Prague Medical REPORT

(Sborník lékařský)

Multidisciplinary Biomedical Journal
of the First Faculty of Medicine,
Charles University

Vol. 120 (2019) No. 2–3

Reviews

Primary Scientific Studies

Case Reports


D
E
C
Fig. 7.
B
C
A
Fig. 6.
B
A
Fig. 8.
C
B
A
B
Fig. 2.
D
A
D
E
Fig. 9.
A
E

Prague Medical Report (Prague Med Rep) is indexed and abstracted by Index-medicus, MEDLINE, PubMed, CNKI, DOAJ, EBSCO, and Scopus.

Abstracts and full-texts of published papers can be retrieved from the World Wide Web (https://pmr.lf1.cuni.cz).

Engraving overleaf: Laurentius Heister, Institutiones chirurgicae, Amsterdam 1750.
Illustration provided by the Institute for History of Medicine and Foreign Languages.

Lipoprotein(a) – Link between Atherogenesis and Thrombosis

Danica Labudovic, Irena Kostovska, Katerina Tosheska Trajkovska, Svetlana Cekovska, Julijana Brezovska Kavrakova, Sonja Topuzovska
Department of Medical and Experimental Biochemistry, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, Skopje, North Macedonia

Received April 23, 2019; Accepted September 4, 2019.

Key words: Lp(a) – Atherogenicity – Thrombogenicity – Cardiovascular disease

Abstract: Lipoprotein(a) – Lp(a) – is an independent risk factor for cardiovascular disease (CVD). Indeed, individuals with plasma concentrations of Lp(a) > 200 mg/l carry an increased risk of developing CVD. Circulating levels of Lp(a) are remarkably resistant to common lipid lowering therapies, currently available treatment for reduction of Lp(a) is plasma apheresis, which is costly and labour intensive. The Lp(a) molecule is composed of two parts: LDL/apoB-100 core and glycoprotein, apolipoprotein(a) – Apo(a), both of them can interact with components of the coagulation cascade, inflammatory pathways and blood vessel cells (smooth muscle cells and endothelial cells). Therefore, it is very important to determine the molecular pathways by which Lp(a) affect the vascular system in order to design therapeutics for targeting the Lp(a) cellular effects. This paper summarises the cellular effects and molecular mechanisms by which Lp(a) participate in atherogenesis, thrombogenesis, inflammation and development of cardiovascular diseases.

Mailing Address: Irena Kostovska, MD., PhD., Bul "Treta Makedonska Brigada" 31b/14, 1000 Skopje, North Macedonia; Phone: +389 782 089 90; e-mail: irenakostovska22@yahoo.com

https://doi.org/10.14712/23362936.2019.9

Introduction

Since its discovery by Kare Berg in 1963, lipoprotein(a) – Lp(a) molecule, has been the subject of controversy and debate about its physiological role and role in atherogenesis, thrombogenesis and development of cardiovascular diseases (CVD). The exact physiological function of Lp(a) has not been fully elucidated, however in recent years a number of prospective epidemiological and clinical studies have shown that Lp(a) level elevated more than 300 mg/l is an independent risk factor for development of coronary and cerebral atherosclerosis and powerful predictor of premature cardiovascular diseases in people with concomitant hypercholesterolemia. The European Society of Cardiology and European Atherosclerosis Society propose that Lp(a) should be measured once in subjects with intermediate or high risk of CVD such as: subjects with premature CVD, subjects with familial hypercholesterolaemia, a family history of premature CVD and/or elevated Lp(a), subjects with recurrent CVD despite statin treatment, subjects with ≥ 3% 10-year risk of fatal CVD according to the European Guidelines (Graham et al., 2007; Catapano et al., 2016) and subjects with ≥ 10% 10-year risk of fatal and/or non-fatal CVD according to the US Guidelines (Grundy et al., 2004). They also suggest that the risk is significant when Lp(a) levels are > 500 mg/l for European populations. It is emphasized that this threshold is higher than the risk threshold in primary care populations of >200 to 300 mg/l (Nordestgaard et al., 2010). The Canadian Cardiovascular Society Guidelines for the Management of Dyslipidemia for the Prevention of Cardiovascular Disease in the adult had similar recommendations, but they used a cut-off of Lp(a) > 300 mg/l as an abnormal level (Anderson et al., 2016).

Elevated Lp(a) carry an increased risk of occlusive complications following various cardiac interventions (percutaneous transluminal coronary angioplasty, stenting) (Berg, 1963; Danesh et al., 2000; Rifai et al., 2004; Nordestgaard and Langsted, 2016).

The concentration of Lp(a) levels is genetically determined and shows great individual and racial differences but inter-individually is stable throughout life. Elevated levels of Lp(a) are remarkably resistant to common lipid lowering therapies and dietary measures, currently available treatment for reduction of Lp(a) is plasma apheresis, which is costly and labour intensive (Moriarty and Hemphill, 2016; van Capelleveen et al., 2016; Khan et al., 2017). Thus, it is necessary to carefully clarify the physiological role of Lp(a) in the body and pathophysiological role of Lp(a) in the development of atherosclerosis and thrombogenesis in order to design therapeutic modality to reduce the concentration of Lp(a) levels in people with high risk of cardiovascular disease (Kronenberg, 1996a; Lippi and Targher, 2012; Boffa and Koschinsky, 2016; Tsimikas, 2017). In this paper are presented the structure and metabolism of Lp(a) and also are summarized recent literature data on cellular effects and molecular mechanisms by which Lp(a) participate in atherogenesis, thrombogenesis, inflammation and development of cardiovascular diseases.

Structure and metabolism of Lp(a)

Lp(a) molecule is expressed in humans, some primates (rhesus monkeys, baboons), and the European hedgehog (Amer. Barbed pig). Lp(a) is a complex and a unique lipoprotein particle rich in cholesterol. It is constructed from two components of low density lipoproteins (LDL) and apolipoprotein(a) – Apo(a). Apo(a) is structurally unique high glycosylated macromolecule whose size is genetically determined and highly variable. Apo(a) has determined structural and functional characteristics of Lp(a). Apo(a) is linked with a disulphide bond to ApoB-100 of LDL, building Lp(a). Stoichiometric ratio of Apo(a) and ApoB-100 in Lp(a) particle is 1:1. In Figure 1 is presented the structure of Lp(a).

Lp(a) and LDL are very similar in composition and physico-chemical characteristics. Table 1 shows the differences in physic-chemical characteristics and composition between Lp(a) and LDL.

Apo(a) gene is located on the long arm of chromosome 6 and several alleles, resulting in extensive polymorphism in individual expression. Gene Apo(a) is located adjacent to the gene encoding the synthesis of plasminogen which shows high structural similarity; it is assumed that the Apo(a) is a member of the plasminogen gene superfamily (Malgaretti et al., 1992). Apo(a) cDNA contains Kringle domains, autonomous protein domains rich in cysteine, which muster in tangles and are stabilized by disulphide bonds. They are important in protein-protein interactions in the process of blood coagulation. These domains have plasminogen, hepatocyte growth factor, prothrombin and Apo(a). Variations in the number of these domains are responsible for the size polymorphism in the molecular weight of Lp(a). Apo(a)

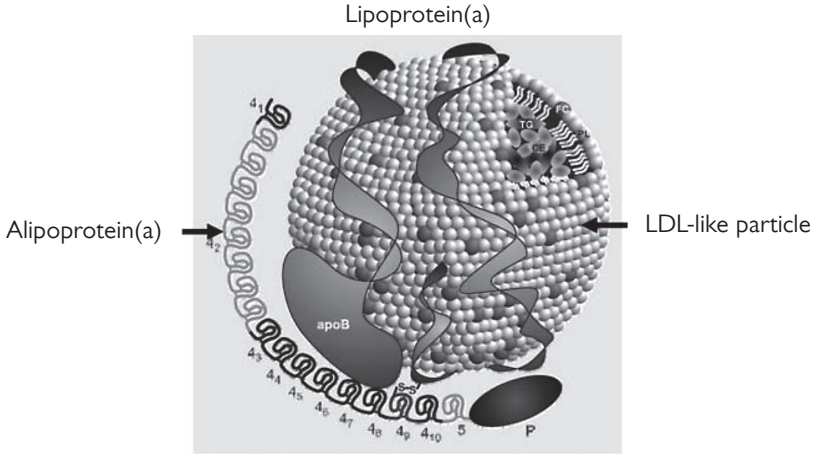

Figure 1 – Structure of lipoprotein(a) – Lp(a). Lp(a) contains low density lipoproteins (LDL) particle connected with apolipoprotein(a) – Apo(a) – with disulphide bridge. LDL is built from a central core rich in cholesterol esters and triglycerides surrounded by phospholipids, free cholesterol and a molecule apolipoproteinB-100 – ApoB-100 (Bdeir et al., 1999).

Table 1 – Differences in physic-chemical characteristics and composition between Lp(a) and LDL

	Lp(a)	LDL
Electrophoretic mobility	pre-β	β
Molecular mass (kDa)	800–1300	549
Density (g/ml)	1069	1045
Proteins %	30.0	22.5
Carbohydrates %	4.5	1.0
Cholesterol esters %	35.3	43.0
Triglycerides %	2.0	3.0
Free cholesterol %	8.5	11.0
Phospholipids %	19.5	19.5

Lp(a) – lipoprotein(a); LDL – low density lipoproteins

cDNA contains inactive serine protease domain, a copy of plasminogen Kringle V domain, and 10 types Kringle IV domains labelled Kringle IV-(1–10). Kringle IV-1 and Kringle IV-(3–10) are single domain while Kringle IV-2 is responsible for multiple domain size polymorphisms in the molecular weight of Apo(a) (300–800 kDa). Kringle IV-9 domain of Apo(a) has a specific binding site for ApoB-100 of LDL, found that it is associated with cell proliferation and smooth-muscle migration. Kringle IV-(6–7) domains mediate formation of foam cells in atherosclerosis, KIV-(7–8) domains are rich in lysine binding sites, Kringle IV-10 is responsible for the increased vascular permeability. cDNA contains the plasminogen Kringle domains I to V, and the active form of serine protease domain, which is connecting with tissue plasminogen activator and urokinase plasminogen activator. This serine protease domain plays a key role in endogenous fibrinolysis (McLean et al., 1987). Principal place of synthesis of Lp(a) is liver. Association of Apo(a) with LDL in Lp(a) probably happens on the surface of hepatocytes. The synthesis of Lp(a) is carried out in two steps. First step is approaching sulfhydryl groups of Apo(a) and ApoB-100 and the second step is binding of Cys4057 of Apo(a) (Kringle domains IV-9) with Cys4326 of ApoB-100 by disulphide bond. The first step is inhibited by lysine and lysine analogues, such as lysine analogue tranexamic acid (Cyclocapron) which reduces the concentration of Lp(a) levels in humans (Frank et al., 1995). Binding of Apo(a), ApoB-100 occurs in the vicinity of the binding site of ApoB-100 on LDL receptor, which leads to poor affinity of Lp(a) the LDL receptor.

Catabolism of Lp(a) is not fully studied. Lp(a) has a longer half-life in plasma compared with LDL, suggesting that catabolism of Lp(a) does not take place entirely via the LDL receptor, but that there are other metabolic pathways for degradation of Lp(a), as through LDL receptor protein – megalin gp330, VLDL (very low density lipoprotein) receptor, galactose specific asialoglycoprotein receptor (ASGPR), plasminogen receptor and by macrophage receptors (Hrzenjak et al., 2003).

The liver and kidney are the major tissues involved in Lp(a) clearance, but the pathways for Lp(a) uptake are still under investigation. Biochemical studies have revealed an exceptional array of receptors that associate with Lp(a) either via its ApoB, Apo(a), or oxidized phospholipids (OxPL) components, such as: namely "classical" lipoprotein receptors, toll-like and scavenger receptors, lectins, and plasminogen receptors. The importance of these receptors in catabolism of Lp(a) from the circulation are still unclear. The *LPA* gene encoding Apo(a) has an exceeding effect on Lp(a) levels which avert any clear associations between potential Lp(a) receptor genes and Lp(a) levels in population studies. Targeted approaches and selection of unique Lp(a) phenotypes within populations has normally allowed for some associations to be made. Few of the suggested Lp(a) receptors can specifically be manipulated with current drugs, but it is not clear whether any of these receptors could provide relevant targets for therapeutic manipulation of Lp(a) levels (McCormick and Schneider, 2019).

Evidence indicated that Lp(a) is not a metabolic product of other lipoproteins, VLDL or LDL, nor is it metabolized to other lipoproteins. A few studies suggested that the variations in Lp(a) plasma concentration in individuals with different isoforms was due to the production rate of Apo(a) rather than by its clearance rate. The low-density lipoprotein receptor (LDLR) was considered to be a possible site for the uptake and degradation of Lp(a), however reports are controversial regarding the significance of the role of the LDLR in Lp(a) catabolism (Kraft et al., 2000), which reported that for Lp(a) with the same allele size the concentration is dependent on the gene dose of the LDLR in familial hypercholesterolemia and deficient subjects have higher values of plasma Lp(a) than heterozygous subjects. However, kinetic studies suggested that the clearance of Lp(a) is not entirely dependent on the LDLR (Reyes-Soffer et al., 2017). LDL from Lp(a) is cleared by the LDLR only after release of Apo(a). It is reported that LDLR or low-density lipoprotein receptor-related protein (LRP) deficient fibroblasts did not alter their uptake and degradation of Lp(a) (Reblin et al., 1997). Although Lp(a) may bind to LDLR and LRP, the binding does not seem to be important for its degradation.

The fact that patients with nephrotic syndrome and chronic renal insufficiency have elevated concentrations of Lp(a), indicate the involvement of the kidney in the catabolism of Lp(a) (Kronenberg et al., 1996b; Schmidt et al., 2016). In one study was observed more Lp(a) deposition in the radial arteries of ESRD (end stage renal disease) patients with high Lp(a) concentration. Filipin and HE (hematoxylin and eosin) staining showed that cholesterol accumulation and foam cell formation are significantly higher in the group with high Lp(a) concentration than in the control group. These findings suggest that high plasma Lp(a) levels might be the main cause of cholesterol accumulation and foam cell formation in the radial arteries of ESRD patients (Ma et al., 2018). As we know, hypercholesterolemia is a main risk factor for the progression of atherosclerosis. Lipoprotein(a), one of the components of plasma lipid profile, was shown similar effects with hypercholesterolemia on promoting

systemic atherosclerosis. Therefore, high-Lp(a) induced radial atherosclerosis means that Lp(a) may contribute to the progression of cardiovascular disease in ESRD patients.

Concentration of Lp(a)

The concentration of Lp(a) among individuals is genetically determined, shows large variations, ranging from 1 to 1,000 mg/l, but remains stable throughout life (Puckey and Knight, 1999). There are variations in the concentration of Lp(a) levels between races due to the existence of polymorphisms in the sequence of Apo(a) isoforms and a lot of additional factors.

Polymorphisms of the Apo(a) gene regulates the concentration of Lp(a) levels by several mechanisms size polymorphism of Apo(a), through a number of iterations in pentanucleotide promoter region of Apo(a) gene and over 93 C/T polymorphisms in non-transcribed region of Apo(a) gene. There is an inverse correlation between the molecular weight of Lp(a), the number of Kringle IV domains in the Apo(a), and individual plasma concentrations of Lp(a). Thus, individuals with high molecular phenotypes of Lp(a) have low concentrations of Lp(a) plasma and those with high molecular phenotypes have high concentrations of Lp(a) plasma because Apo(a) isoforms with high molecular weight are tightly bound and degrade more quickly through endoplasmic reticulum unlike Apo(a) isoforms with a lower molecular weight. Most genetic variations are due to mutations in the Kringle IV domain, the lower part is due to mutations in promoter region (pentanucleotid repetitions) and the coding region (93 C/T polymorphism). For example, mutations in Kringle IV-9 domain participate in the formation of disulphide bridge, which leads to decreased synthesis of Lp(a) and low levels of Lp(a) levels.

Despite the fact that the concentration of Lp(a) is under a strong genetic control and remains stable throughout life, some case control studies found that some exogenous factors can have a small but significant impact on the concentration of Lp(a) levels. Concentration of Lp(a) can be increased by fatty acids of marine origin as elaidic acid, orchiectomy and hypothyroidism. Reducing effect of Lp(a) concentration has palm oil, polyunsaturated fatty acid and hormones replacement therapy in postmenopausal women (de Bruin et al., 1993; Soma et al., 1993; Hermann et al., 1995; Tholstrup et al., 1995; Marcovina et al., 1996).

Because of its similarity with plasminogen, Lp(a) shows thrombogenic and atherogenic properties and may disturb the balance between procoagulant and anticoagulant, anti-inflammatory and proinflammatory, vasodilatation and vasoconstriction and properties of the endothelium. Lp(a) can disrupt the function of endothelium and that makes this molecule not only a link between atherosclerosis and thrombosis, but also a link between endothelial dysfunction and these two processes. There are more mechanisms of atherogenicity and thrombogenicity of Lp(a).

Role of Lp(a) in atherogenesis

Atherosclerosis is a complex process that includes the following events in the arterial wall: deposition of plasma lipoproteins, proliferation of cellular elements, and inflammatory response. The progression of atherosclerosis is conducted in several steps, starting from the foam cell formation to complex atherosclerotic plaque composed of a core rich in lipids and necrotic cell debris, covered with fibrous cap. Lp(a) stimulates atherogenesis by several mechanisms: induction of inflammatory cytokines and adhesion molecules on the surface of vascular endothelial cells, transport of oxidized phospholipids, chemoattraction, inhibition the synthesis of nitrogen monoxide, vascular remodelling, and proliferation of smooth-muscle cells.

Induction of inflammatory cytokines and adhesion molecules on the surface of endothelial cells

The first event in the arterial wall in atherogenesis is the adhesion of mononuclear cells to the endothelium mediated by increased expression of adhesion molecules from endothelial cells as vascular cell adhesion protein-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin. Lp(a) increase the expression of adhesion molecules (VCAM-1, ICAM-1, E-selectin and P-selectin) on the surface of vascular endothelial cells, which initiate atherosclerotic changes in the vessel wall. Lp(a) induces increased expression of interleukin-8 (IL-8), interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α) in macrophages, cytokines – mediators in inflammation process of atherosclerosis (Beisiegel et al., 1990; Linton et al., 2000; Deb and Caplice, 2004).

Chemotaxis

Lp(a) stimulates the secretion of monocyte chemotactic protein (MCP) by endothelial cells which causes chemotaxis of monocytes and their migration through the endothelial barrier. MCP is a potent chemoattractant for monocytes and a key cytokine in the pathogenesis of atherosclerosis. Lp(a) also activate the inflammatory transcription factor – nuclear factor κB leading to the recruitment of inflammatory cells along the arterial wall (Syrovets et al., 1997).

Oxidation of Apo(a), transport of oxidized phospholipids and inhibition of nitrogen monoxide synthase

Apo(a) is a subject to oxidation such LDL. The mechanisms by which Lp(a) accelerates these disorders are not fully understood, but the oxidized phospholipids present on apolipoprotein(a) might have an important role. Lp(a) is the major carrier of oxidized phospholipids in human plasma, and interventions that lower plasma Lp(a) levels also reduce the oxidized phospholipid concentration in plasma (Boffa and Koschinsky, 2019). Oxidized form of Apo(a) facilitates the binding of Lp(a) for macrophage scavenger receptor, triggering the formation of foam cells. Oxidized form of Lp(a) inhibits vasodilatation, stimulates the production

of plasminogen activator inhibitor-1 (PAI-1) by vascular endothelial cells and stimulates the production of superoxide radicals – all these things make oxidized Lp(a) more atherogenic than native Lp(a) molecule (Riis Hansen et al., 1994). Inhibition of nitric oxide synthase by Lp(a) leads to reduction of the concentration of nitrogen monoxide (NO). The reduced concentration of NO leads to oxidative stress and progression of atherosclerotic process, recognizing antiatherogenic role of NO as inhibition of T-cell and smooth-muscle proliferation, neutrophil adhesion, platelet activation, and reduction of endothelial permeability. Lp(a) initiates activation of matrix metalloproteinases MMP-2 and MMP-9. Low concentrations of Lp(a) levels have anti-inflammatory action, binding and removing oxidized phospholipids from the circulation, despite high concentrations of Lp(a) levels leading to excessive accumulation of oxidized phospholipids in the wall of blood vessels actuate the atherosclerotic progression (Bergmark et al., 2008). Lp(a) reduces the activation of latent transforming growth factor-β (TGF-β), which shows a number of cellular effects, such as inhibition of smooth-muscle proliferation and migration, inhibition of expression of adhesion molecules on the surface of endothelial cells – antiatherogenic actions. In the absence of TGF-β, cytokines can induce smooth-muscle proliferation and migration, thus the progression of atherosclerotic lesions (Kojima et al., 1991).

Proliferation of smooth-muscle cells, vascular remodelling and endothelial dysfunction
Atherosclerotic plaques contain Lp(a) proportional to the concentration of Lp(a) levels, unlike normal arterial walls. Plasminogen lysine-binding sites of Apo(a) are probably very important in anchoring the Lp(a) in the extracellular matrix of the arterial wall. It has been proved that mutations of lysine binding sites reduce the affinity of Lp(a) in the artery wall. Apo(a) binds to several extracellular matrix proteins such as fibrin and defensins that are released by neutrophils during the

Table 2 – Atherogenic mechanisms of lipoprotein(a)

Induction of inflammatory cytokines IL-8, IL-1β and TNF-α
Increased expression of adhesion molecules on the surface of endothelial cells VCAM-1, ICAM-1, E-selectin and P-selectin
Increased secretion of MCP and activation of nuclear factor kB with subsequent monocyte chemotaxis
Oxidation of Apo(a) and formation of high atherogenic particles with LDL
Increased transport of oxidized phospholipids in the blood vessel wall
Reduced production of nitrogen monoxide with subsequent vasoconstriction
Reduced activation of latent transforming growth factor-β (TGF-β) with subsequent smooth-muscle proliferation
Increased endothelial permeability through rearrangement of the cytoskeleton

inflammatory process (Bdeir et al., 1999). Lp(a) interacts with β2-integrin Mac-1, which promotes adhesion of monocytes and their transendothelial migration (Sotiriou et al., 2006). Recent experimental studies shown that Apo(a) perform the rearrangement of actin cytoskeleton through increased phosphorylation of myosin light chains by Rho/Rho kinase-dependent signalling pathway (first intracellular signalling path in endothelial cells mediated by Lp(a)), leading to increased contraction and permeability of endothelial cells. This leads to impaired receptor-mediated vasodilation and the endothelial dysfunction. Overall proatherogenic role for this action of Lp(a) has a lysine binding site of Kringle IV type 10 (Riches and Porter, 2012; Riches et al., 2013). Apo(a) induce expression of β-catenin with consequent increased cyclooxygenase-2 (COX-2) expression and prostaglandin E2 (PGE2) secretion, key events in inflammation and vascular remodelling (Dubé et al., 2012). Proteoglycans, as decorin-synthesized by vascular endothelial cells, play an important role in the retention of Lp(a) along the arterial wall (Klezovitch et al., 1998). In Table 2 are summarized the atherogenic mechanisms of Lp(a).

Role of Lp(a) in thrombogenesis

Lp(a) participate in thrombogenesis through several mechanisms: platelet aggregation and activation, inhibition of tissue factor pathway inhibitor (TFPI), decreased production of plasmin and increased expression of plasminogen activator inhibitor-1 (PAI-1).

Aggregation and activation of platelets

Lp(a) and Apo(a) initiate activation of platelets by thrombin receptor-activated hexapeptide (TRAP) and platelet activating factor (PAF). Lp(a) has ability for specifically binding to platelet activating factor – acetyl hydrolase (PAF-AH) and thus inhibits PAF. Lp(a) has antiaggregatory effect mediated by its interaction with the integrin αIIbβ3, which normally binds to fibrinogen to induce platelet aggregation. Apo(a) binds to fibrin in a complex which inhibits the activation of plasminogen (Tsironis et al., 2004).

Inhibition of tissue factor pathway inhibitor (TFPI) and reduced production of plasmin

Many cells have receptors for plasminogen, including endothelial cells and platelets. Lp(a) and Apo(a) inhibit the binding of plasminogen to annexin (plasminogen receptor on the surface of platelets and endothelial cells), thereby preventing the activation of plasminogen to plasmin by the action of tissue factor pathway activation (t-PA). Simultaneously, Lp(a) interferes with the binding sites of t-PA on the surface of endothelial cells. Lp(a) reduces the production of t-PA by the endothelial cells (Kat, 2002). This leads to an antifibrinolytic state. Antifibrinolytic effect of Lp(a) depends on the size of the molecular weight of Apo(a) – those with lower molecular weight have greater antifibrinolytic effect. Lp(a) promotes thrombosis by binding and inhibiting the tissue factor pathway inhibitor (TFPI).

