OSTEOARTHRITIS – A MODERN CONCEPT OF ETIOLOGY AND PATHOGENESIS

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Abstract. Osteoarthritis (OA) is a disease of synovial joints, damaging all the structures of the articular apparatus with a chronic progressive course, resulting in an articular dysfunction. The modern concepts present OA as a “disease of the entire organism” – an active disease process, determined by a group of factors – systemic (genetic, neuro-humoral, hormonal) and local (biomechanical). These factors are “general” for the development of OA, but of different extent of significance in the different articular locations. In the descriptive review are presented the modern concepts of the role and the interaction between the different factors, concerning the aetiology and pathogenesis of the disease. Also a lot of data, referring to the incidence and prevalence of OA all over the world and in our region are pointed out.

Key words: osteoarthritis, pathogenesis, articular cartilage, cytokines, degeneration

INTRODUCTION

Osteoarthritis (OA) is a disease of the synovial joints, which is characterised by deterioration of the synovial fluid qualities, as well as progressive damage and loss of articular cartilage, development of irreversible changes in the underlying bone – bone edema, subchondral bone sclerosis, bony cysts' formation, damage of articular surfaces, osteophytes formation, changes in the articular capsule, as well as in the periarticular tissues – ligaments, insertions and the periarticular muscles [1].

These morphologic changes are the reason for the occurrence of the clinical manifestations of OA – joint pain after certain physical exercises, i.e. mechanical pain, transitory stiffness, lasting less than 30 minutes, crepitus during joint motion, functional deficiency, changes of joint outlook – namely swelling and consecutive typical for OA deformities.

The disease is presented by a cyclic course, when the periods of flares, characterised by superimposed synovial inflammation, low graded osteitis and chondritis, acceleration of structural changes, are followed by periods of “silencing” with a reduction of the clinical manifestations, delay of the structural changes and relative stability of the affected joints [2-4].

The modern conceptions reveal OA not only as a “disease of the entire joint, affecting all the structures of the articular apparatus, developing a consecutive “articular failure”, but as a “disease of the whole organism” – active morbid process, triggered by a

group of factors – systemic (genetic, neurohumoral, hormonal) and local (biomechanical). These above mentioned factors are “common” for OA as an entity, but they have different weight for the course and prognosis of the disease according to the different articular topographic sites [5].

EPIDEMIOLOGY

OA is the most prevailing articular disease, which the most frequent cause for a joint pain and the associated functional deficits, reduction of the quality and even of life duration, a huge burden for the society particularly in the context of the personal suffering of the patients, as well as from the point of view, regarding the treatment expenses [6-7].

DISTRIBUTION

The disease prevalence varies according to the used definition for OA, the topography and the population characteristics (age, sex, BMI etc.) [8].

Clinically defined OA – e.g. the clinical definitions of American College of Rheumatology (ACR) for OA of knee, hip and hands' joints respectively [9-11]. They are based on the presence of the typical for the disease exertional joint pain, short stiffness, limited range of motion, deformities in the absence of inflammation and especially in patients above 50 y/o.

Radiographic defined OA (according to the mostly used Kellgren-Lawrence classification - K/L) [12]. It has been used for decades, but not all the pa-

tients with radiographic features of OA have clinical manifestations, as well as not all of those, who are clinically manifested, have radiographic features of the disease [11, 13].

Symptomatic OA – is defined as a presence of the characteristic for the disease mechanic pain and short termed joint stiffness, found in joints with radiographic confirmed OA.

For the knee joint OA (KOA) the standardized according to the age (> 45 y/o) prevalence of radiographic defined OA in Johnston County Osteoarthritis Project [18] is 27.8%.

For the same age in the above mentioned study the prevalence of radiographic defined hip joint OA (HOA), K/L gradell), is respectively 27%.

Symptomatic KOA in adults > 45 y/o in Johnston County Osteoarthritis Project is 16.7% with sex distribution males/females = 13.5/18.7.

The symptomatic HOA in adults ≥ 45 y/o in the same study is 9,2% with sex distribution males/females = 8.7/9.3.

Based upon the data from the Centre of Disease Control (CDC) in USA [14], the prevalence of the disease in USA is the following: overall OA affects 13.9 % of the adult population above 25 years and 33.6% (12,4 millions) of the population above 65 years of age.

