognition that they are ill, their grasp of the nature of the disorder, and the realism of their judgment about their future).

25. Answer: A

26. Answer: A

27. Answer: D

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Apstrakt i ključne reči. Za originalne radove apstrakt (do 250 reči) treba da ima sledeću strukturu: Uvod, Materijal i metode, Rezultati i Zaključak. U njemu treba navesti pitanje ili problem koji se u radu istražuje, metode koje su korišćene, dobijene rezultate i, na kraju, da se da odgovor da postavljeno pitanje. Za ostale vrste članaka apstrakt se piše u jednom pasusu. Svaki apstrakt treba da pruži jasnu informaciju.

Ispod apstrakta, autori treba da navedu 3—6 ključnih reči ili kratkih fraza prema terminima *Medical Subject Headings*—MeSH (www.nlm.nih.gov/mesh), na srpskom i engleskom jeziku.

Prevod apstrakta, tabela i naslova ilustracija na engleski. Na posebnoj stranici priložiti naslov rada na engleskom jeziku, imena i prezimena autora (bez titular) indeksirana brojevima, zvanični naziv ustanova na engleskom jeziku, *structured Abstract* (Introduction, Methods, Results, Conclusion). Prevesti nazive tabela, slika i celokupni tekst u njima. Treba se pridržavati jezičkog standarda British English. [Radovi koji se u celini dostave na engleskom imaju prednost u objavljivanju. Uputstvo za rukopise na engleskom dato je u *Instructions for contributors*.]

Uvod. U poglavlju Uvod, opisati razlog za istraživanje, dati svrhu studije ili objasniti zašto je ona važna. Cilj istraživanja može biti u formi postavke, istraživačkog pitanja ili hipoteze. Treba samo citirati radove koji su relevantni.

Materijal i metode. Ovo poglavlje opisuje procedure pomoću kojih se izvodi studija; opis treba da omogući drugima, ako žele, da studiju ponove. Ako je metod merenja poznat, treba ga citirati i opisati s par rečenica. Treba prikazati dizajn studije i navesti koje su intervencije preduzimane, da li postoji saglasnost etičkog komiteta o eksperimentima na ljudima i višim životinjama, kako je vršena randomizacija, koliko dugo su praćeni učesnici, kako su prikupljani podaci i kako je vršena statistička analiza podataka. Treba definisati nezavisne i zavisne varijable. Za lekove i hemikalije koristiti generičke nazive, doze

lekova i način davanja. Variabilnost izraziti pomoću srednje vrednosti i standardne devijacije (SD). Pošto su SD i standardna greška srednje vrednosti (SE) pozitivni brojevi, *Council of Science Editors* preporučuje eliminaciju znaka +/- sign; umesto njega SD, kao i SE, se daju u zagradama. Na primer, "sistolni krvni pritisak grupe zdravih studenata iznosio je 129 mm Hg [SD = 6, n = 87]." Vrednost P može se koristiti da se odbaci nulta hipoteza, ali treba navesti procenu snage studije (power of the study) i statistički test korišćen u statističkoj analizi.

U revijskim (preglednim) člancima se opisuju metode kojima su locirani, odabrani, pronađeni i sintetisani podaci. Te metode, takođe, treba navesti u apstraktu.

Rezultati. Dobijene rezultate treba izložiti logičkim tokom korišćenjem teksta, tabela i ilustracija. Sve ilustracije nose naziv "Slike" (Figures). Taj deo rukopisa daje odgovor studije na postavljeno istraživačko pitanje. Ponekad je to najkraći tekstualni deo rukopisa. Detalji se mogu prikazati u jednoj ili više tabela i slika. Ne ponavljati podatke iz tabela ili slika u tekstu. U tekstualnom delu treba samo naglasiti najvažnije rezultate koji direktno odgovaraju na pitanje iz Uvoda.