Table 3 – Thrombogenic mechanisms of lipoprotein(a)

Inhibition of platelet activation factor (PAF)
Inhibition of tissue factor pathway inhibitor (TFPI) and plasmin reduced production
Increased expression of plasminogen activator inhibitor-1 (PAI-1)

Increased expression of plasminogen activator inhibitor-1 (PAI-1)
Lp(a) stimulates the production of PAI-1 by endothelial cells in blood vessels by protein kinase C (PKC)-dependent mechanism. Lp(a) interacts with other proteins, such as prothrombotic α2-macroglobulin (plasmin inhibitor) and serine proteinase inhibitor A1 (SERPINA1) which is t-PA inhibitor. Transforming growth factor-β (TGF-β) is plasmin substrate. Reduced synthesis of plasmin by Lp(a) leads to inhibition of TGF-β and progression of atherosclerosis (Etingin et al., 1991).
In Table 3 are summarized the thrombogenic mechanisms of Lp(a).

Conclusion

In this paper we reviewed the published literature data on atherogenic and thrombogenic role of Lp(a) in development of cardiovascular diseases. Lp(a) has a wide range of functional effects in development of cardiovascular diseases, such as modulation of platelet aggregation, reduced fibrinolysis, recruitment of inflammatory cells, vascular remodelling. Atherogenic and simultaneously thrombogenic function of Lp(a) makes this molecule very powerful in development of atherosclerosis and cardiovascular diseases. Lp(a) act through multiple pathogenic protein molecules and receptors that makes it impossible to find a single therapeutic target, but requires action at multiple levels in the mechanism of pathogenic action of Lp(a). More extensive trials are required in signalling pathways and molecular mechanisms in the action of Lp(a). That will provide in the near future possibility to identify sensitive therapeutic target in reducing Lp(a) levels.

References

Anderson, T. J., Grégoire, J., Pearson, G. J., Barry, A. R., Couture, P., Dawes, M., Francis, G. A., Genest, J. Jr., Grover, S., Gupta, M., Hegele, R. A., Lau, D. C., Leiter, L. A., Lonn, E., Mancini, G. B., McPherson, R., Ngui, D., Poirier, P., Sievenpiper, J. L., Stone, J. A., Thanassoulis, G., Ward, R. (2016) 2016 Canadian Cardiovascular Society Guidelines for the management of dyslipidemia for the prevention of cardiovascular disease in the adult. *Can. J. Cardiol*. **32(11)**, 1263–1282.

Bdeir, K., Cane, W., Canziani, G., Chaiken, I., Weisel, J., Koschinsky, M. L., Lawn, R. M., Bannerman, P. G., Sachais, B. S., Kuo, A., Hancock, M. A., Tomaszewski, J., Raghunath, P. N., Ganz, T., Higazi, A. A., Cines, D. B. (1999) Defensin promotes the binding of lipoprotein(a) to vascular matrix. *Blood* **94**, 2007–2019.

Beisiegel, U., Niendorf, A., Wolf, K., Reblin, T., Rath, M. (1990) Lipoprotein(a) in the arterial wall. *Eur. Heart J*. **11**, 174–183.

Berg, K. (1963) A new serum type system in man: the Lp system. *Acta Pathol. Microbiol. Scand*. **59**, 369–382.

Bergmark, C., Dewan, A., Orsoni, A., Merki, E., Miller, E. R., Shin, M. J., Binder, C. J., Hörkkö, S., Krauss, R. M., Chapman, M. J., Witztum, J. L., Tsimikas, S. (2008) A novel function of lipoprotein [a] as a preferential carrier of oxidized phospholipids in human plasma. *J. Lipid Res.* **49**, 2230–2239.

Boffa, M. B., Koschinsky, M. L. (2016) Lipoprotein (a): Truly a direct prothrombotic factor in cardiovascular disease? *J. Lipid Res.* **57**, 745–757.

Boffa, M. B., Koschinsky, M. L. (2019) Oxidized phospholipids as a unifying theory for lipoprotein(a) and cardiovascular disease. *Nat. Rev. Cardiol.* **16**, 305–318.

Catapano, A. L., Graham, I., De Backer, G., Wiklund, O., Chapman, M. J., Drexel, H., Hoes, A. W., Jennings, C. S., Landmesser, U., Pedersen, T. R., Reiner, Ž., Riccardi, G., Taskinen, M. R., Tokgozoglu, L., Verschuren, W. M. M., Vlachopoulos, C., Wood, D. A., Zamorano, J. L., Cooney, M. T.; ESC Scientific Document Group (2016) 2016 ESC/EAS Guidelines for the management of dyslipidaemias. *Eur. Heart J.* **37(39)**, 2999–3058.

Danesh, J., Collins, R., Peto, R. (2000) Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. *Circulation* **102(10)**, 1082–1085.

Deb, A., Caplice, N. M. (2004) Lipoprotein(a): New insights into mechanisms of atherogenesis and thrombosis. *Clin. Cardiol.* **27(5)**, 258–264.

de Bruin, T. W., van Barlingen, H., van Linde-Sibenius Trip, M., van Vuurst de Vries, A. R., Akveld, M. J., Erkelens, D. W. (1993) Lipoprotein(a) and apolipoprotein B plasma concentrations in hypothyroid, euthyroid and hyperthyroid subjects. *J. Clin. Endocrinol. Metab.* **76**, 121–126.

Dubé, J. B., Boffa, M. B., Hegele, R. A., Koschinsky, M. (2012) Lipoprotein(a): More interesting than ever after 50 years. *Curr. Opin. Lipidol.* **23**, 133–140.

Etingin, O. R., Hajjar, D. P., Hajjar, K. A., Harpel, P. C., Nachman, R. L. (1991) Lipoprotein(a) regulates plasminogen activator inhibitor-1 expression in endothelial cells. A potential mechanism in thrombogenesis. *J. Biol. Chem.* **266(4)**, 2459–2465.

Frank, S., Durovic, S., Kostner, K., Kostner, G. M. (1995) Inhibitors for the *in vitro* assembly of Lp(a). *Arterioscler. Thromb. Vasc. Biol.* **15**, 1774–1780.

Graham, I., Atar, D., Borch-Johnsen, K., Boysen, G., Burell, G., Cifkova, R., Dallongeville, J., De Backer, G., Ebrahim, S., Gjelsvik, B., Herrmann-Lingen, C., Hoes, A., Humphries, S., Knapton, M., Perk, J., Priori, S. G., Pyorala, K., Reiner, Z., Ruilope, L., Sans-Menendez, S., Scholte op Reimer, W., Weissberg, P., Wood, D., Yarnell, J., Zamorano, J. L., Walma, E., Fitzgerald, T., Cooney, M. T., Dudina, A.; European Society of Cardiology (ESC) Committee for Practice Guidelines (CPG) (2007) European guidelines on cardiovascular disease prevention in clinical practice: executive summary: Fourth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (Constituted by representatives of nine societies and by invited experts). *Eur. Heart J.* **28**, 2375–2414.

Grundy, S. M., Cleeman, J. I., Merz, C. N., Brewer, H. B. Jr., Clark, L. T., Hunninghake, D. B., Pasternak, R. C., Smith, S. C. Jr., Stone, N. J.; National Heart, Lung, and Blood Institute; American College of Cardiology Foundation; American Heart Association (2004) Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Arterioscler. Thromb. Vasc. Biol.* **24**, e149–e161.

Hermann, W., Biermann, J., Kostner, G. M. (1995) Comparison of effects of N-3 to N-6 fatty acids on serum levels of lipoprotein(a) in patients with coronary artery disease. *Am. J. Cardiol.* **76**, 459–462.

Hrzenjak, A., Frank, S., Wo, X., Zhou, Y., Van Berkel, T., Kostner, G. M. (2003) Galactose-specific asialoglycoprotein receptor is involved in lipoprotein (a) catabolism. *Biochem. J.* **376**, 765–771.

Kat, H. (2002) Regulation of functions of vascular wall cells by tissue factor pathway inhibitor: basic and clinical aspects. *Arterioscler. Thromb. Vasc. Biol.* **22**, 539–548.

Khan, T. Z., Hsu, L.-Y., Arai, A. E., Rhodes, S., Pottle, A., Wage, R., Gatehouse, P. D., Banya, W., Giri, S., Collins, P., Pennell, D. J., Barbir, M. (2017) Apheresis as novel treatment for refractory angina with raised lipoprotein(a): a randomized controlled cross-over trial. *Eur. Heart J*. **38**, 1561–1569.

Klezovitch, O., Edelstein, C., Zhu, L., Scanu, A. M. (1998) Apolipoprotein(a) binds via its C-terminal domain to the protein core of the proteoglycan decorin. Implications for the retention of lipoprotein(a) in atherosclerotic lesions. *J. Biol. Chem*. **273**, 23856–23865.

Kojima, S., Harpel, P. C., Rifkin, D. B. (1991) Lipoprotein(a) inhibits the generation of transforming growth factor β: an endogenous inhibitor of smooth muscle cell migration. *J. Cell Biol*. **113(6)**, 1439–1445.

Kraft, H. G., Lingenhel, A., Raal, F. J., Hohenegger, M., Utermann, G. (2000) Lipoprotein(a) in homozygous familial hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol*. **20(2)**, 522–528.

Kronenberg, F., Steinmetz, A., Kostner, G. M., Dieplinger, H. (1996a) Lipoprotein(a) in health and disease. *Clin. Lab. Sci*. **33**, 495–543.

Kronenberg, F., Utermann, G., Dieplinger, H. (1996b) Lipoprotein(a) in renal disease *Am. J. Kidney Dis*. **27**, 1–25.

Linton, M. R. F., Yancey, P. G., Davies, S. S., Jerome, W. G., Linton, E. F., Song, W. L., Doran, A. C., Vickers, K. C. (2000) *The Role of Lipids and Lipoproteins in Atherosclerosis*. MDText.com, Inc. Available at: https://www.ncbi.nlm.nih.gov/books/NBK343489/

Lippi, G., Targher, G. (2012) Optimal therapy for reduction of lipoprotein(a). *J. Clin. Pharm. Ther*. **37(1)**, 1–3.

Ma, K. L., Gong, T. K., Hu, Z. B., Zhang, Y., Wang, G. H., Liu, L., Chen, P. P., Lu, J., Lu, C. C., Liu, B. C. (2018) Lipoprotein(a) accelerated the progression of atherosclerosis in patients with end-stage renal disease. *BMC Nephrol*. **19(1)**, 192.

Malgaretti, N. F., Acquati, P. M., Magnaghi, P., Bruno, L., Pontoglio, M., Rocchi, M., Saccone, S., Della Valle, G., D'Urso, M., LePaslier, D. (1992) Characterization by yeast artificial chromosome cloning of the linked apolipoprotein(a) and plasminogen genes and identification of the apolipoprotein(a) 5' flanking region. *Proc. Natl. Acad. Sci. U. S. A*. **89(23)**, 11584–11588.

Marcovina, S. M., Lippi, G., Bagatell, C. J., Bremner, W. J. (1996) Testosterone-induced suppression of lipoprotein(a) in normal men: Relation to basal lipoprotein(a) level. *Atherosclerosis* **122**, 89–95.

McCormick, S. P. A., Schneider, W. J. (2019) Lipoprotein(a) catabolism: a case of multiple receptors. *Pathology* **51(2)**, 155–164.

McLean, J. W., Tomlinson, J. E., Kuang, W. J., Eaton, D. L., Chen, E. Y., Fless, G. M., Scanu, A. M., Lawn, R. M. (1987) cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature* **330(6144)**, 132–137.

Moriarty, P. M., Hemphill, L. (2016) Lipoprotein apheresis. *Endocrinol. Metab. Clin. North Am*. **45**, 39–54.

Nordestgaard, B. G., Langsted, A. (2016) A lipoprotein(a) as a cause of cardiovascular disease: Insights from epidemiology, genetics, and biology. *J. Lipid Res*. **57(11)**, 1953–1975.

Nordestgaard, B. G., Chapman, M. J., Ray, K., Boren, J., Andreotti, F., Watts, G. F., Ginsberg, H., Amarenco, P., Catapano, A., Descamps, O. S., Fisher, E., Kovanen, P. T., Kuivenhoven, J. A., Lesnik, P., Masana, L., Reiner, Z., Taskinen, M. R., Tokgozoglu, L., Tybjaerg-Hansen, A. (2010) Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur. Heart J*. **31**, 2844–2853.

Puckey, L., Knight, B. (1999) Dietary and genetic interactions in the regulation of plasma lipoprotein(a). *Curr. Opin. Lipidol*. **10**, 35–40.

Reblin, T., Niemeier, A., Meyer, N., Willnow, T. E., Kronenberg, F., Dieplinger, H., Greten, H., Beisiegel, U. (1997) Cellular uptake of lipoprotein[a] by mouse embryonic fibroblasts via the LDL receptor and the LDL receptor-related protein. *J. Lipid Res*. **38(10)**, 2103–2110.

Reyes-Soffer, G., Ginsberg, H. N., Ramakrishnan, R. (2017) The metabolism of lipoprotein(a): an ever-evolving story. *J. Lipid Res.* **58**, 1756–1764.

Riches, K., Porter, K. E. (2012) Lipoprotein(a): cellular effects and molecular mechanisms. *Cholesterol* **2012**, 923289.

Riches, K., Franklin, L., Maqbool, A., Peckham, M., Adams, M., Bond, J., Warburton, P., Feric, N. T., Koschinsky, M. L., O'Regan, D. J., Ball, S. G., Turner, N. A., Porter, K. E. (2013) Apolipoprotein(a) acts as a chemorepellent to human vascular smooth muscle cells via integrin αVβ3 and RhoA/ROCK-mediated mechanisms. *Int. J. Biochem. Cell Biol.* **45**, 1776–1783.

Rifai, N., Ma, J., Sacks, F. M., Ridker, P. M., Hernandez, W. J., Stampfer, M. J., Marcovina, S. M. (2004) Apolipoprotein(a) size and lipoprotein(a) concentration and future risk of angina pectoris with evidence of severe coronary atherosclerosis in men. *Clin. Chem.* **50**, 1364–1371.

Riis Hansen, P., Kharazmi, A., Jauhiainen, M., Ehnholm, C. (1994) Induction of oxygen free radical generation in human monocytes by lipoprotein(a). *Eur. J. Clin. Invest.* **24**, 497–499.

Schmidt, K., Noureen, A., Kronenberg, F., Utermann, G. (2016) Structure, function, and genetics of lipoprotein(a). *J. Lipid Res.* **57**, 1339–1359.

Soma, M. R., Osnago-Gadda, I., Paoletti, R., Fumagalli, R., Morrisett, J. D., Meschia, M., Crosignani, P. (1993) The lowering of lipoprotein[a] induced by estrogen plus progesterone replacement therapy in postmenopausal women. *Arch. Intern. Med.* **153**, 1462–1468.

Sotiriou, S. N., Orlova, V. V., Al-Fakhri, N., Ihanus, E., Economopoulou, M., Isermann, B., Bdeir, K., Nawroth, P. P., Preissner, K. T., Gahmberg, C. G., Koschinsky, M. L., Chavakis, T. (2006) Lipoprotein(a) in atherosclerotic plaques recruits inflammatory cells through interaction with Mac-1 integrin. *FASEB J.* **20**, 559–561.

Syrovets, T., Thillet, J., Chapman, M. J., Simmet, T. (1997) Lipoprotein(a) is a potent chemoattractant for human peripheral monocytes. *Blood* **90**, 2027–2036.

Tholstrup, T., Markmann, P., Vessby, B., Sandstorm, B. (1995) Effects of fats high in individual saturated fatty acids on plasma lipoprotein(a) levels in young health men. *J. Lipid Res.* **36**, 1447–1552.

Tsimikas, S. (2017) A test in context: Lipoprotein(a): Diagnosis, prognosis, controversies, and emerging therapies. *J. Am. Coll. Cardiol.* **69**, 692–711.

Tsironis, L. D., Mitsios, J. V., Milionis, H. J., Elisaf, M., Tselepis, A. D. (2004) Effect of lipoprotein(a) on platelet activation induced by platelet-activating factor: role of apolipoprotein(a) and endogenous PAF-acetylhydrolase. *Cardiovasc. Res.* **63(1)**, 130–138.

van Capelleveen, J. C., van der Valk, F. M., Stroes, E. G. (2016) Current therapies for lowering lipoprotein(a). *J. Lipid Res.* **57(9)**, 1612–1618.

 Prague Medical Report / Vol. 120 (2019) No. 2–3, p. 52–63

Pharmacokinetics of Dasatinib

Jana Hořínková, Martin Šíma, Ondřej Slanař
Institute of Pharmacology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Received April 12, 2019; Accepted September 4, 2019.

Key words: Dasatinib – Pharmacokinetics – pH-dependent absorption – Drug interaction

Abstract: Tyrosine kinase inhibitors have recently become an essential tool in management of chronic myeloid leukaemia (CML). Dasatinib, a representative of those drugs, acts by inhibiting key proteins included in CML development, predominantly Bcr-Abl and Src. Its advantage is that it shows activity in many cases where other agents bring no improvement due to resistance. Pharmacokinetics of dasatinib has specific characteristics that may play an important role in achieving sufficient exposure in patients. Therefore, the key pharmacokinetic properties are summarized in this report. For example, dasatinib absorption is significantly influenced by gastric pH and its modulation can be a source of serious interactions, as well as simultaneous administration of drugs affecting cytochrome P450.

This study was supported by the Charles University Project Progres Q25 and a grant no. SVV 260373.

Mailing Address: Mgr. Jana Hořínková, Institute of Pharmacology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Albertov 4, 128 00 Prague 2, Czech Republic; Phone: +420 224 968 035; Fax: +420 224 968 149; e-mail: jana.horinkova@lf1.cuni.cz

https://doi.org/10.14712/23362936.2019.10

Introduction

Possibilities of chronic myeloid leukaemia (CML) therapy have recently been broadening as there are new agents with antitumor activity that can be used to treat this hematologic malignancy. Dasatinib belongs among small molecules inhibiting tyrosine kinases (Cohen, 2002).

The first reports on dasatinib (formerly BMS-354825) in literature reach to 2004 (Lombardo et al., 2004). Two years later, in 2006, it was approved by FDA and EMA (brand name Sprycel®). Indications in adults include Philadelphia chromosome-positive chronic myeloid leukaemia (Ph+ CML) in chronic phase and Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL), it is also approved for treatment of Ph+ CML in chronic phase in paediatric patients (Bristol-Myers Squibb, 2017). Dasatinib shows its benefits in cases where imatinib (Gleevec®, older tyrosine kinase inhibitor firstly approved by FDA in 2001) (Novartis, 2018) fails because of resistance and it has been reported that dasatinib has 325-fold greater activity in inhibiting Bcr-Abl than imatinib (O'Hare et al., 2005). Studies *in vitro* as well as *in vivo* have shown that dasatinib inhibits the kinase activity of 14 out of 15 imatinib-resistant Bcr-Abl isoforms with the only one not responding mutant, T315I (Shah et al., 2004).

The way dasatinib acts against cancer cells is explained by inhibition of several proteins that have their role in cancer pathogenesis as they are employed in cell differentiation, proliferation and survival, most importantly Bcr-Abl, Src, c-kit and PDGFRβ (platelet-derived growth factor receptor β) (Ikeda et al., 1991; Szczylik et al., 1991; Thomas and Brugge, 1997; Lombardo et al., 2004; Yang et al., 2010).

In conducted studies, dasatinib showed variable pharmacokinetic profiles with high intra subject variability (Chandani et al., 2017). This is the reason why the aspects of dasatinib pharmacokinetics are being studied in this review.

Physical/chemical properties

Dasatinib is a white to off-white powder with melting point of 280 to 286 °C (Bristol-Myers Squibb Pharmaceutical Research Institute, 2007). It is a lipophilic substance with partition coefficient (*logP*) between octanol and water 2.71, which means it is able to pass well through cell membranes (Minematsu and Giacomini, 2011).

As a monohydrate, it is insoluble in water (0.008 mg/ml at 24 ± 4 °C), slightly soluble in ethanol, methanol, polyethylene glycol 400 and propylene glycol and very slightly soluble in acetone and acetonitrile (USP definition). It is practically insoluble in corn oil (Bristol-Myers Squibb Pharmaceutical Research Institute, 2007).

Solubility in water is pH dependent. The anhydrous free drug has characteristics of a weak base which is a subject of dissociation when placed into water environment. Two basic ionization constants (pK_a) were determined (6.8 and 3.1) and one weekly acidic pK_a (10.9) (Bristol-Myers Squibb Pharmaceutical Institute, 2007). According to *in vitro* investigation, the solubility surpasses 0.690 mg/ml at pH values lower than

4.0, whereas in higher values it rapidly decreases (0.205 mg/ml at pH 4.28 and less than 0.001 mg/ml at pH 6.99) (Eley et al., 2009).

Another study confirming those findings was performed at the temperature of 37 °C. The results show that the solubility of dasatinib was surprisingly high at pH approximately 1 (49.6 mg/ml), due to full protonation of both basic nitrogen atoms. As expected, the solubility drops down to 3.62 mg/ml at pH 3.64 and it reaches only 1.40 mg/ml at pH 3.81. At higher values dasatinib becomes practically insoluble (Lubach et al., 2013). This fact of pH-dependent solubility plays an important role in absorption from gastrointestinal tract, as the pH values can range variously, and they can be influenced by other concomitantly using drugs.

Pharmacokinetics of dasatinib

Pharmacokinetic parameters of dasatinib are summarized in Table 1.

Absorption

After oral administration, dasatinib is quickly absorbed from gastrointestinal tract. Preclinical testing in various species (mice, rats, dogs and monkeys) showed that maximum plasma concentrations were most frequently reached within 0.6 to 2 h

Table 1 – Pharmacokinetic parameters of dasatinib in preclinical and clinical trials

Parameter	Preclinical data			Clinical data		
T_{max} (h)	0.6–2.0 (Kamath et al., 2008)	1 (Luo et al., 2006)	2 (Lagas et al., 2009)	1.0 (Aplenc et al., 2011)	0.5 (Christopher et al., 2008b)	1.0 (Takahashi et al., 2011)
F (%)	14–34 (Kamath et al., 2008)	45–51 (Luo et al., 2006)	–	–	–	–
Plasma protein binding (%)	92–97 (Kamath et al., 2008)	–	–	94 (Kamath et al., 2008)	–	–
V_{ss} (l/kg)	3.5–6.3 (Kamath et al., 2008)	–	–	–	–	–
V_z/F (l)	–	–	–	606–9113 (Takahashi et al., 2011)	600–9464 (Demetri et al., 2009)	–
$t_{1/2}$ (h)	0.9–4.2 (Kamath et al., 2008)	–	–	1.9–3.6 (Aplenc et al., 2011)	3.6 (Christopher et al., 2008b)	2.2–4.9 (Demetri et al., 2009)

T_{max} – time to reach maximum plasma concentration; F – bioavailability; V_{ss} – apparent volume of distribution at steady state; V_z/F – apparent volume of distribution during terminal phase after non-iv administration; $t_{1/2}$ – half-life

(T_{max} – time to reach maximum plasma concentration) (Luo et al., 2006; Kamath et al., 2008; Lagas et al., 2009). Clinical studies achieved similar results with T_{max} 0.5 to 1.0 h. Among subjects, substantial variability was observed with T_{max} values ranging from 0.28 up to 6.3 h (Christopher et al., 2008b; Aplenc et al., 2011; Takahashi et al., 2011; Bristol-Myers Squibb, 2017).

Bioavailability is known to be variable between subjects, too. First preclinical studies in mice showed bioavailability from 45 to 51% (Luo et al., 2006), whereas other experiments performed in various species led to values between 14 and 34% (14% and 17% mice, 27% rats, 34% dogs, 15.2% monkeys) (Kamath et al., 2008). Bioavailability in humans was not determined because intravenous administration would be risky, but we know that interindividual variability in AUC (area under the curve) can range from 32 to 118% (Dai et al., 2008) and intraindividual variability from 40 to 50% (Chandani et al., 2017).

Experiments, where dasatinib was administered intraportally in rats suggest that first-pass metabolism does not have a significant effect on bioavailability, and it is therefore limited mostly by absorption (Kamath et al., 2008).

Absorption of dasatinib can be influenced by meal, which is taken with the medicine, although the change is not significant. After a single dose of 100 mg, the mean AUC was increased by 14% in subjects with high-fat meal (Bristol-Myers Squibb, 2017).

Another factor impacting dasatinib absorption is gastric pH likely due to alteration of the drug solubility as described above. Dasatinib dissolves better in low pH values, leading to higher amount of drug being absorbed into blood. Gastric pH can be modulated by many substances including medications such as H_2-receptor antagonists (e.g. famotidine, ranitidine), antacids or proton pump inhibitors (e.g. omeprazole, lansoprazole, rabeprazole) which cause increased gastric pH (Pali-Scholl et al., 2010; Mylan Pharmaceuticals, 2011; AstraZeneca Pharmaceuticals, 2012). On the other hand, there are agents that are able to induce gastric acid secretion or by other mechanism decrease gastric pH such as pentagastrin or betaine HCl (Chu et al., 1999; Yago et al., 2013; Šíma et al., 2019).