The prevalence of OA is growing with the advance of ageing, linked with the growth of the duration of life, as well as of the relative number of obese people. All over the world 9.6% from males and 18% from females above 60 years of age have symptomatic OA [10, 15]. For our country is suitable an analogy with neighbouring Greece, where a population study establishes a prevalence of the symptomatic KOA 0,3% in the group 19-44 y/o, and 27,6% in the group 75-79 y/o with sex distribution females/males 0,2/0,5 in the first group, and 33,3/19,9 in the second age group. The prevalence of the symptomatic HOA is 0,1 % in the group 19-44 y/o and 3% in adults 75-79 y/o. With sex distribution females/males – 0,1/0,1 in the first age group and 4,3/0,6 in the second age group [17].

Coxarthrosis (Hip osteoarthrosis) is the third in prevalence of localization OA after KOA, and arthrosis of hand joints – during the life every one of 4 people lived till 85 years state complains of HOA. The prevalence of HOA depending of its definition and target population (ethnos, sex, age) range within 0,7-7,0% for people aged over 55. Unlike KOA and OA of hand joints where females are always affected more often, the prevalence of HOA is more often of males aged up to 55, but after 60 the prevalence is equal for males/females [14, 18].

Incidence

The standardised according to sex and age annual incidence of the symptomatic OA in USA according to CDC [14] is the following: KOA – 240/100 000/annually, hands joints – 100/100 000/year, HOA – 88/100 000/year. The incidence is growing respectively to the age, but a reduction is met in the population, aged about 80 years, females have higher incidence than males, especially after 50 years of age [16].

ETIOLOGY

Based upon the fundamental cause of its appearance, OA is classified in two (2) categories – primary (idiopathic), which is a result from the interaction of a complex of systemic and local factors and a secondary – with a known basic etiologic moment (congenital anomalies, inflammatory, neurologic or metabolic diseases), capable to explain the destructive process, taking place in the joint/joints [19].

Etiology of primary OA

OA is an active disease process, triggered by a group of factors – systemic (acting upon the whole organism) and local (acting upon a certain region – joint). These factors are “common” for OA disease, but with a different significance for the course of the disease in the different articular locations.

Systemic factors – they act as predisposing, increasing the susceptibility of the joint to damages, through direct damage of the articular components or by suppression of the recovering process after articular damages.

✓ Age – strongly associated with OA development. From one point of view the „ageing cartilage“ has a changed chondrocyte function and reacts in a different way to the cytokines and growth factors. On the other hand, the protective role to the joints of the neuro-muscular activity is weakening during the years. In addition, during ageing is also growing the cumulative role of the other risk factors. Not at the last place – the systemic “inflammageing” as a result of growing older is changed and facilitates the development of OA.

✓ Sex and sex hormones – strongly associated with OA development – the females have a higher risk for development of hands joints OA, KOA and generalised OA. HOA is presented with a similar prevalence in both sexes, but the disease is progressing more rapidly in women. The explanations of these fact are complex. They include the presence of estrogen receptors on the chondrocytes with a modulation of their function, depending on

their levels and respective effect upon the cartilage metabolism. On the other hand, should not be underestimated and the circumstances of the gender associated risks of traumatic injuries, occupational, domestic and cumulative stress for the joints.

✓ Race and ethnic origin – The prevalence of the particular regional distribution of OA varies among different races and ethnic groups – hands joints OA and HOA are more rarely met in Asians (Chinese), compared to Caucasians. While the prevalence and progression of KOA is similar, KOA is with a higher prevalence and more rapid progression in Afro-Americans, compared to Caucasians. The reasons are selection and confinement through the years of genetic variants with a common adaptive effect on the ethnic groups, but playing role on the course of the particular articular locations in OA.

✓ Weight and BMI – except as a local („wear and tear”), obesity is also a systemic, fortunately modifiable risk factor for OA of the non-bearing body weight joints, such as the hands joints. Fatty tissue is a powerful endocrine organ, responsible for the secretion of plenty adipokines ($\uparrow$ leptin; $\uparrow$ resistin; $\uparrow$ visfatin; $\uparrow$ lipocalin-2; $\uparrow$ chemerin; $\downarrow$ adiponectin) and cytokines ($\uparrow$ TNF- α ; $\uparrow$ IL-6; $\uparrow$ VEGF), capable of provoking systemic of low intensity, but long lasting inflammatory reaction, known as meta-inflammation [20]. In addition, obesity is accompanied by dyslipidaemia – reduction of the levels of HDLs, higher levels of free fatty acids (FFAs), triglycerides (TGs) and the oxidised low density lipoproteins (ox-LDLs) [21]. On one hand this dyslipidemia is linked with cumulation of lipids by the bone marrow and osteoarthritis chondrocytes [22] and changes in their phenotype – higher expression of katabolic enzymes (MMP-1,3,9,13) parallel with reduced synthesis of proteoglycans and collagen and proinflammatory predisposition with a higher expression of proinflammatory cytokines (IL-1 β ; TNF- α ; COX2 – inductor of PGE2). On the other hand, $\uparrow$ ox-LDLs are present in the synovial fluid (SF), their level in the SF is correlating to BMI [22] and is associated with higher levels and activity of their receptor (LOX-1), which activation is resulting in the increased secretion of vaso-endothelial growth factor (VEGF). On one hand VEGF is well known for its ability to activate the secretion of MMP-1, MMP-3 и MMP-13 by the chondrocytes, on the other hand – for its ability to increase the expression of the proinflammatory cytokines IL-1 β , and TNF- α . In this way $\uparrow$ ox-LDLs can induce and support a cartilage damage and arthritis. $\uparrow$ FFAs on their part can activate the macrophages (MP) by stimulation of Toll-like receptor 2/4 with a final effect MP activation and increased