Tabele. Svaka tabela (4 tabele ili slike su dozvoljene) sa svojim legendama treba da opiše o čemu je reč; redosled im se numeriše arapskim brojevima u tekstu. Naslov treba da je iznad tabele, a objašnjenja, uključujući definicije skraćenica, nalaze se ispod tabele. Sve ovo pisati dvojezično (srpski i engleski). Tabele raditi isključivo u programu *Word* (koristiti *table-insert-table*). U tabeli, u iste ćelije uneti tekst na srpskom i engleskom jeziku. (Nikako ne praviti dve dabele na dva različita jezika.)

Slike. Sve ilustracije (fotografije, grafikoni, sheme) treba numerisati arapskim brojevima redosledom njihovog pominjanja u tekstu (maksimum 4 slike ili tabele su dozvoljene). Sve ilustracije nose naziv "Slike". Slova su tamna na beloj podlozi a veličina treba da je čitjiva kada se štampanjem umanje. Originalne crteže, EKG zapise i sl. treba skenirati sa barem 300 DPI (JPG ili TIF). Legende za slike kucati s dvostrukim proredom na posebnom listu označene Arapskim brojem koji odgovara slici. Simbole, strelice, brojeve ili slova koji su na slici objasniti u legendi. Interna skala treba da se pojavi na mikrosnimku, a metodi bojenja se opisuju u legendi. Tekst legende i sva objašnjenja pišu se dvojezično.

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Curtis MJ, Shattock MJ. The role of the manuscript assessor. In: Hall GM, ed. How to write a paper. London: BMJ Publishing Group; 1994:89-95.

Electronic publications (ove citate treba izbegavati):

International Society of Scientometrics and Informatics Web site. Available at: http://www.issi-society.info (accessed March 20, 2012).

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Revijski članci

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Rešavanje različitih kliničkih problema, uključujući kliničke studije, treba da sadrži sledeće delove: Apstrakt (na srpskom i engleskom), Uvod, Metode ili Prikaz(e) bolesnika, Diskusija, Reference (do 20). Apstrakt se piše u jednom pasusu (nestrukturisano) na do 150 reči. Ovaj tip rukopisa ne sme imati više od 1400 reči, ne računajući reference, tabele i ilustracije. Autori moraju dobiti pismenu saglasnost od pacijenta, bliskog rođaka ili staratelja. U propratnom pismu treba navesti da je takav dokumenat pribavljen. Izjavu o doprinosu autora (ukoliko ih je dva ili više) i izjavu o konfliktima interesa moraju potpisati autori.

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- PROPRATNO PISMO
- IZJAVA O AUTORSTVU
- IZJAVA O KONFLIKTIMA INTERESA

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1. Manuscripts should be submitted in the .DOC format (Microsoft Word), using the Times New Roman font. The text should be single spaced 11 point. The main heading should be 12 point **bold**. Subheadings should be 11 point **bold**. Tables must be 10 point, single spaced; headings within tables should be 10 point **bold**; the main table heading should be 12 point **bold**; legends should be single spaced in 11 point. Illustrations can be submitted in either JPG or TIFF format (300 dpi or higher resolution).

2. Drugs and chemicals should be indicated by generic names. Instruments, apparatus or other devices are indicated by trade names, with the producer's name and place of production indicated in brackets.

3. Numbers in text and tables should be provided if expressed as %; means should be accompanied by SDs, and medians by interquartile range (IQR). In text, use following rule: spell out numbers up to ten and then use numerical designation for 10 and above.

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6. Units of measure, length, height, weight and volume are to be expressed in metric units (e.g., meter—m, kilogram—kg, liter—l) or subunits. Temperature should be in degrees Celsius (^{o}C); quantities of substances are given in moles (mol), and blood pressure is expressed as millimeters of mercury (mm Hg). All values of hematological, clinical and biochemical measurements use the metric system according to the International System of Units (SI units).