Effect of pentagastrin (0.25 mg/kg, sc) and famotidine (10 mg/kg, iv) on dasatinib absorption was investigated in a preclinical study in rats. Both substances were administered 2 h prior to dasatinib administration. Unexpectedly, pentagastrin led to a slight decrease in AUC (from 0.421 μg×h/ml in control group to 0.297 μg×h/ml). That could have been caused by rapid onset of pentagastrin action and too early dosing 2 h before dasatinib since the measured gastric pH after pentagastrin administration was the same as in control group and thus it probably stayed without effect on dasatinib absorption. Famotidine had a significant impact with dasatinib AUC decrease to 0.094 μg×h/ml (Lubach et al., 2013).

There was another interaction study in dogs with famotidine (40 mg, orally, 3 h prior to dasatinib), pentagastrin (6 μg/kg, im, 30 min prior to dasatinib) and betaine HCl (750 mg, orally, either 5 min, or 5 and 20 min prior to dasatinib), in which

probably due to administration only 30 min before dasatinib, pentagastrin led to a doubling of dasatinib AUC (measured gastric pH was also lower than in control group). Two tablets of betaine HCl (1,500 mg in total) had the same effect on absorption as pentagastrin while when dasatinib was given with famotidine and petagastrin the negative effect of famotidine was mitigated (Pang et al., 2013).

In humans, the influence of famotidine and antacids (Maalox® – aluminium/magnesium hydroxides) was examined in a study where dasatinib (50 mg) was administered twice a day, famotidine (40 mg) was given 2 h after dasatinib (and therefore 10 h before another dose of dasatinib) and the antacid (30 ml) was given firstly 2 h before and then at the same time as dasatinib. Although famotidine didn't change first dasatinib dose absorption, the next dasatinib dose 10 h after famotidine was absorbed significantly worse (mean AUC decreased from 136 to 40.2 ng×h/ml). Concomitant use of antacid similarly decreased dasatinib exposure (mean AUC of 46.3 ng×h/ml), however, when separated by 2 h, absorption remained unaltered (Eley et al., 2009).

Decrease of dasatinib exposure was also confirmed in a study in which pharmacokinetic profiles of Japanese patients treated with or without H_2-receptor antagonists (famotidine 20-40 mg/day, nizatidine 300 mg/day) or proton pump inhibitors (lansoprazole 30 mg/day) were analysed. Results were variable but acid suppressants caused decrease in the extent of dasatinib absorption (mean AUC decreased from 3.51 to 1.47 ng×h/ml/mg) (Takahashi et al., 2012).

The effect of famotidine on dasatinib absorption has been also indicated in a case report suggesting more than 3fold decrease in dasatinib exposure when given concomitantly (Matsuoka et al., 2012).

Another study conducted in humans receiving dasatinib (100 mg) alone, with rabeprazole (20 mg twice a day) or altogether with betaine HCl (1,500 mg) confirmed that proton pump inhibitors have negative effect on dasatinib absorption. However, betaine HCl mitigated this interaction (Yago et al., 2014).

Table 2 – The effect of gastric pH modulators on dasatinib AUC

Comedication	AUC (% of dasatinib monotherapy AUC)
Pentagastrin	↓ (71%) (Lubach et al., 2013); ↑ (223%) (Pang et al., 2013)
Famotidine	↓ (22%) (Lubach et al., 2013); ↓ (39%) (Eley et al., 2009)
Rabeprazole	↓ (14.7%) (Yago et al., 2014)
Any H2RA or PPI	↓ (42%) (Takahashi et al., 2012)
750 mg betaine HCl	≈ (119%) (Pang et al., 2013)
1,500 mg betaine HCl	↑ (229%) (Pang et al., 2013)
Famotidine + 750 mg betaine HCl	≈ (113%) (Pang et al., 2013)
Famotidine + 1,500 mg betaine HCl	↑ (149%) (Pang et al., 2013)
Rabeprazole + 1,500 mg betaine HCl	≈ (96%) (Yago et al., 2014)
Antacid (2 h prior to dasatinib)	≈ (104%) (Eley et al., 2009)
Antacid (concomitantly with dasatinib)	↓ (45%) (Eley et al., 2009)

AUC – area under the curve

The effect of gastric pH modulators on bioavailability of dasatinib is summarized in Table 2.

To conclude, combination of dasatinib with acid suppressants is problematic due to decreased dasatinib bioavailability. If necessary, it is advisable to use antacids separated from dasatinib at least by 2 h. A wish to increase dasatinib bioavailability lead to an attempt to synthesize a compound that could act like a dasatinib prodrug. Thus, a compound called JLTN was synthesized with oral bioavailability increase to 150% of the original value. However, no additional development of the compound has followed so far (Liu et al., 2013).

Distribution

When absorbed into blood, most of dasatinib molecules bind to serum proteins (>90%). The volume of distribution is very high, suggesting that dasatinib distributes well from vascular system to other tissues. A preclinical study performed in various species came with mean Vd values ranging from 3.5 to 6.3 l/kg and human volume of distribution was predicted to be around 4.2 l/kg (using scaling by body weight) (Kamath et al., 2008). Later experiments in men confirmed high volume of distribution with mean values from 600 to 9,464 l and with large variability. After repeated administration, dasatinib does not show any signs of accumulation in the body (Demetri et al., 2009; Takahashi et al., 2011).

In breast-feeding females, dasatinib, as a basic molecule, reaches high concentrations in milk. First estimations considering only passive diffusion predicted lacteal distribution to be rather mild. However, dasatinib is a substrate of BCRP (breast cancer resistance protein) and since this protein is also expressed in mammary gland, we can suppose that BCRP is employed in active transport to the milk (He et al., 2008).

Although dasatinib crosses placental barrier, foetal plasma concentrations reach lower values than those in maternal blood. Concentrations measured in foetal plasma, brain, kidneys and liver were similar, suggesting that distribution in foetus occurs mostly by passive diffusion without specific transporters, which may not be fully evolved yet (He et al., 2008).

Elimination (metabolism and excretion)

Dasatinib half-life was determined in four different species – mice, rats, dogs and monkeys. Mice exhibited the lowest value (0.9 h), the longest half-life was observed in dogs (4.2 h) (Kamath et al., 2008). Human half-life values based on three clinical studies range from 2.2 to 4.9 h (Christopher et al., 2008b; Demetri et al., 2009; Aplenc et al., 2011).

Dasatinib undergoes several routes of metabolism, particularly oxidative and conjugative. Hydroxylation, N-dealkylation, N-oxidation, alcohol oxidation and direct glucuronide or sulphate conjugation seem to be the most employed reactions, leading to formation of many metabolites of which nineteen have been identified.

Dasatinib represents the major circulating moiety in a mass balance study, whereas metabolites are accountant for 40 to 60% of total radioactivity (Christopher et al., 2008a; Kamath et al., 2008).

Although the most abundant metabolite observed in rats is piperazine-N-oxide (M5), its concentrations in human plasma are low. On the other hand, the most frequent metabolites in monkeys and humans are M20 and M24, the products of hydroxylation. In spite of the fact that all of these compounds hold certain activity, they don't significantly contribute to total dasatinib efficacy, due to their low potency (Christopher et al., 2008a, b).

The bile of duct cannulated rats contains mainly N-oxides and conjugates, while dasatinib and oxidative metabolites other than N-oxides are found in faeces of intact rats. That can be caused by a hydrolysis of conjugates and a reduction of N-oxides by microorganisms in the course of passage through gastrointestinal tract before being excreted (Christopher et al., 2008a).

When various metabolism of dasatinib via CYP enzymes or flavin-containing monooxygenase 3 (FMO3) were tested *in vitro*, it turned out that all of them are able to metabolize dasatinib with CYP3A4 showing to be the most potent enzyme (Kamath et al., 2008).

Dasatinib exposure was increased when coadministred with ketoconazole in man (Johnson et al., 2010). Conversely, rifampicin, a CYP3A4 inducer, led to a decrease in dasatinib exposure, confirming that dasatinib is a CYP3A4 substrate (Bristol-Myers Squibb, 2017). Therefore, it is recommended to avoid simultaneous administration with strong CYP3A4 inhibitors or inducers such as grapefruit juice because of possible drug interactions. If necessary, dasatinib doses can be modified in order to maintain adequate plasma concentrations. Concomitant administration with other CYP3A4 substrates should be employed with caution as dasatinib itself acts as a CYP3A4 inhibitor (Bristol-Myers Squibb, 2006).

Dasatinib is mainly excreted in the form of metabolites, as only 15 to 19% remains unchanged. The excretion occurs into faeces (particularly bile), the amount of drug being excreted in urine is very low (Christopher et al., 2008a, b; Kamath et al., 2008).

Impact of drug transporters

The role of dasatinib transport through cell membranes has been investigated in order to map possible influence of some efflux proteins and transporters on dasatinib distribution. Unlike imatinib, dasatinib cell uptake isn't dependent on the activity of human organic cation transporter 1 (hOCT1) (Giannoudis et al., 2008; Hiwase et al., 2008). This transporter was found to be important for imatinib active transport into cells and its lower expression can contribute to treatment resistance (Thomas et al., 2004).

ATP-binding cassette transporters (ABC transporters) have major importance on dasatinib influx/efflux. For example, ABCC4 participates in its gastric absorption

(Furmanski et al., 2013). There are other important transporters belonging to this family, like ABCB1 (P-glycoprotein – P-gp) and ABCG2 (breast cancer resistance protein – BCRP). Those proteins are expressed at various barriers, such as intestinal epithelium and blood-brain barrier or blood-testis barrier, where they are able to transport endogenous substances as well as xenobiotics through membranes. Sometimes, their efflux function can cause drug resistance as they prevent the drug to reach its intracellular target (Borst and Elferink, 2002).

Dasatinib may be transported by both of these proteins (Giannoudis et al., 2008; Hiwase et al., 2008; Chen et al., 2009; Hegedus et al., 2009). First findings comparing wild-type and P-gp knockout mice suggested that although dasatinib is a P-gp substrate, it doesn't contribute to low bioavailability (Kamath et al., 2008). Nevertheless, later investigations brought results saying that P-gp can limit dasatinib absorption after oral administration (Lagas et al., 2009). The importance of both transporters (P-gp and BCRP) is supported by results of a study with double knocked-out rats for P-gp and BCRP, which had two-fold higher dasatinib AUC compared with wild-type rats (Tang et al., 2013). Dasatinib can also act as an inhibitor of both of these proteins in higher concentrations and thus it could influence transport of another substances (Hegedus et al., 2009). Nevertheless, the practical impact of those findings on a clinical use in patients with CML has not yet been reliably elucidated.

P-gp also plays a major role in restricting dasatinib accumulation in central nervous system. Although absence of BCRP didn't affect dasatinib brain concentration, inhibition of both these transporters together resulted in considerably higher brain concentration compared to inhibition of only P-gp (Chen et al., 2009; Lagas et al., 2009; Tang et al., 2013). Possible explanations are that P-gp might be able to compensate BCRP loss, or that BCRP can partly take over P-gp's function in its absence.

Pharmacokinetics in special populations

Pharmacokinetic properties of dasatinib were also studied in paediatric settings. Overally, the pharmacokinetics is very similar to that observed in adults and there were no substantial differences. The doses should be reduced and adjusted by body weight or by occurred adverse reactions (Aplenc et al., 2011; Bristol-Myers Squibb, 2017).

Although dasatinib is metabolised through liver, it is not recommended to reduce the dose in patients with mild to moderate hepatic dysfunction (Bristol-Myers Squibb, 2006; Sasaki et al., 2016).

Relationship of pharmacokinetics and pharmacodynamics

Dasatinib exhibits time-dependent effect where plasma concentrations above inhibitory concentration ($IC50_{CD34+cells}$) for more than 12.8 h led to a better clinical response (Ishida et al., 2016). Dasatinib shows many adverse effects like

thrombocytopenia, neutropenia, leucopenia, anaemia, asthenia, pleural effusion, fatigue, nausea or diarrhoea (Visani et al., 2010). The toxicity increases with higher plasma concentrations and so it can be useful to monitor plasma levels of dasatinib in patients in order to prevent serious side effects, particularly in patients with decreased clearance (Demetri et al., 2009).

Conclusion

Dasatinib is a drug with an important role in the management of CML. Its absorption is strongly dependent on gastric pH as it only dissolves at low pH values. For that reason, it is important to pay attention to concomitant use of medications that could modulate gastric pH (e.g. antacids, H_2-receptor antagonists, proton pump inhibitors). After being absorbed into circulatory system, dasatinib binds to plasma proteins to a high degree and it is well distributed to the organs as well as to the breast milk of lactating females. Before being excreted mostly by faeces, dasatinib undergoes predominantly oxidative metabolism mediated by cytochrome P450 and inhibitors or inducers of this enzymatic system can alter dasatinib pharmacokinetics.

References

Aplenc, R., Blaney, S. M., Strauss, L. C., Balis, F. M., Shusterman, S., Ingle, A. M., Agrawal, S., Sun, J., Wright, J. J., Adamson, P. C. (2011) Pediatric phase I trial and pharmacokinetic study of dasatinib: A report from the children's oncology group phase I consortium. *J. Clin. Oncol*. **29**, 839–844.

AstraZeneca Pharmaceuticals (2012) Prilosec – Full prescribing information. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/019810s096lbl.pdf

Borst, P., Elferink, R. O. (2002) Mammalian ABC transporters in health and disease. *Annu. Rev. Biochem*. **71**, 537–592.

Bristol-Myers Squibb (2006) Sprycel: Summary of product characteristics. Available at: https://www.ema.europa.eu/en/documents/product-information/sprycel-epar-product-information_en.pdf

Bristol-Myers Squibb (2017) Sprycel: Full prescribing information. Available at: https://packageinserts.bms.com/pi/pi_sprycel.pdf

Bristol-Myers Squibb Pharmaceutical Research Institute (2007) Investigator brochure: Dasatinib (BMS-354825). Available at: http://spirit-cml.org/isf/referencesafetyinformation/1.2%20Dasatinib%20RSI%20-%20Dasatinib%20IB%20(Nov07)%20superseded.pdf

Chandani, R., He, J., Trabelsi, F. (2017) Atypical pharmacokinetic profiles observed with dasatinib reference listed drug product in bioequivalence studies. *Presented at the AAPS Annual Meeting*, San Diego. Available at: https://www.biopharmaservices.com/wp-content/uploads/2018/06/Poster-1-Dasatinib-final-AAPS-2017.pdf

Chen, Y., Agarwal, S., Shaik, N. M., Chen, C., Yang, Z., Elmquist, W. F. (2009) P-glycoprotein and breast cancer resistance protein influence brain distribution of dasatinib. *J. Pharmacol. Exp. Ther*. **330**, 956–963.

Christopher, L. J., Cui, D., Li, W., Barros, A. Jr., Arora, V. K., Zhang, H., Wang, L., Zhang, D., Manning, J. A., He, K., Fletcher, A. M., Ogan, M., Lago, M., Bonacorsi, S. J., Humphreys, W. G., Iyer, R. A. (2008a) Biotransformation of [14C]dasatinib: *in vitro* studies in rat, monkey, and human and disposition after administration to rats and monkeys. *Drug Metab. Dispos*. **36**, 1341–1356.

Christopher, L. J., Cui, D., Wu, C., Luo, R., Manning, J. A., Bonacorsi, S. J., Lago, M., Allentoff, A., Lee, F. Y., McCann, B., Galbraith, S., Reitberg, D. P., He, K., Barros, A. Jr., Blackwood-Chirchir, A.,

Humphreys, W. G., Iyer, R. A. (2008b) Metabolism and disposition of dasatinib after oral administration to humans. *Drug Metab. Dispos*. **36**, 1357–1364.

Chu, S., Tanaka, S., Kaunitz, J. D., Montrose, M. H. (1999) Dynamic regulation of gastric surface pH by luminal pH. *J. Clin. Invest*. **103**, 605–612.

Cohen, P. (2002) Protein kinases – The major drug targets of the twenty-first century? *Nat. Rev. Drug Discov*. **1**, 309–315.

Dai, G., Pfister, M., Blackwood-Chirchir, A., Roy, A. (2008) Importance of characterizing determinants of variability in exposure: Application to dasatinib in subjects with chronic myeloid leukemia. *J. Clin. Pharmacol*. **48**, 1254–1269.

Demetri, G. D., Lo Russo, P., MacPherson, I. R., Wang, D., Morgan, J. A., Brunton, V. G., Paliwal, P., Agrawal, S., Voi, M., Evans, T. R. (2009) Phase I dose-escalation and pharmacokinetic study of dasatinib in patients with advanced solid tumors. *Clin. Cancer Res*. **15**, 6232–6240.

Eley, T., Luo, F. R., Agrawal, S., Sanil, A., Manning, J., Li, T., Blackwood-Chirchir, A., Bertz, R. (2009) Phase I study of the effect of gastric acid pH modulators on the bioavailability of oral dasatinib in healthy subjects. *J. Clin. Pharmacol*. **49**, 700–709.

Furmanski, B. D., Hu, S., Fujita, K. I., Li, L., Gibson, A. A., Janke, L. J., Williams, R. T., Schuetz, J. D., Sparreboom, A., Baker, S. D. (2013) Contribution of ABCC4-mediated gastric transport to the absorption and efficacy of dasatinib. *Clin. Cancer Res*. **19**, 4359–4370.

Giannoudis, A., Davies, A., Lucas, C. M., Harris, R. J., Pirmohamed, M., Clark, R. E. (2008) Effective dasatinib uptake may occur without human organic cation transporter 1 (hOCT1): Implications for the treatment of imatinib-resistant chronic myeloid leukemia. *Blood* **112**, 3348–3354.

He, K., Lago, M. W., Iyer, R. A., Shyu, W. C., Humphreys, W. G., Christopher, L. J. (2008) Lacteal secretion, fetal and maternal tissue distribution of dasatinib in rats. *Drug Metab. Dispos*. **36**, 2564–2570.

Hegedus, C., Ozvegy-Laczka, C., Apati, A., Magocsi, M., Nemet, K., Orfi, L., Keri, G., Katona, M., Takats, Z., Varadi, A., Szakacs, G., Sarkadi, B. (2009) Interaction of nilotinib, dasatinib and bosutinib with ABCB1 and ABCG2: Implications for altered anti-cancer effects and pharmacological properties. *Br. J. Pharmacol*. **158**, 1153–1164.

Hiwase, D. K., Saunders, V., Hewett, D., Frede, A., Zrim, S., Dang, P., Eadie, L., To, L. B., Melo, J., Kumar, S., Hughes, T. P., White, D. L. (2008) Dasatinib cellular uptake and efflux in chronic myeloid leukemia cells: therapeutic implications. *Clin. Cancer Res*. **14**, 3881–3888.

Ikeda, H., Kanakura, Y., Tamaki, T., Kuriu, A., Kitayama, H., Ishikawa, J., Kanayama, Y., Yonezawa, T., Tarui, S., Griffin, J. D. (1991) Expression and functional role of the proto-oncogene c-kit in acute myeloblastic leukemia cells. *Blood* **78**, 2962–2968.

Ishida, Y., Murai, K., Yamaguchi, K., Miyagishima, T., Shindo, M., Ogawa, K., Nagashima, T., Sato, S., Watanabe, R., Yamamoto, S., Hirose, T., Saitou, S., Yonezumi, M., Kondo, T., Kato, Y., Mochizuki, N., Ohno, K., Kishino, S., Kubo, K., Oyake, T., Ito, S. (2016) Pharmacokinetics and pharmacodynamics of dasatinib in the chronic phase of newly diagnosed chronic myeloid leukemia. *Eur. J. Clin. Pharmacol*. **72**, 185–193.

Johnson, F. M., Agrawal, S., Burris, H., Rosen, L., Dhillon, N., Hong, D., Blackwood-Chirchir, A., Luo, F. R., Sy, O., Kaul, S., Chiappori, A. A. (2010) Phase 1 pharmacokinetic and drug-interaction study of dasatinib in patients with advanced solid tumors. *Cancer* **116**, 1582–1591.

Kamath, A. V., Wang, J., Lee, F. Y., Marathe, P. H. (2008) Preclinical pharmacokinetics and *in vitro* metabolism of dasatinib (BMS-354825): A potent oral multi-targeted kinase inhibitor against SRC and BCR-ABL. *Cancer Chemother. Pharmacol*. **61**, 365–376.

Lagas, J. S., van Waterschoot, R. A., van Tilburg, V. A., Hillebrand, M. J., Lankheet, N., Rosing, H., Beijnen, J. H., Schinkel, A. H. (2009) Brain accumulation of dasatinib is restricted by P-glycoprotein

(ABCB1) and breast cancer resistance protein (ABCG2) and can be enhanced by elacridar treatment. *Clin. Cancer Res*. **15**, 2344–2351.

Liu, F., Lang, L. W., Jiang, J., Lu, H. J., Wang, J. M., Wang, S. C. (2013) Synthesis and biopharmaceutical studies of JLTN as potential dasatinib prodrug. *Chem. Pharm. Bull. (Tokyo)* **61**, 877–881.

Lombardo, L. J., Lee, F. Y., Chen, P., Norris, D., Barrish, J. C., Behnia, K., Castaneda, S., Cornelius, L. A., Das, J., Doweyko, A. M., Fairchild, C., Hunt, J. T., Inigo, I., Johnston, K., Kamath, A., Kan, D., Klei, H., Marathe, P., Pang, S., Peterson, R., Pitt, S., Schieven, G. L., Schmidt, R. J., Tokarski, J., Wen, M. L., Wityak, J., Borzilleri, R. M. (2004) Discovery of N-(2-chloro-6-methyl-phenyl)-2-(6-(4-(2-hydroxyethyl) -piperazin-1-yl)-2-methylpyrimidin-4-ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J. Med. Chem*. **47**, 6658–6661.

Lubach, J. W., Chen, J. Z., Hau, J., Imperio, J., Coraggio, M., Liu, L., Wong, H. (2013) Investigation of the rat model for preclinical evaluation of pH-dependent oral absorption in humans. *Mol. Pharm*. **10**, 3997–4004.

Luo, F. R., Yang, Z., Camuso, A., Smykla, R., McGlinchey, K., Fager, K., Flefleh, C., Castaneda, S., Inigo, I., Kan, D., Wen, M. L., Kramer, R., Blackwood-Chirchir, A., Lee, F. Y. (2006) Dasatinib (BMS-354825) pharmacokinetics and pharmacodynamic biomarkers in animal models predict optimal clinical exposure. *Clin. Cancer Res*. **12**, 7180–7186.

Matsuoka, A., Takahashi, N., Miura, M., Niioka, T., Kawakami, K., Matsunaga, T., Sawada, K. (2012) H2-receptor antagonist influences dasatinib pharmacokinetics in a patient with Philadelphia-positive acute lymphoblastic leukemia. *Cancer Chemother. Pharmacol*. **70**, 351–352.

Minematsu, T., Giacomini, K. M. (2011) Interactions of tyrosine kinase inhibitors with organic cation transporters and multidrug and toxic compound extrusion proteins. *Mol. Cancer Ther*. **10**, 531–539.

Mylan Pharmaceuticals (2011) Mylan-famotidine – Product monograph. Available at: https://pdf.hres.ca /dpd_pm/00014548.PDF

Novartis (2018) Gleevec: Full prescribing information. Available at: https://www.pharma.us.novartis.com /sites/www.pharma.us.novartis.com/files/gleevec_tabs.pdf

O'Hare, T., Walters, D. K., Stoffregen, E. P., Jia, T., Manley, P. W., Mestan, J., Cowan-Jacob, S. W., Lee, F. Y., Heinrich, M. C., Deininger, M. W., Druker, B. J. (2005) *In vitro* activity of Bcr-Abl inhibitors AMN107 and BMS-354825 against clinically relevant imatinib-resistant Abl kinase domain mutants. *Cancer Res*. **65**, 4500–4505.

Pali-Scholl, I., Herzog, R., Wallmann, J., Szalai, K., Brunner, R., Lukschal, A., Karagiannis, P., Diesner, S. C., Jensen-Jarolim, E. (2010) Antacids and dietary supplements with an influence on the gastric pH increase the risk for food sensitization. *Clin. Exp. Allergy* **40**, 1091–1098.

Pang, J., Dalziel, G., Dean, B., Ware, J. A., Salphati, L. (2013) Pharmacokinetics and absorption of the anticancer agents dasatinib and GDC-0941 under various gastric conditions in dogs – Reversing the effect of elevated gastric pH with betaine HCl. *Mol. Pharm*. **10**, 4024–4031.

Sasaki, K., Lahoti, A., Jabbour, E., Jain, P., Pierce, S., Borthakur, G., Daver, N., Kadia, T., Pemmaraju, N., Ferrajoli, A., O'Brien, S., Kantarjian, H., Cortes, J. (2016) Clinical safety and efficacy of nilotinib or dasatinib in patients with newly diagnosed chronic-phase chronic myelogenous leukemia and pre-existing liver and/or renal dysfunction. *Clin. Lymphoma Myeloma Leuk*. **16**, 152–162.