liberation of proinflammatory mediators, such as TNF- α . On one hand this paracrine interaction between adipocytes and MP supports and aggravates the inflammation in the fatty tissue. From the other point of view (since MP are present in synovia) – can induce and support local inflammatory process [22]. These mechanisms shed a light over previously proven connection between obesity, the prevalence and progression of OA, referring either to load bearing joints (KOA, HOA) or to the non-loadbearing, such as the hands joints.

✓ Bone mineral density (BMD) – radiographic defined HOA in different studies, concerning osteoporosis, is associated with increased BMD. The same correlation is present and in cases of radiographic defined KOA, except with their grade of progression – a negative association is established. The observations reveal that along with BMD loss and development of osteoporosis, acceleration of the prevalence and the speed of progression of OA in postmenopausal females is a proven fact [23]. The interaction between BMD and OA is really more complex. Estrogene receptors are present in the key cells, responsible for the changes in OA – chondrocytes, subchondral osteoblasts and synoviocytes. Depending on the concentration of estrogene derivatives the activation of the estrogene receptors have controversial effects – inhibition or amplified expression and secretion. These processes concern either the secretion of proinflammatory cytokines (IL-1 β ; IL-6; TNF- α), or the secretion of growth factors – VEGF; the family of transforming growth factor beta (TGF- β); bone morphogenetic protein (BMP); IHH-induced Hedgehog pathway (Indian Hedge Hog = IHH); the signal pathway NOTCH (notch homolog); IGF-1 (Insulin like Growth Factor-1) signal pathway and Wnt (wingless-type MMTV integration site family) – signal pathway. In this way on one hand are observed amplified processes of synthesis/repair of agrekan, chondrocyte hypertrophy, increased synthesis of collagen type II, increased bone mineralisation, on the other hand – increased degeneration of the extracellular matrix and of collagen type II, low graded, self-supporting synovitis, neoangiogenesis, eburnation of subchondral bone, osteoneogenesis and osteophytes growth.

✓ Diet and alimentary factors – the diet along with the physical activity are basic factors, modifying the influence of obesity and ageing. Despite the fact that some studies report of a reduction of the frequency of occurrence and of the speed of OA progression after a long intake of antioxidants, carotenoids, vit.C and vit. D, this association is of poor

significance, except of the correlation between KOA – vit. D deficiency.

√ Physical activity – This factor is considered by many authors as local, based on the accumulation of micro- and macrotraumatism and the overloading in certain professions and sport activities, which exert a negative effect over the joints.

√ However, the general physical activity by modifying BMI, favourably influencing the neuro-muscular protective mechanism and the proprioception, is definitely a factor with a positive effect on the development and the OA progression.

√ Genetic and epigenetic factors – Despite the opinion that OA is a multifactorial and polygenetic determined disease is non disputable, in the recent 10 years (2010-2020) the number of the studies, dealing with the influence of genetic, epigenetic factors, genomics, proteomics has grown rapidly [24]. In genetic studies two groups of genes are mostly investigated:

√ The first group of genes associated with the inflammation cascade – variants of COX2 gene; HLA cluster variants and variants of IL-1 gene cluster. For the variations in IL-1 gene cluster is established an association either with low bioavailability of IL-1 β , or with OA with various locations – KOA, HOA, hands.