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9. Financial disclosure. A disclosure statement declaring any potential conflict of interest must be signed by each author. (See the policy statement on conflict of interest issued by the World Association of Medical Editors, WAME, www.wame.org or ICMJE uniform disclosure form for potential conflicts of interest, www.icmje.org.) This disclosure includes all affiliations or financial involvement (e.g., employment, consulting fee or honorarium, gifts, stock ownership or options, travel/accomodations expenses, grants or patents received or pending, and royalties) with any organization having a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This information will be held in confidence while the paper is under review. If the manuscript is accepted for publication, the editors will discuss with the author how such information is communicated to the reader in the section "Conflicts of interest."

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c. Assertion that written acknowledgments, consent statements and/or permission by the institutional ethics committee were obtained.

This letter should be signed by corresponding author.

13. Submission of manuscripts. Manuscripts and all enclosures (cover letter, authorship statement and financial disclosures) should be sent by e-mail to editor@scriptamedica.com, preferably in one file. Signed copies of the cover letter and various statements may be faxed to +387 (51) 329-100. Submissions that do not comply with these instructions will be returned, unread.

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16. For further information, please contact us at the following address:

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Specific instructions for a manuscript

Title page. The title page of the manuscript contains the title of the article, the full name of each author (without titles), and the departments and institutions of the author(s) in the order they are listed. The title page must also include the name of the corresponding author, (along with address, phone and fax numbers and e-mail address) to which the work should be attributed. A short running title should have no more than 40 characters, including spaces. The word count should be indicated as well. Original articles may have up to 2.500 words, excluding references and abstract.

The title should identify the main topic or the message of the paper. The standard title of a research paper is a phrase (rarely a sentence) that identifies the topic of the paper; it should be concise and precise, informative and descriptive.

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Abstract and key words. Structured abstracts should be included in papers that report original research. Abstracts are limited to 250 words in four labeled paragraphs: Introduction, Materials and Methods, Results, and Conclusion. The abstract should state concisely the question that was asked or the objectives of the study, the methods that were used, the results obtained, and adequately answer the question posed in the introduction. The abstract should provide pertinent information when read alone.

Below the abstract, authors should provide 3-6 key words or short phrases, according to terms from the Medical Subject Headings—MeSH (www.nlm.nih.gov/mesh).

Introduction. Generally, this section provides the motivation for the paper (i.e., what is missing or unknown in the research literature at this time), an overview of the scientific theory or conceptual models on which the research was based, and the purpose of the study and why it is important. Cite only relevant references.

Materials and methods. This section accurately describes the procedures used to carry out the study; it should be complete enough to permit others to replicate the study. Describe the methodological design, subjects, data sources, data collection methods, and any statistical and analytical procedures. These five parts may not be needed in all papers. Short papers may include these details in different paragraphs, but titled subsections may be used in longer papers. The Methods section should describe how the research was structured, how subjects or groups of subjects (defined by sex, age, and other characteristics) and how the subjects were chosen and assigned to these groups. Identify all drugs and chemicals by generic names, exact drug dosages and routes of administration. Variability should be expressed in terms of means and standard deviations

(SD). Because SD and SEM are positive numbers, we recommend elimination of a +/- sign; instead, the SD may be given in brackets. For example, "systolic blood pressure in group of healthy students was 129 mm Hg [SD = 6, n = 87]." A p-value can be used to disprove the null hypothesis, but the authors should also give an estimate of the power of the study and state the exact tests used for statistical analysis.

Results. This section presents findings in logical sequence using the text, tables and illustrations. This section should show how the results of the study answer the research question. This may be shortest part of the entire paper. Details may be presented concisely in one or more tables or figures. Do not repeat the data presented in tables or illustrations in the text. Emphasize or summarize only important observations and how these answer the question posed in the introduction.

Tables. Each table (4 tables or figures are permitted) with its legends, should be self-explanatory and numbered in Arabic numerals in order of their mention in the text. The title should be typed above the table, and any explanatory text, including definitions of abbreviations, is placed below the table.