Shah, N. P., Tran, C., Lee, F. Y., Chen, P., Norris, D., Sawyers, C. L. (2004) Overriding imatinib resistance with a novel ABL kinase inhibitor. *Science* **305**, 399–401.

Šíma, M., Kutinová-Canová, N., Ryšánek, P., Hořínková, J., Moškořová, D., Slanař, O. (2019) Gastric pH in rats: Key determinant for preclinical evaluation of pH-dependent oral drug absorption. *Prague Med. Rep*. **120**, 5–9.

Szczylik, C., Skorski, T., Nicolaides, N. C., Manzella, L., Malaguarnera, L., Venturelli, D., Gewirtz, A. M., Calabretta, B. (1991) Selective inhibition of leukemia cell proliferation by BCR-ABL antisense oligodeoxynucleotides. *Science* **253**, 562–565.

Takahashi, N., Miura, M., Niioka, T., Sawada, K. (2012) Influence of H2-receptor antagonists and proton pump inhibitors on dasatinib pharmacokinetics in Japanese leukemia patients. *Cancer Chemother. Pharmacol*. **69**, 999–1004.

Takahashi, S., Miyazaki, M., Okamoto, I., Ito, Y., Ueda, K., Seriu, T., Nakagawa, K., Hatake, K. (2011) Phase I study of dasatinib (BMS-354825) in Japanese patients with solid tumors. *Cancer Sci*. **102**, 2058–2064.

Tang, S. C., de Vries, N., Sparidans, R. W., Wagenaar, E., Beijnen, J. H., Schinkel, A. H. (2013) Impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) gene dosage on plasma pharmacokinetics and brain accumulation of dasatinib, sorafenib, and sunitinib. *J. Pharmacol. Exp. Ther*. **346**, 486–494.

Thomas, J., Wang, L., Clark, R. E., Pirmohamed, M. (2004) Active transport of imatinib into and out of cells: implications for drug resistance. *Blood* **104**, 3739–3745.

Thomas, S. M., Brugge, J. S. (1997) Cellular functions regulated by Src family kinases. *Annu. Rev. Cell Dev. Biol*. **13**, 513–609.

Visani, G., Breccia, M., Gozzini, A., Specchia, G., Montefusco, E., Morra, E., Annunziata, M., Camera, A., Cavazzini, F., Stagno, F., Pregno, P., Usala, E., Santini, V., Piccaluga, P. P., Isidori, A. (2010) Dasatinib, even at low doses, is an effective second-line therapy for chronic myeloid leukemia patients resistant or intolerant to imatinib. Results from a real life-based Italian multicenter retrospective study on 114 patients. *Am. J. Hematol*. **85**, 960–963.

Yago, M. R., Frymoyer, A. R., Smelick, G. S., Frassetto, L. A., Budha, N. R., Dresser, M. J., Ware, J. A., Benet, L. Z. (2013) Gastric reacidification with betaine HCl in healthy volunteers with rabeprazole-induced hypochlorhydria. *Mol. Pharm*. **10**, 4032–4037.

Yago, M. R., Frymoyer, A., Benet, L. Z., Smelick, G. S., Frassetto, L. A., Ding, X., Dean, B., Salphati, L., Budha, N., Jin, J. Y., Dresser, M. J., Ware, J. A. (2014) The use of betaine HCl to enhance dasatinib absorption in healthy volunteers with rabeprazole-induced hypochlorhydria. *AAPS J*. **16**, 1358–1365.

Yang, J., Liu, X., Nyland, S. B., Zhang, R., Ryland, L. K., Broeg, K., Baab, K. T., Jarbadan, N. R., Irby, R., Loughran, T. P. Jr. (2010) Platelet-derived growth factor mediates survival of leukemic large granular lymphocytes via an autocrine regulatory pathway. *Blood* **115**, 51–60.

 Prague Medical Report / Vol. 120 (2019) No. 2–3, p. 64–73

Evaluation of Complications Following a Trans-masseteric Antero-parotid Approach for Patients with Sub-condylar Fractures of Their Temporomandibular Joint. A Retrospective Study

Vladimír Machoň, Avni Desai, Jitka Levorová, Dušan Hirjak, Eitan Brizman, René Foltán

Department of Dental Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Received February 17, 2019; Accepted September 4, 2019.

Key words: Sub-condylar fractures – Mandible – Trans-masseteric antero-parotid approach

Abstract: Sub-condylar fractures of the temporomandibular joint can be treated by an extraoral or intraoral approach. Trans-masseteric antero-parotid approach (TMAP) is an extraoral approach utilising a retromandibular incision. The authors evaluated patients' status and any complications of using TMAP from the years 2013–2017. There were 39 patients (44 fractures). When using TMAP, in 43 fractures the fragments were favourably positioned, in one case the position was compromised. Of the complications, postoperative palsy of the facial nerve was reported 6.8% – in all cases this was only temporary. Late occlusion had an equal number of complications (in 2 cases this was as a result of an infectious complication of the wound, and in 2 cases due to resorption of the proximal fragment). Muscular pain and dysfunction of the temporomandibular joint following trauma were observed consistently in 6.8% of patients. Sialocoele, a non-conforming scar, and infectious complications were observed in 4.5% of patients. TMAP allows rapid surgical performance, with a good view for perfect repositioning and fixation of fragments of sub-condylar fractures of the temporomandibular joint. The complications associated with this approach are, for the most part, temporary, the aesthetic handicap of a scar is considered by patients to be acceptable. Overall, it is possible to evaluate retromandibular TMAP as safe, and the authors recommended it for treatment of sub-condylar fractures of the mandible.

Mailing Address: Vladimír Machoň, MD., Department of Dental Medicine, First Faculty of Medicine, Charles University and General University Hospital in Prague, U Nemocnice 2, 120 00 Prague 2, Czech Republic; e-mail: machonv@seznam.cz

https://doi.org/10.14712/23362936.2019.11

Introduction

Temporomandibular joint fractures belong to the most common fractures of the facial skeleton, forming 25 to 35% of all fractures of the lower jaw. They can be classified as fractures of the articular head (intra-articular), the articular neck, and sub-condylar fractures. Treatment of joint fractures can be conservative (non-surgical) or surgical. Conservative treatment comprises of a soft diet, temporary intermaxillary fixation and rehabilitation of mouth opening. The principle of surgical treatment consists of open reduction of the fragments into their anatomical position and then stable fixation by osteosynthesis (ORIF). Access for open reduction and internal fixation may include, pre-auricular, end-aural, posterior-auricular, retromandibular, subangular, submandibular and intraoral approaches (Eckelt and Loukota, 2010; Leiser et al., 2013; Spinzia et al., 2014).

The authors have evaluated the use of a retromandibular trans-masseteric antero-parotid approach in the surgical treatment of sub-condylar fractures.

Material and Methods

The group consisted of patients with sub-condylar fractures of their joints, treated by open reduction and internal fixation between January 2013 and May 2017, all by a retromandibular trans-masseteric antero-parotid approach (TMAP).

There was a total of 44 fractures in 39 patients (22 men and 17 women) with an average age of 39.5 years (age 18 to 73 years). Four patients had bilateral sub-condylar fractures together with fractures of their mandible, one case was a bilateral sub-condylar fracture, 9 patients had unilateral sub-condylar fractures together with a fracture of their mandible, 2 patients had sub-condylar fractures along with a mid-face fracture. 23 patients had isolated sub-condylar fractures. In two cases the sub-condylar fracture was comminuted.

In 15 cases, the proximal fragment was displaced medially, in 22 cases the mandible was shortened, with lateral displacement of the proximal fragment, in 4 cases the mandible had medial displacement of the proximal fragment and was truncated, in 2 cases the proximal fragment was dislocated in front of the articular prominence.

Aetiological factors contributing to the development of the sub-condylar fractures included, falling onto their face (21 patients), assault (11 patients), bicycle accident (4 patients), one fracture from a car crash, one head clash whilst playing football, one blow from a dog's head.

All patients were examined and treated by the same surgical team at First Faculty of Medicine, Charles University and General University Hospital in Prague.

The aim of this work was to evaluate the complications related to this relatively rarely used surgical approach.

Operative technique (Figures 1–5)

All operations were performed under general anaesthetic via nasotracheal intubation.

Transmasseter Anteroparotideal Approach

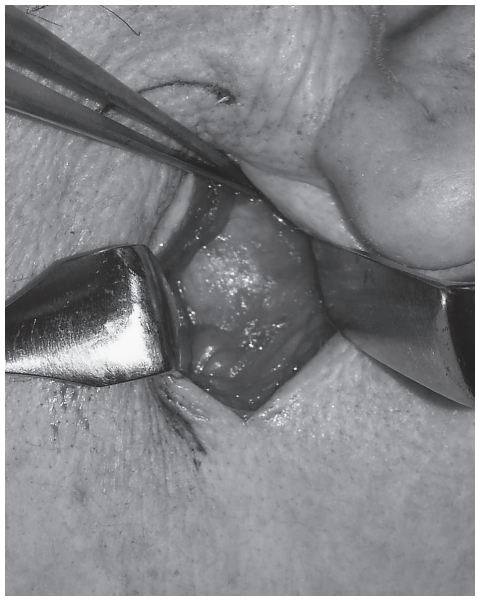

Figure 1 – Retromandibular incision.

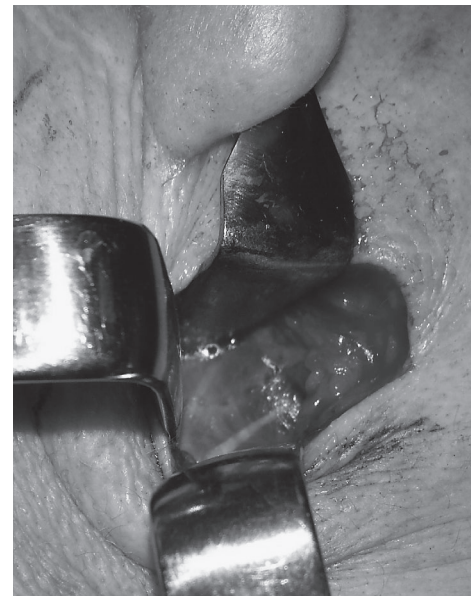

Figure 2 – Masseter muscle and facial nerve branches.

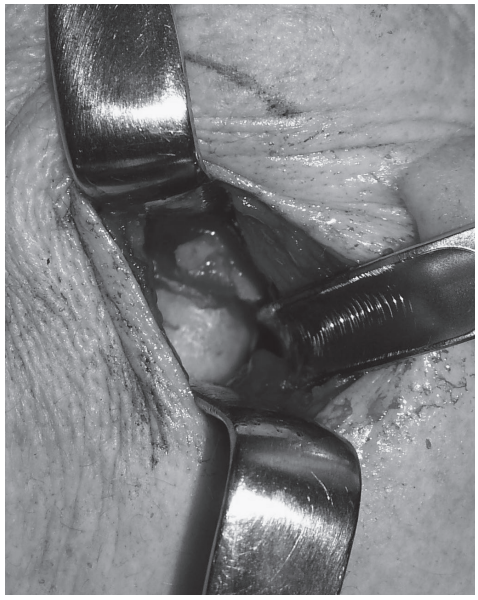

Figure 3 – Reposition of fracture.

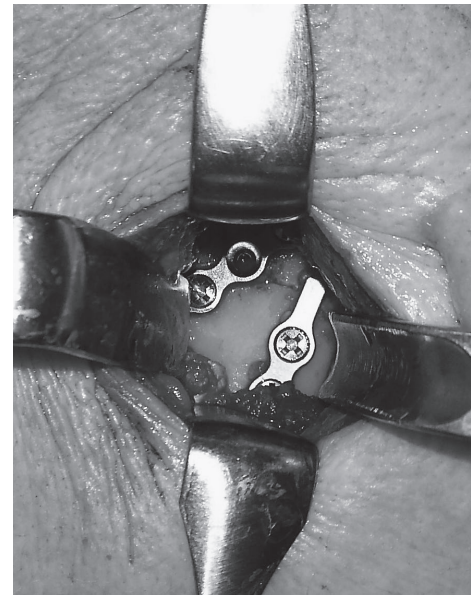

Figure 4 – Osteosynthesis with two miniplates.

The operation began with the placement of IMF (intermaxillary fixation) screws (3 into each jaw) followed by inter-maxillary fixation with elastic bands (between the opposing MMF – mandibulo-maxillary fixation screws and then crossed between

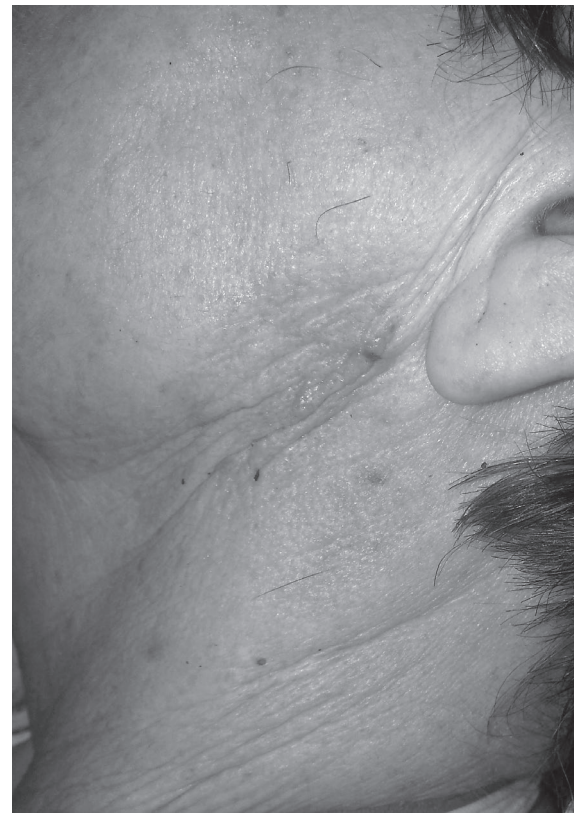

Figure 5 – Retromandibular incision, 2 months after surgery.

the screws). Next a 2-cm vertical retromandibular skin incision was done – behind the edge of the mandible, revealing the capsule of the parotid gland. Blunt dissection superficial to the capsule to expose the anterior edge of the gland, which was then retracted posteriorly. The masseter muscle was then penetrated (between visualized branches of the facial nerve) leading to the external surface of the mandible, which would be chased to the location of the sub-condylar fracture. DePuy Synthes osteosynthesis material (Johnson and Johnson, USA) was used to fix the fragments utilising: 2 straight plates (25 fractures), a trapezoidal plate (TCP – 12 fractures), an L-shaped plate (in 7 fractures).

After fixation of the fracture and irrigation of the surgical wound, a suction drain was sutured in place for all cases, followed by closure of the wound – sutured in layers (Wilson et al., 2005; Biglioli and Colletti, 2008; Eckelt and Loukota, 2010).

Finally, the elastic intermaxillary fixation was removed and the occlusion was confirmed. Simple elastic fixation was left on for patients with complex (multiple) fractures for 10–14 days, as well as for patients with comminuted fractures. In patients with isolated sub-condylar fractures, the intermaxillary fixation was removed at the end of the operation.

Postoperative care

Drains were removed 24 hours after surgery. A mixed diet was recommended for patients for the first 4 weeks, and a soft diet from the 4th to 6th week. Antibiotics were prescribed for the first week after surgery (Co-Amoxiclav, or for patients who were allergic to penicillin – Clindamycin).

For the 1st week after surgery, patients were recommended to open their mouths as little as possible. From the next week they were advised – to gradually and increasingly open, until it starts to become painful. Intense rehabilitation began if limited opening (less than 30 mm) was seen in the 5th postoperative week. In cases of facial nerve weakness, facial functional rehabilitation started from the 1st postoperative day.

Patient check-up

10 to 14 days after surgery (for removal of stitches), then one month, 3 months, 6 months, and 1 year after surgery. At each check-up, an assessment of the patients' mouth-opening, presence of pain, surgical wound condition, facial nerve function, temporomandibular joint (TMJ) function and occlusion status were made. Radiographic examination from 2 different projections was performed on the 2nd postoperative day and then at 3 months and 12 months after surgery. From 2015, cone beam CT (CBCT) was routinely used instead of plain-film (digital) X-rays.

Results

Upon clinical examination, correct occlusion was achieved for all patients immediately following surgery. However, according to the X-ray or CBCT images, on the 2nd postoperative day one case did not appear to have an ideal proximal fragment position (but as their occlusion was functional, there was no indication for further reposition). In all other cases, though, replication of the ideal position for the proximal fragment was achieved.

The average operating time from the start of IMF screw insertion until completion of suturing of the surgical wound was 56.34 minutes.

Pain rating: one month after surgery, pain was present in 3 patients (VAS > 2), pain was not otherwise noted in any other patients.

Assessment of mouth-opening: one month after surgery, 9 patients experienced mouth opening of less than 30 mm, by 6 weeks after surgery the mouth-opening of all patients had improved to more than 35 mm, as was noted in subsequent inspections.

Postoperative complications

- Facial nerve dysfunction was observed after surgery in 3 fractures (6.8%), all of these were resolved 1 week after surgery. Permanent dysfunction did not occur. Resolution always occurred after targeted facial nerve functional rehabilitation.

- Sialocoele was observed in 2 fractures (4.5%). This was resolved by repetitive drainage, leading to gradual resolution within 2 weeks.
- Masseter muscle pain – present in 3 fractures (6.8%), in all cases this was resolved with targeted relaxation massage, thermotherapy (application of dry heat 3× daily for 5 minutes). All patients improved their condition, eliminating muscle pain within 2 months of their surgery.
- Inflammatory complications at the surgical site were observed in 2 fractures – associated with redness, swelling and laboratory-proven inflammatory markers. In both cases, the complications were treated with surgical wound drainage and prolonged use of antibiotics. The patients' intermaxillary fixation were reinstated (for the duration of inflammation).
- "Increased" scarring was present after the treatment of 2 fractures, both were the cases when the patients had been treated for inflammatory complications.
- Malocclusion was observed postoperatively in 4 fractures (9%). In one case, there was non-union of the proximal fragment, in one case progressive resorption of the proximal fragment occurred (the patient began to see a change in their bite 3 months after the trauma). In 2 cases, the osteosynthetic material got loosen due to an inflammatory process. This subsequently led to dislocation of the proximal fragment. In 2 cases the situation was resolved by total joint replacement, in the remaining 2 cases the patients refused further surgery.
- TMJ dysfunction in the sense of pain-free clicking (locked closed), was reported post-operatively after 3 fractures (6.8%). It was addressed by TMJ physiotherapy.

Discussion

Low sub-condylar fractures usually require an external (submandibular, subangular, retromandibular) approach, or an intraoral (endoscopically assisted) approach. The choice of access to the fracture is determined by operative experience, and available equipment – especially the specific instruments required for an intraoral endoscopically assisted approach (Eckelt and Loukota, 2010).

The use of an external approach (versus an intraoral approach) brings with it a large number of possible complications, including dysfunction of the facial nerve, sialocoele, salivary fistula, Frey's syndrome and, last but not least, the presence of a postoperative scar. Risk of haematoma, infectious complications, or fragment malunion are equally common for both approaches (Eckelt and Loukota, 2010; Al-Moraissi et al., 2018; Rozeboom et al., 2018).

The retromandibular approach was first described in 1967 as an external approach to vertical sub-condylar osteotomy (Hinds and Girotti, 1967), and was subsequently used as an approach for the treatment of sub-condylar fractures. The length of cut in the retromandibular approach may vary. The cut is guided by the jaw edge under the earlobe (Eckelt and Loukota, 2010), and the incision can be extended pre-auricularly (Wilson et al., 2005; Salgarelli et al., 2013) or to the posterior auricular region (Choi, 2015). The authors have only utilised the retromandibular acces for TMAP

as published by Biglioli and Colletti (2008). This approach enabled the authors to have an adequate operative field for repositioning and fixation of the fragments; in the authors' work, an unsatisfactory anatomical reduction only occurred perioperatively in one case.

Retromandibular access can be gained in three ways – trans-masseteric antero-parotid, trans-masseteric subcutaneous or the most commonly used – trans-parotid approach (Eckelt and Loukota, 2010; Al-Moraissi et al., 2018; Bruneau et al., 2018; Rozeboom et al., 2018). According to a review published by Rozeboom et al. (2018) (70 studies, 2,783 patients), 59.4% used the trans-parotid retromandibular approach and 12.5% a non-trans-parotid approach.

The most frequent risk of external approach to TMJ fractures is injury to the facial nerve – which is located directly within the operative field. Al-Moraissi et al. (2018), in the systemic review, evaluated facial nerve injuries in 96 studies (a total of 3,873 patients with articular fractures); the incidence of temporary hypofunction of the facial nerve was between 0 and 19%, with 0.3–2.2% reporting persistent hypofunction of the facial nerve. In the review of Al-Moraissi et al. (2018), the risk of temporary facial nerve injury in sub-condylar fractures accessed from a retromandibular incision using a trans-parotid approach with facial nerve preparation was 11.8%, using a trans-parotid approach without nerve preparation 10.5%, trans-masseteric antero-parotid approach 3.3%, and in a trans-masseteric antero-parotid approach extending pre-auricularly 2.3%. As the branches of the facial nerve are well visualized within the operative field, transient paresis is most often caused by postoperative swelling or retraction of the nerve perioperatively (Wilson et al., 2005; Eckelt and Loukota, 2010). Kanno et al. (2016) showed a significantly higher risk of transient paralysis of the facial nerve in dislocated fractures. Only TMAP was used in the authors' work, and facial nerve hypofunction was recorded in 3 cases (6.8%), with hypofunction in all cases temporary, and complete function restored within 1 week. The higher percentage of temporary facial nerve palsy may be related to the fact that a mini-retromandibular approach was used in all cases, necessitating a greater pressure by retractors on the surrounding tissues (including the branches of the facial nerve).

Other postoperative complications are related to the parotid gland. Rozeboom et al. (2018) presented a review of the risk of sialocoele as 2.33% and salivary fistula as 4.3% when using external access, these risks being mainly associated with the trans-parotid approach. For the antero-parotid trans-masseteric approach, this complication is referred to as zero by a series of authors (Wilson et al., 2005; Trost et al., 2009; Narayanan et al., 2012; Leiser et al., 2013; Salgarelli et al., 2013), as access by careful preparation occurs superficially to the glandular capsule. However, even during this preparation, the integrity of the capsule may be compromised. In the authors' work, sialocoele was a postoperative complication in 2 patients (4.5%).

28.6% of patients in the review by Rozeboom et al. (2018) suffer from the postoperative complication of an (aesthetically) unsatisfactory scar. In the authors'

work, an unsatisfactory scar was reported in 2 patients (4.5%), but in both cases, the surgical wounds had been complicated by infection. In all other cases (where there was physiological healing), the scar was assessed by the patients to be satisfactory. Similar results are reported by Bruneau et al. (2018) (wound dehiscence occurred in 1 patient out of 43 operated – 2.3%).

Infectious complications were noted in the authors' work in 2 patients (4.5%), in both cases comminuted fractures. Similar results were reported by Trost et al. (2009). Rozeboom et al. (2018) then gave an overall incidence of inflammatory complications as 2.7%, with the retromandibular approach being most at risk of this. The onset of infection may be related to haematoma retention at the surgical wound site, prolonged operating time, and infection of the surgical wound by the perioperative introduction of intermaxillary fixation (Eckelt and Loukota, 2010).

Similar to other authors presenting TMAP (Wilson et al., 2005; Trost et al., 2009; Narayanan et al., 2012; Leiser et al., 2013), Frey's syndrome was not reported in the authors' work. However, Rozeboom et al. (2018) indicated a total incidence of 0.74% for external approaches (most often in approaches using a retromandibular incision).

Another complication related to TMAP is muscle pain – resulting from postoperative muscle contraction or muscle scarring (Eckelt and Loukota, 2010). In the authors' work, muscle pain was observed in 3 patients (6.8%), eliminated by targeted postoperative muscular rehabilitation. Postoperative muscle contraction was related to a limitation of jaw movement. In the authors' work, an abduction limitation (MIO below 30 mm) was seen in 9 patients (23%) 6 weeks after surgery. In later check-ups, opening had been improved by physiotherapy and rehabilitation. These results are in agreement with other authors (Trost et al., 2009; Leiser et al., 2013).

The other complications reported in the results were not directly related to the operational approach used. These included a postoperative occlusion disorder, infection, and a functional TMJ disorder.

The most common cause of an unsatisfactory postoperative occlusion was the insufficient use of perioperative intermaxillary fixation or insufficient reduction of the proximal fragment. These occlusion disorders were noticed by the patient immediately after surgery (Eckelt and Loukota, 2010). Disorders of occlusion occurred in 2 cases, 3 weeks after surgery; in both cases, the patients had suffered postoperative wound infection, followed by loosening of the osteosynthesis and dislocation of the proximal fragment. In one case, resorption occurred after fragment union, and the patient experienced a change in their occlusion 3 months after surgery. In one case, there was non-union between the proximal fragment and the mandible, and its subsequent resorption. Avascular necrosis of the joint head, leading to its' resorption, often presents after extensive stripping of tissues from the proximal fragment, or even by its removal from the surgical wound and subsequent re-insertion. However, damage to the bony structure initially occurs due

to the force of impact from their original trauma (Eckelt and Loukota, 2010). The cause of necrosis was not demonstrated by the authors of this study – the proximal fragment was never removed from the wound and the authors were not aware of any extensive stripping of the muscles.

TMJ dysfunction detected by audible TMJ phenomena was due to dislocation and reposition of the disc. One of the aetiological factors behind disc dislocation is trauma (Laskin et al., 2006), however, disc dislocation with a fracture of the articular joint cannot be objectively assessed because it relies purely on patients informing us that they did not suffer from this prior to their trauma. Overall, TMJ pathological conditions are reported as uncommon complications (Eckelt and Loukota, 2010).

Conclusion

A trans-masseteric antero-parotid approach facilitates rapid surgical performance with a good visual field, enabling an accurate reduction and fixation of sub-condylar fractures of the TMJ. The complications associated with this approach are, for the most part, temporary, and the relatively poor aesthetics of a scar is considered acceptable by patients. Overall, it is possible to evaluate the TMAP approach as safe, and the authors recommended it for the treatment of sub-condylar fractures of the mandible.

References

Al-Moraissi, E. A., Louvrier, A., Colletti, G., Wolford, L. M., Biglioli, F., Ragaey, M., Meyer, C., Ellis, E. 3rd (2018) Does the surgical approach for treating mandibular condylar fractures affect the rate of seventh cranial nerve injuries? A systematic review and meta-analysis based on a new classification for surgical approaches. *J. Craniomaxillofac. Surg*. **46(3)**, 398–412.

Biglioli, F., Colletti, G. (2008) Mini-retromandibular approach to condylar fractures. *J. Craniomaxillofac. Surg*. **36(7)**, 378–383.

Bruneau, S., Courvoisier, D. S., Scolozzi, P. (2018) Facial nerve injury and other complications following retromandibular subparotid approach for the management of condylar fractures. *J. Oral Maxillofac. Surg*. **76(4)**, 812–818.

Choi, M. G. (2015) Transmasseteric antero-parotid facelift approach for open reduction and internal fixation of condylar fractures. *J. Korean Assoc. Oral Maxillofac. Surg*. **41(3)**, 149–155.

Eckelt, U., Loukota, R. (2010) *Fractures of the Mandibular Condyle: Approaches and Osteosynthesis*. Eberl Medien GmbH, Immenstadt im Allgäu.

Hinds, E. C., Girotti, W. J. (1967) Vertical subcondylar osteotomy: a reappraisal. *Oral Surg. Oral Med. Oral Pathol*. **24(2)**, 164–170.

Kanno, T., Sukegawa, S., Tatsumi, H., Karino, M., Nariai, Y., Nakatani, E., Furuki, Y., Sekine, J. (2016) Does a retromandibular transparotid approach for the open treatment of condylar fractures result in facial nerve injury? *J. Oral Maxillofac. Surg*. **74(10)**, 2019–2032.

Laskin, D. M., Greene, C. S., Hylander, W. L. (2006) *Temporomandibular Disorders: An Evidenced-based Approach to Diagnosis and Treatment*, 1st Edition. Quintessence Pub. Co., Batavia.

Leiser, Y., Peled, M., Braun, R., Abu-El Naaj, I. (2013) Treatment of low subcondylar fractures – a 5-year retrospective study. *Int. J. Oral Maxillofac. Surg*. **42(6)**, 716–720.

Narayanan, V., Ramadorai, A., Ravi, P., Nirvikalpa, N. (2012) Transmasseteric anterior parotid approach for condylar fractures: experience of 129 cases. *Br. J. Oral Maxillofac. Surg*. **50(5)**, 420–424.

Rozeboom, A. V. J., Dubois, L., Bos, R. R. M., Spijker, R., de Lange, J. (2018) Open treatment of condylar fractures via extraoral approaches: A review of complications. *J. Craniomaxillofac. Surg*. **46(8)**, 1232–1240.

Salgarelli, A. C., Anesi, A., Bellini, P., Pollastri, G., Tanza, D., Barberini, S., Chiarini, L. (2013) How to improve retromandibular transmasseteric anteroparotid approach for mandibular condylar fractures: our clinical experience. *Int. J. Oral Maxillofac. Surg*. **42(4)**, 464–469.

Spinzia, A., Patrone, R., Belli, E., Dell'Aversana Orabona, G., Ungari, C., Filiaci, F., Agrillo, A., De Riu, G., Meloni, S. M., Liberatore, G., Piombino, P. (2014) Open reduction and internal fixation of extracapsular mandibular condyle fractures: a long-term clinical and radiological follow-up of 25 patients. *BMC Surg*. **14**, 68.

Trost, O., Trouilloud, P., Malka, G. (2009) Open reduction and internal fixation of low subcondylar fractures of mandible through high cervical transmasseteric anteroparotid approach. *J. Oral Maxillofac. Surg*. **67(11)**, 2446–2451.

Wilson, A. W., Ethunandan, M., Brennan, P. A. (2005) Transmasseteric antero-parotid approach for open reduction and internal fixation of condylar fractures. *Br. J. Oral Maxillofac. Surg*. **43(1)**, 57–60.

 Prague Medical Report / Vol. 120 (2019) No. 2–3, p. 74–83

The Role of Magnetic Resonance Spectroscopy Imaging Parameters to Predict Early Biochemical Recurrence after Radical Prostatectomy

Mehdi Kardoust Parizi[1], Ali Razi[1], Soheil Alizadeh[1], Amir Kasaeian[2]
[1]Department of Urology, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran;
[2]Department of Biostatistics and Epidemiology, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran

Received December 9, 2018; Accepted September 4, 2019.

Key words: Prostatectomy – Recurrence – MR spectroscopy – Prostate cancer

Abstract: To evaluate the role of magnetic resonance spectroscopy imaging (MRSI) parameters to predict early biochemical recurrence (BCR) after radical prostatectomy (RP) in patients with non-metastatic prostate cancer (PCa). Between November 2010 and March 2012, 60 consecutive patients with clinically non-metastatic biopsy confirmed PCa underwent RP after MRSI assessment in a prospective study. Demographic, clinicopathological, magnetic resonance imaging (MRI) staging, MRSI parameters, and postoperative serum prostate-specific antigen were recorded. The univariate and multivariate Cox regression analyses were used to assess the association between potential prognosticators and early BCR (BCR less than 12 months after RP). In univariate Cox regression, preoperative serum PSA (prostate-specific antigen) (HR – hazard ratio = 1.016, p=0.003), surgical Gleason score > 7 (HR = 5.034, p=0.006) and MRSI risk score (HR = 4.061, p=0.0001); and in multivariate model, preoperative serum PSA (HR = 1.012; p=0.046), surgical GS > 7 (HR = 4.196; p=0.017) and MRSI risk score (HR = 3.256; p=0.013) were associated with early BCR. The greatest AUC (area under the curve) was related to MRSI risk score (AUC = 0.733) and the AUC of the multivariate model was 0.776. MRI/MRSI parameters specially MRSI risk score might be acceptable predictors of early BCR. These parameters can improve the accuracy of predictive nomograms to assess the risk of BCR after RP.

Mailing Address: Soheil Alizadeh, MD., Shariati Hospital, North Karegar Street, Tehran, Iran; Phone: +982 184 901 000; e-mail: s.alizadeh17@gmail.com

https://doi.org/10.14712/23362936.2019.12

Introduction

Prostate cancer (PCa) is the second most common cancer in men (Parkin et al., 2005), that consists of 15% of all new cancers in men (Ferlay et al., 2015). Most of the prognosticators that predict oncological outcomes after radical prostatectomy (RP) are related to the surgical specimen (Kattan et al., 1998; Han et al., 2003; Stephenson et al., 2006). Therefore, pre-operative predictive factors could be valuable for prediction of RP outcomes and also help urologists and patients to perform appropriate decision-making.

Recently, it has been demonstrated that magnetic resonance imaging (MRI) and magnetic resonance spectroscopy imaging (MRSI) are useful to predict oncological outcomes after RP (D'Amico et al., 2000; Pucar et al., 2004; Shukla-Dave et al., 2007).

In this study, we assess MRSI and MRI related prognosticators to predict the early biochemical recurrence (BCR) and time to early BCR after RP in patients with non-metastatic PCa.

Material and Methods

This study was conducted in agreement with the Declaration of Helsinki and it was approved by the local Ethics Committee. Between November 2010 and March 2012, 60 consecutive patients with biopsy-confirmed non-metastatic PCa were included in this prospective study after signing informed consent. Patients underwent MRI and MRSI six weeks after prostate biopsy. All cases have been subjected to standard open radical retropubic prostatectomy by one surgeon. The mean duration between MRI/MRSI and surgery was 29 days. Patients were followed-up by prostate-specific antigen (PSA) 45 days after surgery and every 3 months for the first year and then every 6 months. BCR has been defined as a postoperative PSA ≥ 0.4 ng/ml followed by an increasing PSA. Early BCR was defined as BCR less than one year after RP. All patients with confirmed metastasis on bone scan or chest/abdominal computed tomography scan before RP or patients with a positive surgical margin after RP were excluded from the study. Demographic, clinicopathological and imaging data including patient's age, preoperative serum PSA, biopsy cores number, malignant biopsy cores percentage, biopsy Gleason score (GS), prostate weight, prostate involvement percentage (in the surgical specimen), and surgical GS were recorded. This study was approved by the ethics committee of the Tehran University of Medical Sciences.

MRSI technique and interpretation

Patients underwent MRI (T1W, T2W and dynamic contrast-enhanced [DCE]) and multivoxel MRSI with endorectal coil using Siemens Magneto Avanto 1.5 T, 18 Channel T-Class magnetic resonance machine prior to surgery. In MRI, lesions with a hypointense signal on T2W without a hyperintense signal on T1W that have been enhanced earlier than or contemporaneously with an enhancement of adjacent normal prostatic tissues were defined as malignant and as "index lesion" (Zakian

et al., 2010; Weinreb et al., 2016). In index lesion of each patient, the ratio of choline plus creatine to citrate (Ch + Cr/Ci) was measured in voxels with 0.216 ml volume. In addition, the voxels according to the quantitative ratio were divided into two groups; healthy voxels (ratio < 0.5) and non-healthy voxels (ratio ≥ 0.5). Non-healthy voxels were categorized into three groups; low-grade voxels (0.5 ≤ ratio ≤ 0.6), intermediate grade voxels (0.7 ≤ ratio ≤ 3), and high-grade voxels (ratio > 3). Equation: $\frac{(1\times LG)+(2\times IG)+(3\times HG)}{100}$ was used to calculate "MRSI risk score" for each patient (LG: low-grade voxels percentage; IG: intermediate grade voxels percentage; HG: high-grade voxels percentage) (Weinreb et al., 2016). For MRI grouping, a three-point staging (as same as pathologic T stage definition) was used, as follows: 1) tumour confined within prostate (T2); 2) tumour extends through the prostate capsule (T3); 3) tumour is fixed or invades adjacent structures other than seminal vesicles, such as external sphincter, rectum, bladder, levator muscles, and/or pelvic wall (T4).

The MRI and MRSI of patients were interpreted by a radiologist with 10 years of experience in prostate imaging interpretation. We matched tumour locations on MRSI and location on step-section pathology maps.

Statistical analysis

Data were analysed by the statistical package for the social sciences (SPSS Inc., Chicago, IL, USA) software version 22. The overall BCR-free survival graph and BCR-free survival graphs by categorical variables were designed. The univariate Cox regression analysis was used to assess the association between potential prognosticators and early BCR. Continuous variables with a hazard ratio (HR) > 1 and $p<0.05$ in univariate Cox regression analysis and categorical variables with $p<0.05$ in the log-rank test were considered for a multivariate Cox regression model. AUCs (AUC – area under the curve) were calculated and the ROC (receiver operating characteristic) curve of the multivariate model was plotted. A p-value less than 0.05 was considered statistically significant.

Results

Demographic, clinical and pathological characteristics of patients are demonstrated in Table 1. The mean patients' age and follow-up duration were 64.2 years (range 46–78 years) and 32.3 months (range 12–90 months), respectively. Nineteen patients (31.7%) had BCR during 12 months after RP, with the mean time to early BCR of 5.9 months (range 1.5–12 months). BCR-free survival at 3rd, 6th and 12th month were 91.67% (81.13–96.44%), 81.65% (69.32–89.39%) and 69.68% (56.27–79.70%), respectively.

The mean of MRSI risk scores was 0.91 (range 0–2.37). The mean of Ch + Cr/Ci means of non-healthy voxels was 1.93 (range 0.58–8.44). The mean of maximum Ch + Cr/Ci was 4.83 (range 0.46–19.80). The mean of high-grade voxels

Table 1 – Demographic, clinical and pathological characteristics of non-metastatic prostate cancer patients treated with radical prostatectomy

Characteristic		Value
Age, mean ± SD (range), years		64.2 ± 7.6 (46–78)
Preoperative serum PSA, mean ± SD (range), ng/ml		15.2 ± 24.1 (2.3–160)
Biopsy cores number, mean ± SD (range)		11.3 ± 5.2 (6–27)
Malignant biopsy cores percentage, mean ± SD (range)		39.0 ± 17.8 (7.5–100)
Biopsy Gleason score, No. of patients (%)	<7	20 (33.3)
	7	30 (50.0)
	>7	10 (16.7)
Prostate weight, mean ± SD (range), g		50.6 ± 17.3 (13–121)
Prostate involvement percentage, mean ± SD (range)		22.2 ± 15.4 (1.5–90)
Surgical Gleason score, No. of patients (%)	<7	29 (48.3)
	7	22 (36.7)
	>7	9 (15.0)

PSA – prostate-specific antigen; SD – standard deviation

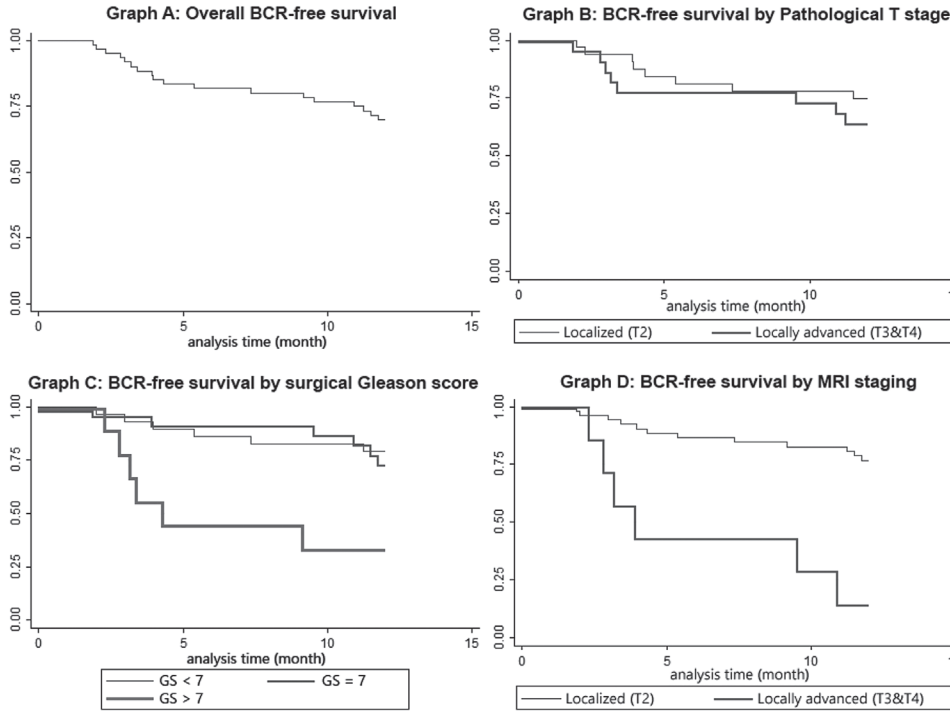

Figure 1 – Kaplan-Meier graphs present overall biochemical recurrence (BCR) free survival and BCR-free survival by three categorical variables.

Table 2 – BCR-free survival by categorical prognosticators of prostate cancer patients treated with radical prostatectomy

	3rd month (95% CI)	6th month (95% CI)	9th month (95% CI)	12th month (95% CI)	Log-rank test (P-value)
BCR-free survival by surgical Gleason score					
<7	93.10 (75.14–98.23)	86.14 (67.16–94.56)	82.55 (63.02–92.34)	78.88 (58.87–89.92)	0.004
=7	95.45 (71.87–99.35)	90.91 (68.30–97.65)	90.91 (68.30–97.65)	72.73 (49.10–86.71)	
>7	77.78 (36.48–93.93)	44.44 (13.59–71.93)	33.33 (7.83–62.26)	33.33 (7.83–62.26)	
BCR-free survival by pathological T stage					
Localized (T2)	93.75 (77.25–98.40)	81.19 (62.84–91.08)	77.94 (59.21–88.83)	74.63 (55.57–86.43)	0.369
Locally advanced (T3 and T4)	86.36 (63.44–95.39)	77.27 (53.74–89.85)	77.27 (53.74–89.85)	63.64 (40.29–79.88)	
BCR-free survival by MRI staging					
Localized (T2)	94.23 (83.17–98.10)	86.52 (73.79–93.33)	84.55 (71.47–91.96)	76.61 (62.49–85.99)	0.0001
Locally advanced (T3 and T4)	71.43 (25.82–91.98)	42.86 (9.78–73.44)	42.86 (9.78–73.44)	14.29 (0.71–46.49)	

BCR – biochemical recurrence; MRI – magnetic resonance imaging; CI – confidence interval

percentage was 14.6% (range 0–50%). The mean of non-healthy voxels percentage and number were 45.6% and 6.1, respectively.

The overall BCR-free survival graph and BCR-free survival graphs by categorical variables are shown in Figure 1. There was a statistically significant difference between the surgical GS groups (p=0.003) and MRI staging groups (p=0.0001), in the log-rank test (Table 2).

Table 3 presents associations between MRI/MRSI parameters and early BCR in univariate and multivariate analyses and AUCs. Preoperative serum PSA (HR = 1.016, p=0.003), surgical GS > 7 (HR = 5.034, p=0.006) and MRSI risk score (HR = 4.061, p=0.0001) were associated with early BCR in univariate Cox regression analysis. In the multivariate Cox regression model, preoperative serum PSA (HR = 1.012; p=0.046), surgical GS > 7 (HR = 4.196; p=0.017) and MRSI risk score (HR = 3.256; p=0.013) were associated with early BCR. Figure 2 illustrates the ROC curve of the multivariate model predicting early BCR. The greatest AUC was related to MRSI risk score (AUC = 0.733). The AUC for the multivariate model was 0.776, which confirms a greater predictive value of multivariate model than each variable. Figure 3 shows a sample of index lesion consisted of 3 voxels with high Ch + Cr/Ci ratio.

Table 3 – The univariate and multivariate Cox regression analyses and areas under curves of clinicopathological and MRI/MRSI parameters for predicting early BCR

	Hazard ratio (95% CI)	P-value (univariate Cox regression analysis)	Hazard ratio (95% CI)	P-value (multivariate Cox regression analysis)	Area under curves (univariate)	Area under curve (multivariate)
Preoperative serum PSA	1.016 (1.005–1.027)	0.003	1.012 (1.000–1.024)	0.046	0.699	
Surgical Gleason score						
<7	ref.	–	–	–	–	0.776
=7	1.268 (0.408–3.932)	0.681	–	–	–	
>7	5.034 (1.608–15.760)	0.006	4.196 (1.294–13.603)	0.017	0.638	
MRSI risk score	4.061 (1.864–8.845)	0.000	3.256 (1.289–8.226)	0.013	0.733	
Pathological T stage	1.560 (0.585–4.161)	0.373				
Ch + Cr/Ci mean of non-healthy voxels	1.156 (0.896–1.491)	0.264				

BCR – biochemical recurrence; MRI – magnetic resonance imaging; MRSI – magnetic resonance spectroscopy imaging; PSA – prostate-specific antigen; Ch + Cr/Ci – choline + creatine/citrate; CI – confidence interval

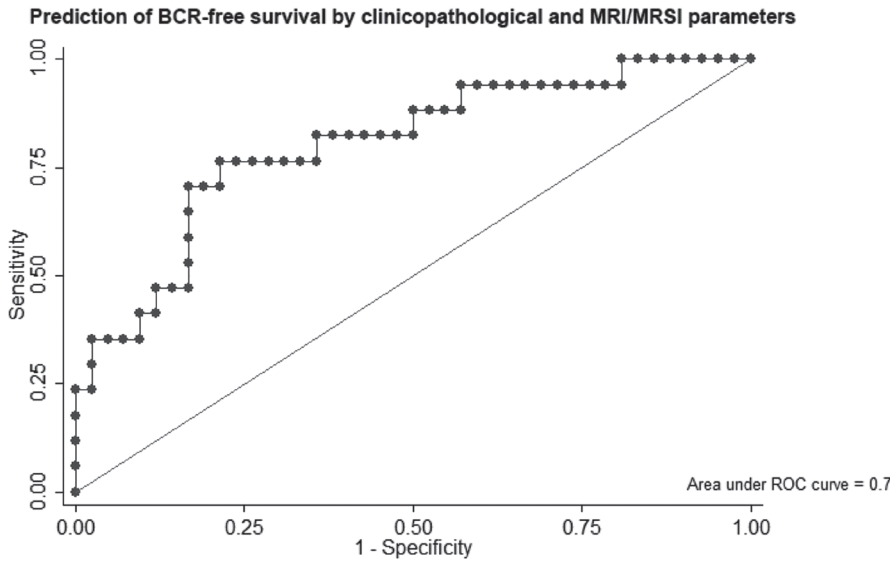

Figure 2 – ROC (receiver operating characteristic) curve of clinicopathological and MRI/MRSI parameters (preoperative serum PSA, surgical Gleason score and MRSI – magnetic resonance spectroscopy imaging risk score) in the prediction of biochemical recurrence (BCR) free survival in a multivariate analysis.

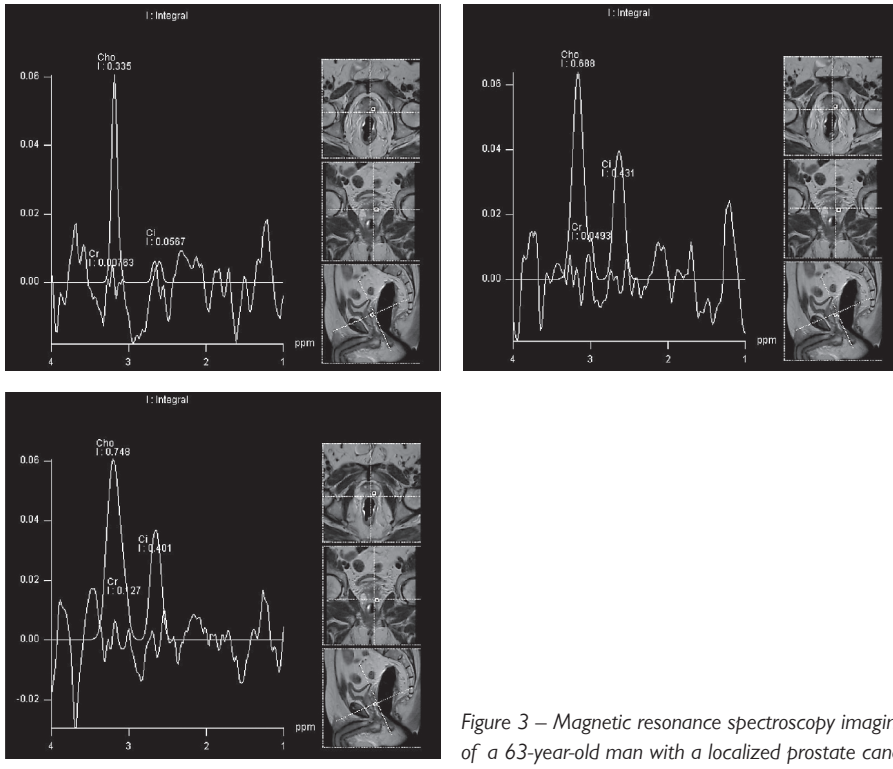

Figure 3 – Magnetic resonance spectroscopy imaging of a 63-year-old man with a localized prostate cancer.

Discussion

BCR after RP represents a local or distant cancer control failure and has a critical role in the patient's management. The incidence of post-RP BCR is estimated up to 45% (Loeb et al., 2011; Liesenfeld et al., 2017). Both early and late BCR are related to PCa specific death (Ferlay et al., 2015). Pound et al. (1999) showed that time to BCR was significantly associated with the development of metastatic disease in patients with PCa. Therefore, accurate prognostic models predicting early disease recurrence might help to perform a proper decision-making process such as neoadjuvant treatments.

Several parameters including patient's age, preoperative PSA, surgical GS, and pathological stage, are frequently used variables to predict biochemical failure after RP (Liesenfeld et al., 2017). Some validated nomograms have been designed to predict BCR and relapse after definitive treatment of PCa (Kattan et al., 1998; Han et al., 2003; Stephenson et al., 2006).