Illustrations (Figures). All figures (photographs, graphs, or schemes) should be numbered with Arabic numerals in the order of their mention in the text (a maximum of 4 figures or tables may be submitted). All lettering should be dark against a white background and of sufficient size to be legible when reduced for publication. Do not send original artwork, x-ray films, or ECG tracings but rather photographs of such material. Images need to be at least 300 DPI (JPG or TIF files). Figure legends should be typed double-spaced on a separate page with Arabic numerals corresponding to the figure. All symbols, arrows, numbers, or letters should be explained in the legend. An internal scale should appear on photomicrographs, and methods of staining should be described in the legend.

Discussion. Briefly state the principal finding that relates to the purpose or research question posed in the Introduction and follow with an interpretation of the results obtained. Compare your findings with work reported previously by others. Discuss the implications of your findings and their limitations with respect to the methods used.

Acknowledgments. List all persons as well as financial and material supporters who helped to realize the project, even if they did not meet the criteria for authorship.

References. The reference list is the responsibility of the authors. List all the papers or other sources cited in describing previous or related research. Cite references in the text sequentially in the Vancouver numbering style, as superscripted number after any punctuation mark. For example: ...as reported by Vulić and colleagues.[12] When two references are cited, they should be separated by comma, with no space. Three or more consequtive references are given as a range with an en rule. References in tables and figures should be in numerical order according to where the item is cited in the text. For citations according to the Vancouver style, see Uniform Requirements for Manuscripts Submitted to Biomedical Journals; this source gives the rules and formats established by the International Committee of Medical Journal Editors (www.icmje.org). If there are six authors or fewer, list all six by last name, space, initials, comma. If there are seven or more, list the first three in the same way, followed by et al. For a book, list the editors and the publisher, the city of publication, and year of publication. For a chapter or section of a book, give the authors and title of the section, and the page numbes. For online material, please cite the URL and the date you accessed the website.. Online journal articles can be cited using the DOI number. Do not put references within the Abstract section. All titles should be in English (the name of the original language should appear in brackets). See examples below that conform to the Uniform Requirements for Manuscripts Submitted to Biomedical Journals:

De Lacey G, Record C, Wade J. How accurate are quotations and references in medical journals. BMJ 1985; 291:884-6.

International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Croat Med J 2003; 44:770-83.

Huth EJ. How to write and publish papers in the medical sciences. Philadelphia: ISI Press, 1982.

Davidović L, Marković M, Čolić M, et al. Treatment of traumatic rupture of the thoracic aorta. Srp Arh Celok Lek 2008; 136: 498-504.

Curtis MJ, Shattock MJ. The role of the manuscript assessor. In: Hall GM, ed. How to write a paper. London: BMJ Publishing Group; 1994: 89-95.

Electronic publications:

International Society of Scientometrics and Informatics Web site. Available at: http://www.issi-society.info Accessed March 20, 2012.

Lock SP. Journalology: are the quotes needed? CBE Views. 1989:1257-9. Available at: http://garfield.libraryupenn. edu/essays/v13p019y1990.pdf. Accessed April 25, 2012.

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Review articles are written by individuals who have studied a particular subject or area extensively, and who are considered experts. For these reviews, the word count may not exceed 2.500 words, excluding references and abstract. The manuscript may have up to 4 tables or illustrations, and as many as 50 references.

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Case reports are most likely to be published if they describe any of the following: an unreported drug side effects (adverse

or beneficial), drug interactions; a new, unexpected, or unusual manifestation of a disease; previously unsuspected causal association between two diseases; presentations, diagnosis and/or management of new and emerging diseases; an unexpected association between diseases or symptoms; an unexpected event in the course of observing or treating a patient, findings that shed new light on the possible pathogenesis of a disease or an adverse effect; a previously unknown disease. *Scripta Medica* does not publish instructive case reports, that is, presentations that make important teaching point of what is already well known but often forgotten.

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- Authorship statement
- Financial disclosure statement