Stephenson et al. (2006) evaluated the long-term post RP recurrence in a predictive nomogram including clinical stage, serum PSA, and biopsy Gleason grade, number of positive and negative biopsy cores, and year of surgery. The authors concluded that these parameters might improve the accuracy of the previously explained nomogram to predict 1 to 10 years' progression-free survival after RP.

Zakian et al. (2010), assessed the prognosticative value of MRI/MRSI findings to predict BCR after RP in 132 patients with PCa. They presented the MRSI index lesion volume and the presence of high-grade MRSI voxels as valuable parameters to predict time to BCR after RP. These new modalities can be used to improve the accuracy of oncological outcomes predictor nomograms (Zhang et al., 2017).

In this study, we evaluated several MRI/MRSI parameters in details to find a reasonable correlation between these prognosticators and early BCR. Kaplan-Meier survival graphs for biochemical recurrence-free survival demonstrated a significant association between surgical GS and MRI staging and early BCR. MRSI risk score has a greater AUC (0.733) in comparison with other predictive factors. Moreover, surgical Gleason score > 7 was a significant factor to predict early BCR in the multivariate analysis. The statistical value of MRSI parameters to predict the oncological outcome after RP has been analysed by some investigators. Nomograms including MRI/MRSI parameters might be more accurate to predict oncological outcomes of patients after RP in comparison with popular nomograms such as D'Amico and cancer of the prostate risk assessment (CAPRA) score (Zhang et al., 2015, 2017).

Zhang et al. (2017) in an MRI-based analysis of predictors assessed the oncologic outcomes after RP in 205 patients with PCa in a retrospective study. They compared the performance of MRI-based nomogram with D'Amico and CAPRA schemes in term of 3 years BCR after RP and found a greater AUC for MRI-based nomogram (AUC 0.909 for MRI-based nomogram versus 0.901 and 0.894 for D'Amico and CAPRA, respectively).

We excluded patients with a positive surgical margin to modify cofounder effect of such an important predictor affecting early BCR. No patient received any neoadjuvant or adjuvant therapy before BCR. So, these predictors might be helpful to find cases with clinically organ-confined PCa that may benefit from neoadjuvant or early adjuvant treatments.

The present study has some limitations. First, short-term follow-up of 32.3 months could be considered a limitation of our study. As we analysed early BCR rate (biochemical recurrence less than 12 months), this follow-up period can be regarded sufficient. Second, the small sample size of the present study makes robust recommendation difficult. The results of this study may be confirmed by future prospective large sample studies.

Conclusion

MRSI/MRI parameters might be useful to find high-risk patients for early BCR. These potential prognosticators can influence the surgeon and the patient's decision-making process to select an appropriate treatment strategy. Moreover, MRSI/MRI parameters might be included in predictive nomograms to increase the accuracy of these prognostic tools in patients with PCa.

References

D'Amico, A. V., Whittington, R., Malkowicz, B., Schnall, M., Schultz, D., Cote, K., Tomaszewski, J. E., Wein, A. (2000) Endorectal magnetic resonance imaging as a predictor of biochemical outcome after radical prostatectomy in men with clinically localized prostate cancer. *J. Urol*. **164(3)**, 759–763.

Ferlay, J., Soerjomataram, I., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D. M., Forman, D., Bray, F. (2015) Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer* **136(5)**, E359–E386.

Han, M., Partin, A. W., Zahurak, M., Piantadosi, S., Epstein, J. I., Walsh, P. C. (2003) Biochemical (prostate specific antigen) recurrence probability following radical prostatectomy for clinically localized prostate cancer. *J. Urol*. **169(2)**, 517–523.

Kattan, M. W., Eastham, J. A., Stapleton, A. M., Wheeler, T. M., Scardino, P. T. (1998) A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. *J. Natl. Cancer Inst*. **90(10)**, 766–771.

Liesenfeld, L., Kron, M., Gschwend, J. E., Herkommer, K. (2017) Prognostic factors for biochemical recurrence more than 10 years after radical prostatectomy. *J. Urol*. **197(1)**, 143–148.

Loeb, S., Feng, Z., Ross, A., Trock, B. J., Humphreys, E. B., Walsh, P. C. (2011) Can we stop prostate specific antigen testing 10 years after radical prostatectomy? *J. Urol*. **186(2)**, 500–505.

Parkin, D. M., Bray, F., Ferlay, J., Pisani, P. (2005) Global cancer statistics, 2002. *CA Cancer J. Clin*. **55(2)**, 74–108.

Pound, C. R., Partin, A. W., Eisenberger, M. A., Chan, D. W., Pearson, J. D., Walsh, P. C. (1999) Natural history of progression after PSA elevation following radical prostatectomy. *JAMA* **281(17)**, 1591–1597.

Pucar, D., Koutcher, J. A., Shah, A., Dyke, J. P., Schwartz, L., Thaler, H., Kurhanewicz, J., Scardino, P. T., Kelly, W. K., Hricak, H., Zakian, K. L. (2004) Preliminary assessment of magnetic resonance spectroscopic imaging in predicting treatment outcome in patients with prostate cancer at high risk for relapse. *Clin. Prostate Cancer* **3(3)**, 174–181.

Shukla-Dave, A., Hricak, H., Kattan, M. W., Pucar, D., Kuroiwa, K., Chen, H. N., Spector, J., Koutcher, J. A., Zakian, K. L., Scardino, P. T. (2007) The utility of magnetic resonance imaging and spectroscopy for predicting insignificant prostate cancer: an initial analysis. *BJU Int*. **99(4)**, 786–793.

Stephenson, A. J., Scardino, P. T., Eastham, J. A., Bianco, F. J. Jr., Dotan, Z. A., Fearn, P. A., Kattan, M. W. (2006) Preoperative nomogram predicting the 10-year probability of prostate cancer recurrence after radical prostatectomy. *J. Natl. Cancer Inst*. **98(10)**, 715–717.

Weinreb, J. C., Barentsz, J. O., Choyke, P. L., Cornud, F., Haider, M. A., Macura, K. J., Margolis, D., Schnall, M. D., Shtern, F., Tempany, C. M., Thoeny, H. C. (2016) PI-RADS Prostate Imaging – Reporting and Data System: 2015, Version 2. *Eur. Urol*. **69(1)**, 16–40.

Zakian, K. L., Hricak, H., Ishill, N., Reuter, V. E., Eberhardt, S., Moskowitz, C. S., Shukla-Dave, A., Wang, L., Scardino, P. T., Eastham, J. A., Koutcher, J. A. (2010) An exploratory study of endorectal MRI and spectroscopy of the prostate as pre-operative predictive biomarkers of biochemical relapse after radical prostatectomy. *J. Urol*. **184(6)**, 2320.

Zhang, Y. D., Wang, Q., Wu, C. J., Wang, X. N., Zhang, J., Liu, H., Liu, X. S., Shi, H. B. (2015) The histogram analysis of diffusion-weighted intravoxel incoherent motion (IVIM) imaging for differentiating the Gleason grade of prostate cancer. *Eur. Radiol*. **25(4)**, 994–1004.

Zhang, Y. D., Wu, C. J., Bao, M. L., Li, H., Wang, X. N., Liu, X. S., Shi, H. B. (2017) MR-based prognostic nomogram for prostate cancer after radical prostatectomy. *J. Magn. Reson. Imaging* **45(2)**, 586–596.

 Prague Medical Report / Vol. 120 (2019) No. 2–3, p. 84–94

Sarcoporosis Is a Part of Aging

Petr Broulík
3rd Department of Medicine – Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University and General University Hospital in Prague, Prague, Czech Republic

Received March 8, 2019; Accepted September 4, 2019.

Key words: Aging – Sarcopenia – Osteoporosis – Frailty

Abstract: Ageing is associated with the accumulation of damage to all the macromolecules within and outside cells leading to progressively more cellular and tissue defects and resulting in age-related frailty, disability and disease. As a result of the aging process the bone deteriorates in composition, structure and function. Age-related musculoskeletal losses are a major public health burden because they can cause physical disability and increased mortality. We tried to find out on a small set of old women, without risk factors for osteoporosis, what caused them the loss of bone minerals. All 492 women had just only one risk factor – the old age. Laboratory findings have shown a decreased serum C telopeptide and low serum alkaline phosphatase circulating markers, used to quantify bone resorption and formation, and very low level of vitamin D. Very low level of vitamin D that disrupted calcium absorption through the intestine, and decreased calcemia increased parathyroid hormone levels with resulting bone effect. The manifestation of physiological aging is worsening eyesight, peripheral neuropathy, depression, worsening of physical condition, skin aging, sarcopenia and bone mineral loss. Senile osteoporosis, which is not caused by known risk factors for osteoporosis, does not appear to be a separate disease, but is part of the physiological process of aging. Treatment of senile osteoporosis should be focused on the control of secondary hyperparathyroidism by administration of vitamin D and calcium. The risk of fractures in the advanced age is determined by a large number of factors ranging from hazards in the home environment to frailty and poor balance.

Mailing Address: Prof. Petr Broulík, MD., DSc., 3rd Department of Medicine – Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University and General University Hospital in Prague, U Nemocnice 1, 128 08 Prague 2, Czech Republic; Phones: +420 224 966 686, +420 224 962 954; e-mail: pbrou@lf1.cuni.cz

https://doi.org/10.14712/23362936.2019.13

Introduction

The very old age is difficult to define. It is the last phase of life, in which the involution, the sum of the involutional changes (extinction, "drop", atrophic), with deterioration of the fitness, the resistance and the adaptability of the organism is more pronounced. We are talking about frailty syndrome. Frailty was proposed by Fried and colleagues (2001) using data from the cardiovascular health study and is based on five characteristics: unintentional weight loss, muscle weakness, reduced energy and endurance, slowness of gait and low physical activity.

The body changes with aging because changes occur in individual cells and in whole organs. These changes result in changes in function and in structure. When the number of cells becomes too low, an organ cannot function normally. Thus, most organs during aging start to function sub-optimally. The manifestations of physiological aging are deteriorating vision, peripheral neuropathy, depression, deconditioning, skin aging, sarcopenia, osteoporosis and movement disorders. Age-related musculoskeletal losses are a major public health burden because they can cause physical disability and increased mortality.

Bones become less dense partly because they contain less calcium (which gives bones strength). The amount of calcium decreases because the body absorbs less calcium from foods. Also, levels of vitamin D, which helps the body use calcium, decrease. Certain bones are weakened more than others. Those most affected include the end of the thighbone (femur) at the hip, the ends of the arm bones (radius and ulna) at the wrist, and the bones of the spine (vertebrae).

Osteoporosis (OP) is predominantly a condition of the elderly although the age profile varies for different fractures. Senile osteoporosis is a type of osteoporosis, a disease of bone leading to an increased risk of fracture due to reduced bone density in aging people. There is calcium deficiency which leads to deterioration of the bone structure, involves a thinning of both the trabecular (spongy) and cortical (hard) bone.

The study has been undertaken to find out if the old age without other risk factors for osteoporosis can be the only cause for involutional osteoporosis. The primary efficacy endpoint was to find out if there are women with low bone mineral content who are just only old.

Methods

We studied 492 women who were recruited from 3,000 patients treated at the 3rd Department of Medicine – Department of Endocrinology and Metabolism, First Faculty of Medicine, Charles University and General University Hospital in Prague during the years 2016 and 2017. Women who were 75 years of age or older were considered to be eligible if they had received first time a diagnosis of osteoporosis and did not have known risk factors for osteoporosis and who had been visited on our outpatient ward for first time.

Women were excluded if they had hypercalcemia, hypocalcemia, recent use of drugs affecting bone metabolism or have hysterectomy and adnexotomy or if they had taken estrogen, or glucocorticoids within the preceding 2 years or bisphosphonates at any time.

At the first visit to the outpatient ward medical history was recorded and all patients underwent physical examination. A study questionnaire was used for recording the relevant demographic and clinical data (age, weight, height, abdominal girth, smoking habit, medications and concomitant disease).

All patients provided written informed consent and the protocol was approved by the appropriate ethical review boards.

The research group consists of 492 women aged 75–92 years with an average age of 80 ± 7 years, with a T-score of more than –2.5 SD (standard deviation) at the lumbar spine or total hip or femoral neck were eligible for participation. Clinical risk factors used for the assessment of fracture probability were: previous fragility fracture, glucocorticoid treatment, current smoking, untreated hypogonadism, inflammatory bowel disease, prolonged immobility, thyroid disorders and HIV infection (Kanis et al., 2019).

Standing height and weight measurements were completed with participants wearing lightweight clothing and no shoes. Body mass index (BMI) was calculated. Standard laboratory tests were used to determine levels of calcium, phosphate alkaline phosphatase, gamma glutamyl transferase and cholesterol.

Laboratory parameters used to evaluate changes in bone turnover were serum C-terminal telopeptide of collagen I (βCTX) to assess the rate of bone resorption and serum alkaline phosphatase with GGTP (gamma glutamyl transpeptidase) to assess the rate of bone formation. Assays were performed at the central laboratory.

Bone mineral density (BMD) was measured at the lumbar spine (L1–L4), total femur (TF), femoral neck (FN), in all participants by using a dual energy X-ray absorptiometry (DXA) densitometer (Discovery A, Hologic, Inc., MA, USA, Software 6 version: Apex 3.5.1) or DEXA Lunar Prodigy. Patients and control groups were measured on the same densitometer. BMD was expressed as g/cm^2 of hydroxyapatite and as T-scores (standard deviation from the mean of a healthy population of the same age).

Spine radiographs were evaluated at a central site. Screening spine radiographs were evaluated for the presence or absence of a vertebral fracture using the semi-quantitative scale (Genant et al., 1993). Atraumatic fractures were defined as fracture following insignificant antecedent trauma such as bending, twisting, sneezing or coughing.

Results

All results are given in Table 1. The present study included a total of 492 women with age 80 ± 7 years. The women had low normal calcium, high normal parathyroid hormone, very low concentration of 25(OH)D and normal phosphate.

Table 1 – Clinical and biochemical data from 492 women with osteoporosis (means ± SD)

	Number – 492
Sex (female)	492
Age (years)	80 ± 7
BMI (kg/m^2)	28.8 ± 3.6
S. calcium (mmol/l) (2.0–2.7)	2.34 ± 0.2
S. phosphate (mmol/l) (0.65–1.61)	1.16 ± 0.3
S. PTH (pmol/l) (1.58–6.03)	6.2 ± 2.8
Uric acid (mmol/l) (220–420)	368 ± 45
S. creatinine (μmol/l) (44–110)	74 ± 5
S. total cholesterol (mmol/l) (2.9–5.2)	6.23 ± 0.6
Calcidiol (ng/ml) (30–80)	10.6 ± 5.2
CTx (ug/l) (0–1008)	525 ± 170
Polymorbidity	468 (96%)
Degenerative changes in axial skeleton and supporting joins	482 (98%)
ALP (μkat/l) (0.65–2.20)	1.39 ± 0.14
GGTP (μkat/l) (0.14–0.84)	0.38 ± 0.02
Back pain	482 (98%)
Fracture presented on X-ray	45 (9%)

SD – standard deviation; BMI – body mass index; S. – serum; PTH – parathyroid hormone; CTx – C-terminal telopeptide; ALP – alkaline phosphatase; GGTP – gamma glutamyl transpeptidase

Circulating biochemical marker of bone formation (gamma glutamyl transferase and alkaline phosphatase) was not significantly higher. The marker of bone resorption serum CTx was normal not suggesting increase in bone remodelling.

The women had normal cholesterol and had overweight. 45 women (9%) had one radiologically defined fracture.

Bone mineral density in femoral neck and lumbar spine measured by T-score has been in the range of osteoporosis (T-score < –2.5 SD).

Discussion

As a result of the aging process the bone deteriorates in composition, structure and function (Demontiero et al., 2012). The mineral composition of bone changes with advancing age. Bone matrix, the framework of skeletal cells, becomes weaker and thinner. With advancing age, a negative balance in bone remodelling results in decreased bone mass and alterations in the bone structure. The amount of bone resorbed by the osteoclasts is not fully restored with bone deposited by the osteoblasts and this imbalance leads to bone loss. Loss of bone mass and strength in humans with advancing age, is associated with an increase in the prevalence of apoptotic osteoblasts and osteocytes and a corresponding decrease in osteoblast number and decreased bone formation rate (Almeida, 2012). Several studies have reported considerable variations in the shape of osteocytes and their lacunae

with aging. The osteocytes are considered to be the cells responsible for sensing mechanical signals on the bones and consequently orchestrating the activity of osteoblasts and osteoclasts (Hemmatian et al., 2017). Senile osteoporosis, also referred to as degenerative osteoporosis, occurs as a result of aging on the bones. Most of bone mass in human body is the cortical bone. Typical for very old people is the excessive cortical bone thinning and risk of nonvertebral fractures. Nonvertebral fractures form 75% of all fractures after 60 years of age (Bliuc et al., 2014). Bone is a tissue with continual active metabolic turnover associated with its remodelling (Riggs et al., 2004). Increased bone resorption leads to the initial fall in bone mineral density. With increasing age there is also a significant reduction in bone formation. This is mostly due to a shift from osteoblastogenesis to predominant adipogenesis in the bone marrow. There is predominant differentiation of mesenchymal stem cells (MSCs) into adipocytes at the expense of osteoblasts (Rosen et al., 2009). The lipotoxicity of marrow adipocytes on bone comes from the observation of PPARγ induction by thiazolidenediones (Broulík et al., 2011). With aging there is a lower levels of osteoblast differentiation (Lecka-Czernik, 2006).

As a person ages, especially after crossing the age of 70, there is impairment in the function of the kidneys with decreased absorption and ability to synthetize vitamin D. The decreased concentration of vitamin D hinders the amount of calcium which can be absorbed. Decreased calcium levels trigger the parathyroid hormone to send signals to the body to reabsorb the bone in order to compensate for the deficiency of calcium in the body. All this results in gradual eroding of the spongy and hard bone structure with a resultant increased risk of bone fractures (Demontiero et al., 2012). New bone formation slows down with increasing age, and the rate by which old bone cells are reabsorbed increases. Age-related factors in etiopathogenesis of senile osteoporosis include low intake of vitamin D and reduced capacity to synthesize vitamin D in skin of elderly individuals. At the age of 70, the activity of enzymatic apparatus of the skin synthetizing vitamin D is up to 10-times lower than in young individuals. Vitamin D is not common in food. With increasing age hydroxylation of vitamin D slows down and resistance of target tissue to the active vitamin D metabolite calcitriol increases. Lower levels of active vitamin D metabolite lead to reduced intestinal absorption of calcium and hypocalcemia, stimulating production and release of parathyroid hormone (PTH) by parathyroid glands; the patients' levels of immunoreactive PTH (senile secondary hyperparathyroidism) are increasing. There occurs imbalance in bone remodelling in favour of bone resorption, with marked multiplication of resorption cavities and their inadequate filling with osteoblasts.

Lack of vitamin D and its active metabolites also results in reduced muscle strength, up to 4-times in the quadriceps, and limited neuromuscular coordination. Muscle weakness contribute to fatigue, weakness and reduced activity tolerance. Sarcopenia recently has been defined as reduced skeletal muscle mass associated with low muscle strength poor physical performance or both (Cruz-Jentoft et al.,

2019). The amount of muscle tissue and muscle strength tend to decrease beginning around age 30 and continuing throughout life (Kemp et al., 2018). Sarcopenia and osteoporosis are both processes associated with aging caused by a decrease in mass and quality, with very similar etiological moments and very similar pathogenesis. Analyzes of geriatric patients show that the vast majority of them suffer from both sarcopenia and osteoporosis. Some of people speak of sarcoporosis (Jenšovský, 2012).

Joint problems ranging from mild stiffness to debilitating arthritis have been very common in our women. Additionally, the use of medications and the presence of health problems increases as people grow older, and these things may interfere with how the body uses nutrients needed for bone health. Some medications and health conditions prevent absorption of minerals. Collagen formation may become impaired.

In the aging process, bone is also adversely affected by decrease in production of bone anabolic peptides, such as growth hormone, insulin and insulin growth factor I, and adrenal steroids, such as dehydroepiandrosterone and androstenedione.

An important factor with a negative impact on bone mass is immobilization, even if short-term, when due to insufficient stimulation of bone mechanoreceptors, bone resorption prevails over bone formation. Elderly persons have often their joint apparatus damaged by degenerative diseases limiting their mobility, ability to walk and protracting periods of immobility, which are significant risk factors for development of osteoporosis.

Of a great interest is the relation between blood circulation and bone metabolism. Ischemia caused by ligature of the femoral artery in rabbits resulted in cortical thinning and reduction of the mechanical resistance of the bone. Laroche et al. (1995) found atherosclerotic changes in interosseous arteries that were similar to those in coronary arteries.

Bones receive about 5% of cardiac output. A limiting factor for centrifugal resorption is most probably the demand of most remote cells for adequate supply of oxygen and amino acids, and the possibilities of removing waste products. Of great importance for bone cells and their metabolic activity is the regional blood flow. Our group of women with compression fractures of vertebral bodies exhibited a higher incidence of ischemic heart disease or ischemic disease of lower limbs (Broulik et al., 1982).

BMD decreases with the patient's increasing age, 50% of women in 70–79-year age group have T-score of bone density in the femoral neck less than –2.0 SD and at the age of 80 years and more their number increases up to 70%. In patients over 75 years, Z-score rather than T-score should be taken into account. However, experienced osteologists do not focus merely on densitometry that may be associated with a number of errors (degenerative changes in the spine) especially in the region of the L-spine, but assess all indicators of the disease, including the patient's habitus (Schousboe et al., 2013).

In elderly patients, the predictive value of densitometry assessment of the proximal femur is much higher. BMD measuring of the proximal femur and femoral neck has shown that a higher incidence of fractures in these locations is associated with a lower BMD value. Although there are not many longitudinal studies measuring BMD in the elderly, it seems that in some patients, loss of the cortical, and perhaps also trabecular, bone stops after the age of 70.

Elderly patients with senile osteoporosis should be diagnosed on a case-by-case basis, as in advanced age a number of severe degenerative changes can be found in the spine concomitantly with osteoporosis that may play a significant role in explaining the patients' complaints.

The treatment of involutional osteoporosis is currently much more effective in prevention of bone loss than in its restoration. Treatment of elderly patients is less effective than in the young individuals. Data on treatment of osteoporosis reported in the literature relate almost exclusively to the population younger than 75 years. It should be noted that the speed of loss of the cortical and trabecular bone changes with increasing age and its response to treatment varies. Trabecular bone is metabolically more active than cortical bone and therefore its response to treatment is different (Boonen et al., 2008).

Treatment of senile osteoporosis should be focused on the control of senile secondary hyperparathyroidism by administration of vitamin D and calcium. Patients receive vitamin D3 either in drops (14–20 gtto once a week), or injections of cholecalciferol (vitamin D2) 300,000 IU i.m. once a month that have proved to be effective as they are more regular. However, vitamin D3 is four-time more powerful. The given protocol of administration of vitamin D prevents overdosing because it has been demonstrated that the level of active vitamin D 25(OH)D3 is extremely low in the elderly (Rizzoli et al., 2013). The optimum dose of vitamin D3 in the elderly is 800 IU per day. It is recommended to administer vitamin D to seniors as the prevention (Hin et al., 2017). In addition, it is necessary to ensure the daily intake of calcium, i.e. at least 500–1,000 mg of elemental calcium per day. Some polymorbid patients may have problems with calcium intake (intolerance, constipation, lack of appetite). This condition may be addressed by food high in calcium, milk cheese and white yoghurts. It is also possible to combine calcium supplements with foods rich in calcium. Calcium carbonicum requires an acidic environment, and therefore is administered during meals (Caution is necessary in case of administration of blockers of H2-receptor or proton pump!) (Pfeifer et al., 2009).

Certain cases of severe osteoporosis with pain syndrome were successfully treated with intravenous injections at the dose of 5 vials of 10% calcium gluconicum with one vial of quajacurane or tramal (personal experience). The cycle includes up to 10 such infusions. Combined administration of 1,200 mg of calcium per day with a physiological dose of vitamin D3 significantly decreases the incidence of non-vertebral fractures, including proximal femur fractures, in individuals older than 75 years (Bischoff-Ferrari et al., 2009).

It is also possible to use hydroxyapatitum osseum which contains both inorganic (hydroxyapatite) and organic (ossein) components. A similar preparation is made from eggshells.

The above mentioned preparations together with specialized rehabilitation, exercises, massage and iontophoresis are essential tools in treatment of senile osteoporosis.

Of no less importance is a proper diet with adequate amount of proteins. People with positive protein balance heal much better following surgery, such as total hip replacement. A high intake of salt increases calciuria values, similarly as excessive intake of proteins. A diet rich in salt and animal proteins may increase the patient's calcium requirement (Mangano et al., 2017).

It is important for the patients to follow the rehabilitation plan, including exercises to strengthen lower limb muscles and to improve gait and postural stability. Elderly patients should be adequately informed about all environmental safety hazards in their homes, particularly in the bathroom, leading to potential falls (Scherrington et al., 2008).

A favourable effect of rehabilitation and bone loading exercises is explained by irritation of bone cells responsible for bone formation, namely by electrical current induced by activation of bone crystals. Another favourable effect of exercises is remodelling of bone trabeculae in the direction of the highest loading. Elderly patients experience especially in the region of an osteoporotic bone affected by microfractures, muscle spasms that cause pain syndrome.

It has to be taken into account that individuals aged 80 and more are highly susceptible to fractures resulting from falls, even if they receive adequate treatment. The risk of fracture of the arm, forearm, femoral neck or vertebral body increases between the age of 45 and 85 years 8-times in women and 5-times in men. About 30% of persons older than 65 years and 50% of persons older than 80 years fall at least once a year. A total of 90% of all proximal femur fractures are caused by fall. It is necessary to control factors leading to falls, i.e. gait and stability disorders (arthropathy, peripheral neuropathy, impairment of the ocular or vestibular system), postural hypotension (treatment of hypertension by diuretics), arrhythmia and the use of hypnotics, sedatives, anxiolytics and antidepressants (Kannus et al., 2004).

Hormone therapy should not be used in senile osteoporosis, as recommended by the recent Women Health Initiative (WHI) study. In elderly women there occurs pain in atrophic mammary gland, proliferation of atrophic endometrium with bleeding; the risk of ischemic heart disease, phlebothrombosis, hypertension and stroke are increasing.

Reasonable doses of anabolics have proved to be beneficial in senile osteoporosis. They considerably strengthen the muscle corset of the axial skeleton and their androgenic component has a favourable effect on osteoblasts. A certain role is played also by their euphorizing effect. Currently the market offers second-

generation bisphosphonates – alendronate sodium, and third-generation bisphosphonates – ibandronate and zoledronate. These medications have a powerful antiresorptive effect targeted directly at osteoclasts, resulting in marked decrease of bone resorption, however our old patients suffering from senile osteoporosis do not have increased markers of osteoresorption. Data from Axelsson et al. (2017) show that in old patients with prior fracture alendronate reduce the risk of hip fracture. The study has limitation there is lack of data regarding bone densitometry and fracture trauma type. Data from Vandenbroucke et al. (2017) are overview of all possibilities how to treat osteoporosis. Paper support our idea that nonpharmacological interventions such as fall prevention play an essential role in the management of osteoporosis. These medications require a good knowledge of the patients in terms of their ability to comply with the prescribed use of medications and of their other comorbidities that should not be so severe to decrease the therapeutic efficacy of bisphosphonates. The approach must be strictly individualized, based on the knowledge of the patient's medical history, social status, possible polymorbidities, hearing and sight abilities and finally the ability to adhere to therapy (Black et al., 2012).

Pain caused by both osteoporosis and degenerative changes of the spine or weight bearing joints should be managed by a reasonable analgesic therapy that should relieve pain without affecting the patients' mobility and sense of orientation.

From our observation of 492 women with senile osteoporosis, which is not caused by known risk factors for osteoporosis, does not appear to be a separate disease, but is part of the physiological process of aging. Treatment of senile osteoporosis should be focused on the control of senile secondary hyperparathyroidism by administration of vitamin D and calcium.

References

Almeida, M. (2012) Aging mechanisms in bone. *Bonekey Rep.* **1**, 102.

Axelsson, K. F., Wallander, M., Johansson, H., Lundh, D., Lorentzon, M. (2017) Hip fracture risk and safety with alendronate treatment in the oldest-old. *J. Intern. Med.* **282**, 546–559.

Bischoff-Ferrari, H. A., Kiel, D. P., Dawson-Hughes, B., Orav, J. E., Li, R., Spiegelman, D., Dietrich, T., Willett, W. C. (2009) Dietary calcium and serum 25-hydroxyvitamin D status in relation to BMD among US adults. *J. Bone Miner. Res.* **24**, 935–942.

Black, D. M., Bauer, D. C., Schwartz, A. V., Cummings, S. R., Rosen, C. J. (2012) Continuing bisphosphonate treatment for osteoporosis – For whom and for how long? *N. Engl. J. Med.* **366**, 2051–2053.

Bliuc, D., Nguyen, T. V., Eisman, J. A., Center, J. R. (2014) The impact of nonhip nonvertebral fractures in elderly women and men. *J. Clin. Endocrinol. Metab.* **99**, 415–423.

Boonen, S., Dejaeger, E., Vanderschueren, D., Venken, K., Bogaerts, A., Verschueren, S., Milisen, K. (2008) Osteoporosis and osteoporotic fracture occurrence and prevention in the elderly: a geriatric perspective. *Best Pract. Res. Clin. Endocrinol. Metab.* **22**, 765–785.

Broulik, P., Kragstrup, J., Mosekilde, L., Melsen, F. (1982) Osteon cross-sectional size in the iliac crest: Variation in normals and patients with osteoporosis, hyperparathyroidism, acromegaly, hypothyroidism and treated epilepsia. *Acta Pathol. Microbiol. Immunol. Scand. A* **90**, 339–344.

Broulík, P., Sefc, L., Haluzík, M. (2011) Effect of PPAR-γ agonist rosiglitazone on bone mineral density and serum adipokines in C57BL/6 male mice. *Folia Biol. (Praha)* **57**, 133–138.

Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A. A., Schneider, S. M., Sieber, C. C., Topinkova, E., Vandewoude, M., Visser, M., Zamboni, M. (2019) Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* **48**, 16–31.

Demontiero, O., Vidal, C., Duque, G. (2012) Aging and bone loss: new insights for the clinician. *Ther. Adv. Musculoskelet. Dis.* **4**, 61–76.

Fried, L. P., Tangen, C. M., Walston, J., Newman, A. B., Hirsch, C., Gottdiener, J., Seeman, T., Tracy, R., Kop, W. J., Burke, G., McBurnie, M. A.; Cardiovascular Health Study Collaborative Research Group (2001) Frailty in older adults: evidence for a phenotype. *J. Gerontol. A Biol. Sci. Med. Sci*. **56**, M146–M156.

Genant, H. K., Wu, C. Y., van Kuijk, C., Nevitt, M. C. (1993) Vertebral fracture assessment using a semiquantitative technique. *J. Bone Miner. Res*. **8**, 1137–1148.

Hemmatian, H. I., Bakker, A. D., Klein, N., van Lenthe, G. H. (2017) Aging, osteocytes, and mechanotransduction. *Curr. Osteoporos. Rep*. **15**, 401–411.

Hin, H., Tomson, J., Newman, C., Kurien, R., Lay, M., Cox, J., Sayer, J., Hill, M., Emberson, J., Armitage, J., Clarke, R. (2017) Optimum dose of vitamin D for disease prevention in older people. BEST-D trial of vitamin D in primary care. *Osteoporos. Int*. **28**, 841–851.

Jenšovský, J. (2012) Bone care in older age. *Interní Med*. **14**, 199–202. (in Czech)

Kanis, J. A., Cooper, C., Rizzoli, R., Reginster, J. Y.; Scientific Advisory Board of the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) and the Committees of Scientific Advisors and National Societies of the International Osteoporosis Foundation (IOF) (2019) Executive summary of the European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Calcif. Tissue Int.* **104**, 235–238.

Kannus, P., Sievanen, H., Palvanen, M., Järvinen, T., Parkkari, J. (2004) Prevention of falls and consequent injuries in elderly people. *Lancet* **366**, 1885–1893.

Kemp, G. J., Birrell, F., Clegg, P. D., Cuthbertson, D. J., De Vito, G., van Dieën, J. H., Del Din, S., Eastell, R., Garnero, P., Goljanek-Whysall, K., Hackl, M., Hodgson, R., Jackson, M. J., Lord, S., Mazzà, C., McArdle, A., McCloskey, E. V., Narici, M., Peffers, M. J., Schiaffino, S., Mathers, J. C. (2018) Developing a toolkit for the assessment and monitoring of musculoskeletal ageing. *Age Ageing* **47**, iv1–iv19 (Suppl. 4).

Laroche, M., Ludor, I., Thiechart, M., Arlet, J., Pieraggi, M., Chiron, P., Moulinier, L., Cantagrel, A., Puget, J., Utheza, G. (1995) Study of the intraosseous vessels of the femoral head in patients with fractures of the femoral neck or osteoarthritis of the hip. *Osteoporos. Int.* **5**, 213–217.

Lecka-Czernik, B. (2006) PPARs and bone metabolism. *PPAR Res*. **2006**, 18089.

Mangano, K. M., Sahni, S., Kiel, D. P., Tucker, K. L., Dufour, A. B., Hannan, M. T. (2017) Dietary protein is associated with musculoskeletal health independently of dietary pattern: the Framingham Third Generation Study. *Am. J. Clin. Nutr.* **105**, 714–722.

Pfeifer, M., Begerow, B., Minne, H., Tucker, K. L., Dufour, A. B., Hannan, M. T. (2009) Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. *Osteoporos. Int.* **20**, 315–322.

Riggs, B. L., Melton, L. J. III., Robb, R. A., Camp, J. J., Atkinson, E. J., Peterson, J., Roulean, P. A., McCollough, C. H., Bouxsein, M. L., Khosla, S. (2004) A population-based study of age and sex differences in bone volumetric density, size, geometry and structure at different skeletal sites. *J. Bone Miner. Res.* **19**, 1945–1954.

Rizzoli, R., Boonen, S., Brandi, M. L., Bruyère, O., Cooper, C., Kanis, J. A., Kaufman, J. M., Ringe, J. D., Weryha, G., Reginster, J. Y. (2013) Vitamin D supplementation in elderly or postmenopausal women:

a 2013 update of the 2008 recommendations from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO). *Curr. Med. Res. Opin.* **29**, 305–313.

Rosen, C., Ackert-Bicknell, C., Rodriguez, J., Pino, A. (2009) Marrow fat and the bone microenvironment: Developmental, functional, and pathological implications. *Crit. Rev. Eukaryot. Gene Expr.* **19**, 109–124.

Schousboe, J. T., Shepherd, J. A., Bilezikian, J. P., Baim, S. (2013) Executive summary of the 2013 International Society for Clinical Densitometry Position Development Conference on bone densitometry. *J. Clin. Densitom.* **16**, 455–466.

Sherrington, C., Whitney, J. C., Lord, S. R., Herbert, R. D., Cumming, R. G., Close, J. C. (2008) Effective exercise for prevention of falls: a systemic review and meta-analysis. *J. Am. Geriatr. Soc.* **56**, 2234–2243.

Vandenbroucke, A., Luyten, F. P., Flamaing, J., Gielen, E. (2017) Pharmacological treatment of osteoporosis in the oldest old. *Clin. Interv. Aging* **12**, 1065–1077.

Cerebellar Squamous Cell Carcinoma Due to Malignant Transformation of Cerebellopontine Angle Epidermoid Cyst, Report an Interesting Case and Review the Literature

Naderafshar Fereydonyan[1], Morteza Taheri[2], Farid Kazemi[3]
[1]Department of Neurosurgery, Arad General Hospital, Tehran, Iran;
[2]Department of Neurosurgery, Iran University of Medical Sciences, 7Tir Hospital, Tehran, Iran;
[3]Department of Neurosurgery, Iran University of Medical Sciences, Rasool Akram Hospital, Tehran, Iran

Received September 24, 2018; Accepted September 4, 2019.

Key words: Squamous cell carcinoma – Cerebellopontine angle – Epidermoid cyst – Middle cerebellar peduncle

Abstract: Malignant transformation of an epidermoid tumour is a rare entity that in almost all patients occurs at the same site of the primary lesion. We report a case of an epidermoid tumour with malignant transformation to squamous cell carcinoma (SCC) at the adjacent site but without any relation to the primary site of the tumour. A 30-year-old patient with a history of cranial surgery and resection of cerebellopontine (CP) angle epidermoid cyst five years ago, presented with a headache, nausea, and vomiting. Physical examination showed no neurological deficit. The brain magnetic resonance imaging (MRI) demonstrated a well-defined lesion within left middle cerebellar peduncle with no relation to CP angle cistern (the previous tumour site). It was isointense on T1, isointense on T2 and had a rim enhancement on gadolinium (GD) injection. Via retrosigmoid and transcortical approach, total resection of the tumour was performed. During the surgery, there was no visible relationship between the current lesion and the previously resected lesion site. Histopathology revealed squamous cell carcinoma. The systemic survey to finding a probable origin of the tumour was negative and the patient referred for performing brain radiotherapy. We are reporting a case of malignant transformation of epidermoid cyst separate from primary location. Moreover, malignant transformation can occur years after index surgery even after gross total resection.

Mailing Address: Morteza Taheri, MD., Department of Neurosurgery, Iran University of Medical Sciences, 7Tir Hospital, Tehran, Iran; Phones: +989 120 194 908, +982 155 228 580; Fax: +982 155 217 901; e-mail: drtaheri38@yahoo.com

https://doi.org/10.14712/23362936.2019.14

Introduction

The intracranial epidermoid tumour is a rare and benign tumour. Malignant transformation of an epidermoid tumour is a rare entity that in almost all patients occurs at the same site of the primary lesion (Lakhdar et al., 2011). Now we report a case of an epidermoid tumour with malignant transformation to squamous cell carcinoma (SCC) at the adjacent to the surgical site but without direct relation to the primary site of the tumour.

Case report

A 30-year-old patient presented with left hemifacial spasm. The physical examination demonstrated decreased left side hearing loss, decreased sensation in V1 and V2 territory and deviation of the tongue to right and uvula to the left. The brain magnetic resonance imaging (MRI) revealed an extraaxial cystic mass in the left cerebellopontine (CP) angle compressing cerebellum, cerebellar peduncle and brain stem (Figure 1). With a diagnosis of an epidermoid tumour, retrosigmoid craniectomy and near-total resection of tumour was performed. Only a very small part of tumour adhesive to the facial nerve remained in place to prevent nerve damage. Histopathology confirmed the diagnosis of the epidermoid tumour. The patient had only short time follow-up postoperatively. About five years later, he returned because of a headache, nausea, and vomiting. Physical examination showed no neurological deficit. The brain MRI demonstrated a well-defined lesion within left middle cerebellar peduncle with no relation to CP angle cistern that was isointense

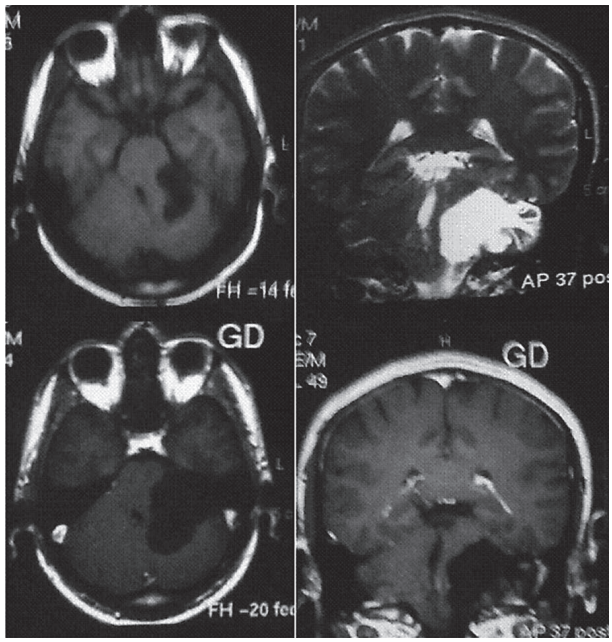

Figure 1 – The brain magnetic resonance imaging (T1, T2 and T1 with gadolinium injection) demonstrated an extraaxial cystic mass in cerebellopontine angle.

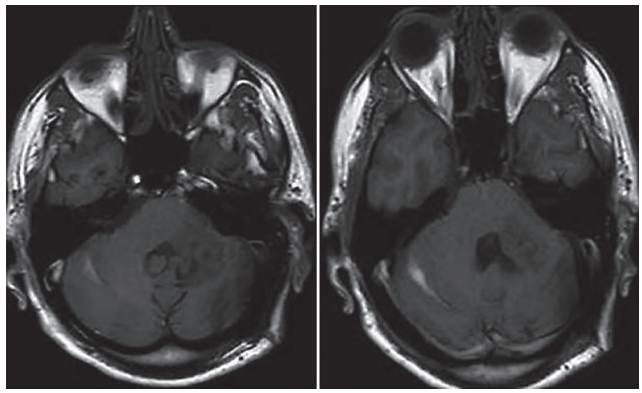

Figure 2 – The T1 images sequences of brain magnetic resonance imaging revealed the lesion.

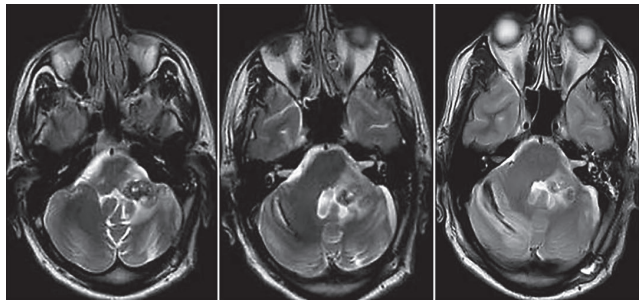

Figure 3 – The T2 images sequences of brain magnetic resonance imaging revealed the lesion within middle cerebellar peduncle.

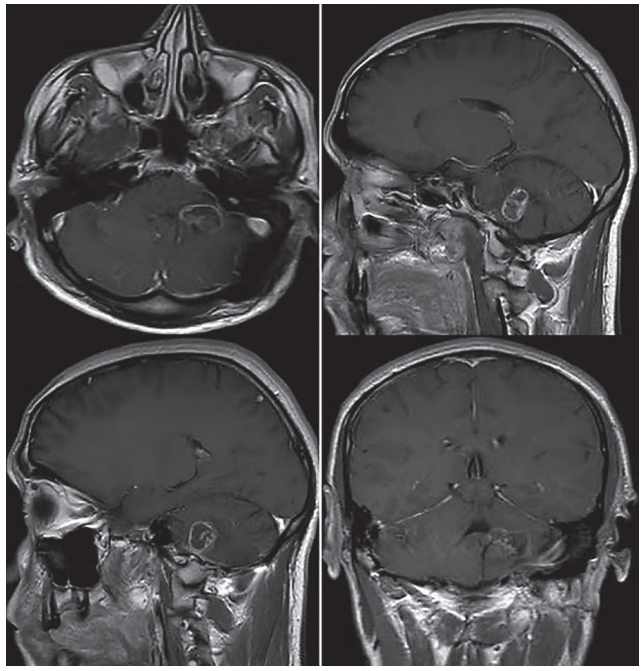

Figure 4 – The brain magnetic resonance imaging with gadolinium injection demonstrated the ring enhancement of the middle cerebellar peduncle lesion.

Table 1 – Summarizes the most information of some studies have reported malignant transformation epidermoid cyst to squamous cell carcinoma

Study	Sex	Age	Location	Interval to MT	Location of SCC	Final diagnosis	Treatment	Outcome	Follow-up
Hamlat et al. (2003)	F	54	parietotemporal	10 m	operation field	SCC transformed from the EC/ leptomeningeal carcinomatosis	–/IT chemotherapy	expire	16 m
Lakhdar et al. (2011)	M	50	CP angle	6 m	operation field	SCC transformed from the EC	surgery/ radiotherapy	good	3 m
Tamura et al. (2006)	F	56	CP angle	8 y	operation field	SCC transformed from the EC	surgery/ radiosurgery	good	4 m
Kodama et al. (2007)	M	67	CP angle	2 m	operation field	SCC transformed from the EC/ leptomeningeal carcinomatosis	surgery/ radiosurgery	expire	13 m
Kim and Kim (2008)	F	72	CP angle	2 m	operation field	SCC transformed from the EC	surgery/ radiotherapy	good	12 m
Chon et al. (2012)	M	43	CP angle	5 m	outside of operation field	SCC transformed from the EC	surgery/ radiosurgery	recurrence	2 y
Hao et al. (2010)	F	61	CP angle	6 y	MT over the primary lesion	SCC transformed from the EC	surgery/–	expire	36 d
Ge et al. (2009)	M	44	temporal lobe	6 y	operation field	SCC transformed from the EC	surgery/?	?	?
Nakao et al. (2010)	F	74	CP angle	20 y	operation field	SCC transformed from the EC	surgery/ radiotherapy	good	17 m
Kano et al. (2010)	F	64	left parapontine extension to medial temporal	14 y	operation field	SCC transformed from the EC	surgery/ radiotherapy	death	2 y
Nishiura et al. (1989)	M	38	CP angle	7 m	operation field	SCC transformed from the EC	surgery/ chemotherapy	alive	2 y
Murase et al. (1999)	F	50	CP angle	11 y	operation field	SCC transformed from the EC	surgery/ chemotherapy/ radiosurgery	good	5 y

Study	Sex	Age	Location	Interval to MT	Location of SCC	Final diagnosis	Treatment	Outcome	Follow-up
Knorr et al. (1991)	M	74	CP angle	13 m	operation field	SCC transformed from the EC	surgery/ radiotherapy	death	7 w
Feng et al. (2014)	M	42	CP angle	–	first presentation	malignant epidermoid cyst	surgery/ radiotherapy	good	6 m
Fox and South (1965)	M	43	anterior temporal	7 y	operation field	SCC transformed from the EC	surgery/–	death	1.5 m
Ozutemiz et al. (2017)	M	64	posterior horn of the left lateral ventricle	23 y	adjacent to tumour	SCC transformed from the EC	surgery/–	recurrence	3 m
Ding et al. (2016)	F	55	temporal region and prepontine area	7 m	operation field	SCC transformed from the EC	surgery/–	death	6 m
Nosaka et al. (1979)	M	46	CP angle	5 m	adjacent to tumour within brain stem	SCC transformed from the EC	–/–	death	2 m
Asahi et al. (2001)	F	55	CP angle	13 y	operation field	SCC transformed from the EC	surgery/–	death	3 m
Our study	M	30	CP angle	5 y	middle cerebellar peduncle	SCC transformed from the EC	surgery/ radiotherapy	good	1 y

SCC – squamous cell carcinoma; EC – epidermoid cyst; MT – malignant transformation; CP angle – cerebellopontine angle; IT – intrathecal; M – male; F – female; d – day; w – week; m – month; y – year

on T1, isointense on T2 and had a rim enhancement on gadolinium (GD) injection (Figures 2–4). The patient underwent total resection of the tumour via retrosigmoid and transcortical cerebellar hemispheric approach. During the surgery, there was no visible relationship between the current lesion and the previously resected lesion site. Histopathology revealed squamous cell carcinoma with the origin of the epidermoid tumour. The systemic survey to find a probable origin of the tumour was negative and the patient referred for performing brain radiotherapy. On last follow-up about 2 years later, the patient demonstrated no significant neurological sign and symptom, and no tumour recurrence.

Discussion

The intracranial epidermoid tumour is a rare and benign tumour that has a slow growth and includes 0.2–1.8% all intracranial tumours (Tamura et al., 2006; Lakhdar et al., 2011). The remnant of the squamous epithelial include in the neural tube at the time of last separation phase between three to five weeks of fetus life (Hao et al., 2010). A malignant transformation from an epidermoid tumour is a rare entity and was reported for the first time by Ernst in 1912 (Lakhdar et al., 2011). There is controversy about the exact mechanism of malignant transformation of an epidermoid tumour, but inflammation due to reaction to a foreign body, *in situ* carcinoma, chronic inflammation due to frequent cyst rupture and subtotal resection cyst wall may be the reasons (Lakhdar et al., 2011). Malignant transformation usually occurs within the primary location of the lesion (Tamura et al., 2006; Lakhdar et al., 2011). Lakhdar et al. (2011) reported the mean age 53 years and female predominance in the cases with malignant transformation of an epidermoid tumour. The mean time to malignant transformation was 14 years (2 to 33 years) (Lakhdar et al., 2011). Tamura and colleagues (2006) reported aged from 36 to 67 years for these patients associated with a mean time of 8.4 years (3 months to 33 years) to malignant transformation.

Rapid development of signs and symptoms is the main clinical feature of malignant transformation, and focal enhancement within the lesion and leptomeningeal metastasis are the main radiologic features indicating the malignant transformation of an epidermoid tumour (Kodama et al., 2007; Lakhdar et al., 2011). Epidermoid tumour is a well-defined lesion that its irregular and nodular surface gives the shiny mother of pearl appearance to this tumour (Chon et al., 2012). This tumour is hypo signal on T1 and hyper signal on T2, and usually without enhancement on GD injection (Tamura et al., 2006). Although in rare cases, it can have minimal rim enhancement (Lakhdar et al., 2011) but appearing of a significant rim or nodular enhancement in epidermoid tumours should alert the physician for a malignant transformation (Kodama et al., 2007).

Malignant transformation can be classified to five groups: 1) primary malignant transformation from an epidermoid cyst, 2) malignant transformation from remnant part of epidermoid tumour, 3) malignant transformation associated with

leptomeningeal carcinomatosis, 4) SCC originated from other benign cysts, and 5) other malignancies from benign cysts (Kim and Kim, 2008).

Malignant transformation in almost all patients occurs at the same site of the primary lesion within surgical field (Fox and South, 1965; Nishiura et al., 1989; Knorr et al., 1991; Murase et al., 1999; Hamlat et al., 2003; Tamura et al., 2006; Kodama et al., 2007; Ge et al., 2009; Hao et al., 2010; Kano et al., 2010; Nakao et al., 2010; Lakhdar et al., 2011; Feng et al., 2014). Tow study reported the malignant transformation in a site adjacent to the primary lesion rather than exactly within the surgical field (Nosaka et al., 1979; Ozutemiz et al., 2017). But only in our study, it occurred within the site away from the primary site and only one case similar to our case has been reported, yet (Chon et al., 2012).

Table 1 summarized the features of some previous studies in this setting.

Conclusion

Although malignant transformation of an epidermoid tumour is a rare complication, but it can occur. In patients with subtotal resection, rapid and progressive signs and symptoms should be taken seriously. Considering the radiologic finding of malignant transformation, follow-up imaging should be performed either in operated patients or in patients with conservative treatment. Appearing of nodular or rim enhancement in the MRI should warrant for malignant transformation. Our hypothesis is that the malignant transformation can occur away from the primary site of the lesion. But for confirming this hypothesis, it needs more survey of the natural history of the epidermoid tumour. In the setting of occurrence of malignant transformation within the different site from the primary site, complete work up to discover other main probable sites of the tumour including lung and gastrointestinal is mandatory.

References

Asahi, T., Kurimoto, M., Endo, S., Monma, F., Ohi, M., Takami, M. (2001) Malignant transformation of cerebello-pontine angle epidermoid. *J. Clin. Neurosci*. **8(6)**, 572–574.

Chon, K. H., Lee, J. M., Koh, E. J., Choi, H. Y. (2012) Malignant transformation of an epidermoid cyst in the cerebellopontine angle. *J. Korean Neurosurg. Soc*. **52(2)**, 148–151.

Ding, S., Jin, Y., Jiang, J. (2016) Malignant transformation of an epidermoid cyst in the temporal and prepontine region: Report of a case and differential diagnosis. *Oncol. Lett*. **11(5)**, 3097–3100.

Feng, R., Gu, X., Hu, J., Lang, L., Bi, H., Guo, J., Pan, L. (2014) Surgical treatment and radiotherapy of epidermoid cyst with malignant transformation in cerebellopontine angle. *Int. J. Clin. Exp. Med*. **7(1)**, 312–315.

Fox, H., South, E. A. (1965) Squamous cell carcinoma developing in an intracranial epidermoid cyst (cholesteatoma). *J. Neurol. Neurosurg. Psychiatry* **28**, 276–281.

Ge, P., Luo, Y., Fu, S, Ling, F. (2009) Recurrent epidermoid cyst with malignant transformation into squamous cell carcinoma. *Neurol. Med. Chir. (Tokyo)* **49(9)**, 442–444.

Hamlat, A., Hua, Z. F., Saikali, S., Egreteau, J., Guegan, Y. (2003) Malignant transformation of intracranial epidermoid cyst with leptomeningeal carcinomatosis: case report. *Acta Neurol. Belg*. **103(4)**, 221–224.

Hao, S., Tang, J., Wu, Z., Zhang, L., Zhang, J., Wang, Z. (2010) Natural malignant transformation of an intracranial epidermoid cyst. *J. Formos. Med. Assoc.* **109(5)**, 390–396.

Kano, T., Ikota, H., Kobayashi, S., Iwasa, S., Kurosaki, S., Wada, H. (2010) Malignant transformation of an intracranial large epidermoid cyst with leptomeningeal carcinomatosis: case report. *Neurol. Med. Chir. (Tokyo)* **50(4)**, 349–353.

Kim, M. S., Kim, O. L. (2008) Primary intracranial squamous cell carcinoma in the brain stem with a cerebellopontine angle epidermoid cyst. *J. Korean Neurosurg. Soc.* **44(6)**, 401–404.

Knorr, J. R., Ragland, R. L., Smith, T. W., Davidson, R. I., Keller, J. D. (1991) Squamous carcinoma arising in a cerebellopontine angle epidermoid: CT and MR findings. *AJNR Am. J. Neuroradiol*. **12(6)**, 1182–1184.

Kodama, H., Maeda, M., Hirokawa, Y., Suzuki, H., Hori, K., Taki, W., Takeda, K. (2007) MRI findings of malignant transformation of epidermoid cyst: case report. *J. Neurooncol*. **82(2)**, 171–174.

Lakhdar, F., Hakkou el, M., Gana, R., Maaqili, R. M., Bellakhdar, F. (2011) Malignant transformation six months after removal of intracranial epidermoid cyst: a case report. *Case Rep. Neurol. Med*. **2011**, 525289.

Murase, S., Yamakawa, H., Ohkuma, A., Sumi, Y., Kajiwara, M., Takami, T., Sakai, N. (1999) Primary intracranial squamous cell carcinoma – case report. *Neurol. Med. Chir. (Tokyo)* **39**, 49–54.

Nakao, Y., Nonaka, S., Yamamoto, T., Oyama, K., Esaki, T., Tange, Y., Mori, K., Wada, R. (2010) Malignant transformation 20 years after partial removal of intracranial epidermoid cyst – case report. *Neurol. Med. Chir. (Tokyo)* **50(3)**, 236–239.

Nishiura, I., Koyama, T., Handa, J., Amano, S. (1989) Primary intracranial epidermoid carcinoma – case report. *Neurol. Med. Chir. (Tokyo)* **29(7)**, 600–605.

Nosaka, Y., Nagao, S., Tabuchi, K., Nishimoto, A. (1979) Primary intracranial epidermoid carcinoma. Case report. *J. Neurosurg*. **50(6)**, 830–833.

Ozutemiz, C., Ada, E., Ersen, A., Ozer, E. (2017) Imaging findings of an epidermoid cyst with malignant transformation to squamous cell carcinoma. *Turk. Neurosurg*. **27(2)**, 312–315.

Tamura, K., Aoyagi, M., Wakimoto, H., Tamaki, M., Yamamoto, K., Yamamoto, M., Ohno, K. (2006) Malignant transformation eight years after removal of a benign epidermoid cyst: a case report. *J. Neurooncol*. **79(1)**, 67–72.

Kinetics of Myristic Acid Following Accidentally Induced Septic Response

Roman Zazula[1], Miroslav Průcha[2], František Pehal[2], Kseniya Dryahina[3], Michal Moravec[1,4], Martin Müller[1], Tomáš Nejtek[1,4]

[1]Department of Anesthesiology and Intensive Care, First Faculty of Medicine, Charles University and Thomayer Hospital, Prague, Czech Republic;
[2]Department of Clinical Biochemistry, Hematology and Immunology, Na Homolce Hospital, Prague, Czech Republic;
[3]Department of Chemistry of Ions in Gaseous Phase, J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic;
[4]Faculty of Military Health Sciences, University of Defence, Hradec Králové, Czech Republic

Received July 4, 2019; Accepted September 4, 2019.

Key words: Biomarkers – Sepsis – Myristic acid – Kinetics

Abstract: Myristic acid was identified as a metabolite with the highest diagnostic sensitivity and specificity in the metabolome of patients with bacteraemia. Subsequently, its significant decrease was observed in patients in septic shock not responding to treatment. In our study we have captured myristic acid serum level kinetics in 96 hours following accidental intravenous self-administration of eubiotic Hylak forte causing infection-like systemic inflammatory response syndrome (SIRS). To our knowledge, this is the first time the kinetics of myristic acid levels is presented in a septic patient. Myristic acid was evaluated in comparison with other inflammatory biomarkers and with its level in a control group of healthy subjects. Myristic acid levels during septic response were significantly elevated in comparison with the control group. The peak level was recorded almost immediately after the insult with a gradual decrease within 96 hours. Myristic acid appears to be a promising biomarker in sepsis diagnostics, further research by our group into this topic is ongoing.

This study was supported by Ministry of Health of the Czech Republic – DRO (Thomayer Hospital – TN 00064190).

Mailing Address: Assoc. Prof. Roman Zazula, MD., PhD., Department of Anesthesiology and Intensive Care, First Faculty of Medicine, Charles University and Thomayer Hospital, Vídeňská 800, 140 59 Prague 4, Czech Republic; Phone: +420 261 083 811; Fax: +420 261 083 393; e-mail: roman.zazula@ftn.cz

https://doi.org/10.14712/23362936.2019.15

Introduction

Although clinical features are still the cornerstone in diagnosis of sepsis, there are various biomarkers which can help physicians to confirm or question the diagnosis (Prucha et al., 2015). It has been 20 years since Brunkhorst et al. (1998) described the kinetics of procalcitonin (PCT) levels in iatrogenic sepsis. Study of Kauppi et al. (2016) identified myristic acid as a metabolite with the highest sensitivity of 1.00 (95% CI 0.85–1.00) (CI – confidence interval) and specificity of 0.95 (95% CI 0.74–0.99) in the metabolome of septic patients with bacteraemia. Cambiaghi et al. (2017) observed its significant decrease in nonresponders to the treatment of septic shock. We present a rare case of sepsis-like inflammatory response to accidental intravenous administration of the Hylak forte eubiotic preparation (germfree concentrate of metabolites of *Escherichia coli*, *Enterococcus faecalis*, *Lactobacillus acidophils* and *Lactobacillus helvetici*) with evaluation of the kinetics of several biomarkers including myristic acid. To our knowledge, this is the first time the kinetics of myristic acid serum levels following septic insult are being described.

Case report

A 30-year-old woman with no significant medical history was admitted to the Department of Gynaecology with suspected infection from uterine myoma necrosis. She was treated by intravenous antibiotics. On admission her laboratory tests were unremarkable except for C-reactive protein (CRP) of 300 mg/l. She had stable vital signs with no symptoms of SIRS (systemic inflammatory response syndrome). On the second day the patient accidentally self-administered 2 ml of the Hylak forte eubiotic preparation (Merckle GmbH) in her peripheral intravenous cannula. The patient developed infection-like systemic inflammatory response syndrome shortly afterwards, she became febrile (39.6 °C) with tachycardia (125 beats/min) and tachypnoea (22 breaths/min), her blood pressure remained normal. The treatment consisted of fluid resuscitation and corticosteroids, intravenous antibiotic was continued. Remission of the clinical symptoms occurred within 12 hours after the event. The patient had no signs of organ dysfunction and was discharged on day 5. During a period of 96 hours following the accidental injection of Hylak, serum levels of CRP, PCT, interleukin 6 (IL-6), presepsin, copeptin and free myristic acid were evaluated. The myristic acid levels were determined by the gas chromatography/mass spectrometry method. Gas chromatograph with flame ionisation detector was used to eliminate quantification errors of mass spectrometry scanning. Very shortly after the insult, we recorded the peak level of myristic acid reaching 10-fold higher value (230.9 μmol/l) than the reference range, with a gradual decrease in the 96 hours to 26.6 μmol/l. The reference values were determined as the median of myristic acid levels measured in healthy subjects (18.9 μmol/l; min 7.8; max 27.7; n=66). Myristic acid was not detected in the eubiotic itself. All the biomarkers under evaluation showed dynamics typical of septic inflammatory response. CRP levels, significantly elevated at the time of admission due to the underlying disease and

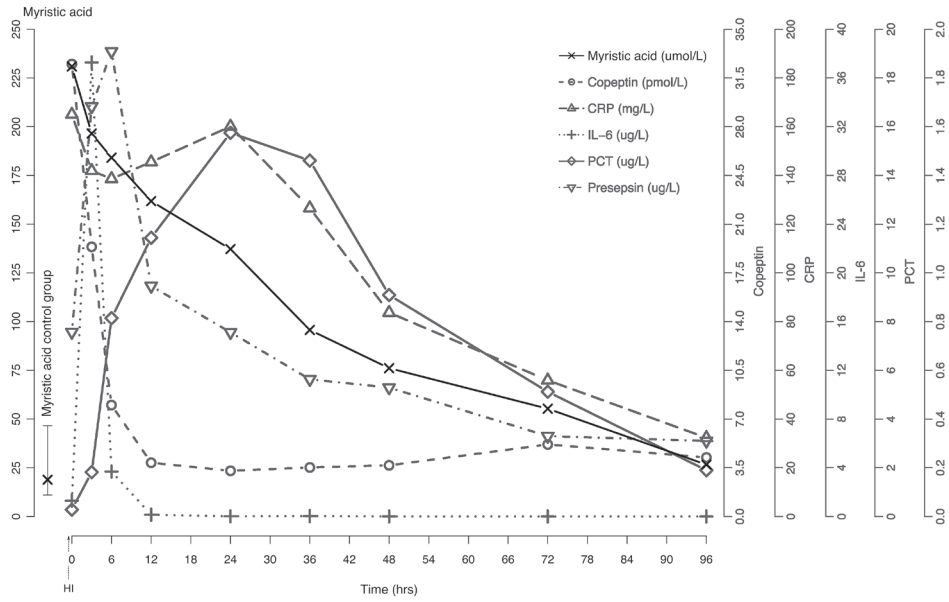

Figure 1 – Myristic acid serum levels compared to serum levels of copeptin, C-reactive protein, interleukin-6, procalcitonin and presepsin during a period 96 hours following accidental injection of Hylak forte (HI – Hylak forte injection 23 minutes before time T0. Reference range in myristic acid control group is presented as median (minimum; maximum).

monitored following the Hylak injection, exhibited a second mild peak 24 hours after the insult (Figure 1).

Discussion

Of interest is the ability of germless concentrate administered intravenously to trigger SIRS with a significant elevation of selected septic biomarkers. While the preparation may be sterile, it undoubtedly contains pathogen-associated molecular patterns (PAMPs) that can evoke a systemic response, and it is well known that infusion of these (for example lipopolysaccharide) can evoke a similar response to sepsis. Arguably, the clinical course was not as severe as one would expect after an intravenous application of live bacteria. The kinetics measurements of selected biomarkers of sepsis and myristic acid after accidentally induced iatrogenic sepsis were inspired by the study of Kauppi et al. (2016), who identified six metabolites in blood samples associated with the diagnosis of sepsis with bacteraemia. Myristic acid stands out of those six as the metabolite with the highest sensitivity and specificity in this metabolomic study. The biomarker alone outperformed even the traditional combination of laboratory findings and SIRS criteria recommended for sepsis diagnosis. The interesting question remains as to the origin and the role of this monosaturated organic acid of linear structure with the already described

atherogenic and thrombogenic potential (Zong et al., 2016). Myristic acid chains are incorporated in lipid A in different quantities in various species of Gram-negative bacteria (Steimle et al., 2016). However, in the same laboratory using the identical method, myristic acid was not detected in the eubiotic Hylak forte. Myristic acid is also a component of cellular membranes, it can be covalently linked to proteins and serve to anchor signal proteins by insertion of the acyl chain into the lipid bilayer (Jennings and Linder, 2009; Stillwell, 2016). Protein N-myristoylation has been shown to be an important evolutionarily conserved modification of proteins implicated in different physiological processes like cell proliferation, differentiation, survival, and cell death (Udenwobele et al., 2017). It is possible, that alterations in lipid metabolism linked to changes in cellular energy production during inflammatory response activation may be responsible for elevated levels of myristic acid in early stages of a septic episode (Kauppi et al., 2016; Cambiaghi et al., 2017).

Conclusion

Myristic acid shows a potential to be a promising marker for early identification of septic patients. The clinical relevance of these findings is currently systematically evaluated, focusing on what new information a marker can provide in describing patients with systemic infections (ClinicalTrials.gov NCT03314831).

References

Brunkhorst, F. M., Heinz, U., Forycki, Z. F. (1998) Kinetics of procalcitonin in iatrogenic sepsis. *Intensive Care Med*. **24**, 888–889.

Cambiaghi, A., Pinto, B. B., Brunelli, L., Falcetta, F., Aletti, F., Bendjelid, K., Pastorelli, R., Ferrario, M. (2017) Characterization of a metabolomic profile associated with responsiveness to therapy in the acute phase of septic shock. *Sci. Rep*. **7**, 9748.

Jennings, B. C., Linder, M. E. (2009) Regulation of G proteins by covalent modification. In: *Handbook of Cell Signaling*, 2nd Edition. Bradshaw, R., Dennis, E., pp. 1629–1634, Academic Press, Cambridge.

Kauppi, A. M., Edin, A., Ziegler, I., Mölling, P., Sjöstedt, A., Gylfe, Å., Strålin, K., Johansson, A. (2016) Metabolites in blood for prediction of bacteremic sepsis in the emergency room. *PloS One* **11**, e0147670.

Prucha, M., Bellingan, G., Zazula, R. (2015) Sepsis biomarkers. *Clin. Chim. Acta* **440**, 97–103.

Steimle, A., Autenrieth, I. B., Frick, J. S. (2016) Structure and function: Lipid A modifications in commensals and pathogens. *Int. J. Med. Microbiol*. **306**, 290–301.

Stillwell, W. (2016) *An Introduction to Biological Membranes: Composition, Structure and Function*. Elsevier Science, London.

Udenwobele, D. I., Su, R. C., Good, S. V., Ball, T. B., Varma Shrivastav, S., Shrivastav, A. (2017) Myristoylation: An important protein modification in the immune response. *Front. Immunol*. **8**, 751.

Zong, G., Li, Y., Wanders, A. J., Alssema, M., Zock, P. L., Willett, W. C., Hu, F. B., Sun, Q. (2016) Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. *BMJ* **355**, i5796.

Instructions to Authors

Prague Medical Report is an English multidisciplinary biomedical journal published quarterly by the First Faculty of Medicine of the Charles University. Prague Medical Report (Prague Med Rep) is indexed and abstracted by Index-medicus, MEDLINE, PubMed, CNKI, DOAJ, EBSCO, and Scopus.

Articles issued in the journal

a) Primary scientific studies on the medical topics (not exceeding 30 pages in standardized A4 format – i.e. 30 lines and 60–65 characters per line – including tables, graphs or illustrations)
b) Short communications
c) Case reports
d) Reviews
e) Lectures or discourses of great interest
f) Information about activities of the First Faculty of Medicine and other associated medical or biological organizations

Layout of the manuscript

a) Title of the study (brief and concise, without abbreviations)
b) Information about the author(s) in the following form:
   - first name and surname of the author(s) (without scientific titles)
   - institution(s) represented by the author(s)
   - full corresponding (mailing) author's reference address (including first name, surname and scientific titles, postal code, phone/fax number and e-mail)
c) Abstract (maximum 250 words)
d) Key words (4–6 terms)
e) Running title (reduced title of the article that will appear at the footer (page break), not more than 50 typewritten characters including spaces)
f) Introduction
   - The use of abbreviations should be restricted to SI symbols and those recommended by the IUPAC-IUB. Abbreviations should be defined in brackets on first appearance in the text. Standard units of measurements and chemical symbols of elements may be used without definition.
g) Material and Methods
h) Results
i) Discussion

j) Conclusion
k) References
   - All the sources of relevant information for the study should be cited in the text (citations such as "personal communication" or "confidential data" are not accepted).
   - It is not permitted to cite any abstract in the References list.
   - References should be listed alphabetically at the end of the paper and typed double-spaced on separate pages. First and last page numbers must be given. Journal names should be abbreviated according to the Chemical Abstract Service Source Index. All co-authors should be listed in each reference (et al. cannot be used).
   - Examples of the style to be used are:
     Yokoyama, K., Gachelin, G. (**1991**) An Abnormal signal transduction pathway in CD4–CD8– double-negative lymph node cells of MRL *lpr/lpr* mice. *Eur. J. Immunol.* **21**, 2987–2992.
     Loyd, D., Poole, R. K., Edwards, S. W. (**1992**) *The Cell Division Cycle. Temporal Organization and Control of Cellular Growth and Reproduction.* Academic Press, London.
     Teich, N. (**1984**) Taxonomy of retroviruses. In: *RNA Tumor Viruses,* eds. Weiss, R., Teich, N., Varmus, H., Coffin, J., pp. 25–207, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
     References in the text should be cited as follows: two authors, Smith and Brown (**1984**) or (Smith and Brown, **1984**); three or more authors, Smith et al. (**1984**) or (Smith et al., **1984**). Reference to papers by the same author(s) in the same year should be distinguished in the text and in the reference list by lower-case letters, e.g. **1980**a, or **1980**a, b.
l) tables, figures, illustrations, graphs, diagrams, photographs, etc. (incl. legends)

Technical instructions

a) Manuscripts (in UK English only) must be delivered in the electronic form via Online Manuscript Submission and Tracking system (http://www.praguemedicalreport.org/). In case of problems, contact the Prague Medical Report Office (medical.report@lf**1**.cuni.cz). The online submission has to include the complete version of the article in PDF format, separately the manuscript as a MS Word file and a cover letter. The detailed version of the Instructions to Authors can be found at: http://www.praguemedicalreport.org/download/instructions_to_authors.pdf.
b) Text should be written in MS WORD only. We accept only documents that have been spell-checked with UK English as a default language.
c) Please, write your text in Times New Roman script, size **1**2, and line spacing **1**.5.
d) Text should be justified to the left, with no paragraph indent (use Enter key only); do not centre any headings or subheadings.

e) Document must be paginated-numbered beginning with the title page.
f) Tables and graphs should represent extra files, and must be paginated too.
g) Edit tables in the following way: Make a plain text, indent by Tab (arrow key) all the data belonging to a line and finish the line by Enter key. For all the notes in table, use letter x, not *.
h) Make your graphs only in black-and-white. Deliver them in electronic form in TIFF or JPG format only.
i) Deliver illustrations and pictures (in black-and-white) in TIFF or JPG format only. The coloured print is possible and paid after agreement with the Prague Medical Report Office.
j) Mark all the pictures with numbers; corresponding legend(s) should be delivered in an extra file. Mark the position of every picture (photo) in the manuscript by the corresponding number, keep the order **1**, 2, 3...

Authors' Declaration

The corresponding (or first author) of the manuscript must print, fill and sign by his/her own hand the Authors Declaration and fax it (or send by post) to the Prague Medical Report Office. Manuscript without this Declaration cannot be published. The Authors' Declaration can be found by visiting our web pages: http://pmr.lf**1**.cuni.cz or web pages of Prague Medical Report Online Manuscript Submission and Tracking system: http://www.praguemedicalreport.org/.

Editorial procedure

Each manuscript is evaluated by the editorial board and by a standard referee (at least two expert reviews are required). After the assessment the author is informed about the result. In the case the referee requires major revision of the manuscript, it will be sent back to the author to make the changes. The final version of the manuscript undergoes language revision and together with other manuscripts, it is processed for printing.

Concurrently, proofs are electronically sent (in PDF format) to the corresponding (mailing) author. Author is to make the proofs in PDF paper copy and deliver it back to the editorial office by fax or as a scanned file by e-mail. Everything should be done in the required time. Only corrections of serious errors, grammatical mistakes and misprints can be accepted. More extensive changes of the manuscript, inscriptions or overwriting cannot be accepted and will be disregarded. Proofs that are not delivered back in time cannot be accepted.

Article processing charge

Authors do not pay any article processing charge.

Open Access Statement

distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the BOAI definition of open access.

Editorial Office
Prague Medical Report
Kateřinská 32, 121 08 Prague 2, Czech Republic
e-mail: medical.report@lf1.cuni.cz
Phone: +420 224 964 570. Fax: +420 224 964 574

Prague Medical REPORT

(Sborník lékařský)

Published by the First Faculty of Medicine, Charles University, Karolinum Press,
Ovocný trh 560/5, 116 36 Praha 1 – Staré Město, Czech Republic, www.karolinum.cz

Editorial Office: Prague Medical Report, Kateřinská 32, 121 08 Prague 2, Czech Republic,
Phone: +420 224 964 570, Fax: +420 224 964 574,
e-mail: medical.report@lf1.cuni.cz
Editor in Chief: Kateřina Jandová, MD., PhD.
Editor: Assoc. Prof. Jan Šváb, MD., PhD.
Foreign Language Editor: Prof. Jaroslav Pokorný, MD., DSc.
Executive Editors: Mgr. Jiří Frühauf, Mgr. Lucie Šulcová
Editorial Board: Prof. Jan Betka, MD., DSc.; Zdeněk Kostrouch, MD., PhD.;
Prof. Emanuel Nečas, MD., DSc.; Prof. František Perlík, MD., DSc.;
Prof. Karel Smetana, MD., DSc.; Prof. Karel Šonka, MD., DSc.;
Assoc. Prof. Jan Tošovský, MD., PhD.; Prof. Jiří Zeman, MD., DSc.

Published as quarterly journal. Typeset and printed by Karolinum Press.
Annual subscription (4 issues) EUR 60,–. Single copy EUR 20,–.
Distribution: Karolinum Press, Ovocný trh 560/5,
116 36 Praha 1, Czech Republic, e-mail: journals@karolinum.cz

ISSN 1214-6994 (Print)
ISSN 2336-2936 (Online)

Reg. No. MK ČR E 796