√ The second group of genes play a role in 2 processes – development of the chondrocyte growth plate, chondral and enchondral ossification during the early skeleton development. Here are included osteoprotegerin (OP), TGF- β /Smad3 and growth differentiating factor 5 (GDF5), also known as a cartilage morphogenetic protein 1, member of BMP/TGF- β family, stimulating the proliferation and differentiation of the chondrocytes. In the field of HOA – a special attention is paid to the identified by Castano Betancourt in 2012 variant of DOT1-like, histone H3 methyl transferase (DOT1L) [24-26]. DOT1L association is interesting in several aspects – DOT1L has a biologic link with Wnt- signal cascade, DOT1L is associated with the human height, joint space width – JSW and HOA development. Not at last place DOT1L gene is linked with the epigenetic regulation of the gene expression – DNA methylation, since the demethylation can lead to increased gene transcription [27]. A true panoply of enzymes, linked with articular cartilage degeneration – metalproteinases (MMP-3; MMP-9; MMP-13; ADAMTS-4) are under epigenetic regulation, as well as IL-1 β -promotor region in chondrocytes and leptin, which regulate the expression of MMP-13. The working hypothesis is that the growth factors and the proinflammatory cytokines regulate the process of meth-

ylation of prodestructive genes. Ageing also changes the ratio methylation/demethylation. The different profiles of methylation/demethylation process play the role of gene amplifiers or promoters, associated with embryogenesis and skeleton formation, as well as with matrix regulation, Wnt-cascade, angiogenesis and inflammation [28-30]. Other mechanisms for epigenetic control are the histone modifications and micro-ribonucleic acids (miRNAs/microRNAs) [24]. Twenty-seven miRNAs (family's miRNAs) by the means of their decreased or increased expression, are associated with the onset and the progression of OA: miR-9; miR-16; miR-21; miR-23a/23b; miR-27a/27b; miR-29a/29b; miR-30a/30b; miR-33a; miR-34a/34b; miR-105; miR-125b; miR-126; miR-127; miR-139; miR-140; miR-145; miR-146a; miR-149; miR-181a/181b; miR-186; miR-210; miR-221; miR-365; miR-411; miR-483; miR-488 [31]. The changed expression of miRNAs interacts with the inflammatory cytokines (IL-1 β ; TNF- α ; IL-6; IL-8; IL-17a) and the signal pathways of key growth factors (FGF2; TGF- β ; WNT-signal pathway; IHH-induced Hedgehog pathway; BMP-signal pathway; NOTCH –signal pathway; IGF-1-; VEGF – signal pathway), provoking an intensive cross-reacting dialogue on the level of protein molecules with a final result – hyper stimulation of IL-6 network and suppression of TGF- β signal pathway, especially in the direction of suppression of the anabolic SMAD3 mediated pathway and dysregulation of MMP ($\uparrow$ MMP-1;2;3;8;9/ $\downarrow$ MMP13) [24, 31].

Local risk factors

1. Body weight – BMI/constitutional type (predilectional distribution sites of fatty tissue – Waist/hip ratio – WHR). Obesity is a basic and fortunately modifiable risk factor for OA of the weight bearing joints – by cumulation of mechanic stress due to the abnormal overloading with a consecutive depletion of cartilage reserve – so called „wear and tear“. The mechanism of „wear and tear“ is complex and includes: increased expression by the „overloaded“ chondrocytes of katabolic enzymes, such as metalproteinases (MMP-1, 3, 9, 13), linked with the proteoglycans and matrix degeneration, along with inhibition of proteoglycans and collagen synthesis [20]. Additionally, exposed to overloading chondrocytes have an increased expression of proinflammatory cytokines, such as IL-1 β ; TNF- α и COX2 – inductor of PGE2 [19, 20, 22]. Moreover, besides of the chondrocytes the cyclic overloading changes also the metabolism and the phenotype of the osteoblasts in subchondral bone towards an inflammatory predisposition [21, 22]. The overload-

ing, caused by obesity, has a dominant influence on the prevalence and the progression of KOA, especially in women with bilateral affection and also influences to a milder extent the prevalence and progression of bilateral HOA.

2. Physical activity – this factor is considered by many authors as a local, based on the accumulation of micro- and macrotraumatism in certain professions and sport's activities with a negative effect upon the joints.

3. Traumatism – the role of the acute trauma in the development of secondary OA is well known and confirmed with animal models (meniscal and ligamentous damages of knee joint, intraarticular fractures, femuro-acetabular impingement in HOA, rupture of the ligaments of the elbow and the ankle with or without intraarticular fractures). The mechanism, leading to OA after an articular damage is complex and includes interaction between the inflammation (liberation of proinflammatory mediators – IL-6, CCL2) and of the deteriorated biomechanics, which act synergistic.

4. Deterioration of biomechanics – hereby are included the axial (angular) misalignment of the lower extremities – coxa/genu varum/valgum, as well as some congenital (acetabular dysplasia, aberrations in the shape of caput femuri) or acquired (aseptic necrosis–Legg-Perthes; Osgood-Schlatter; Kienböck) anomalies of articular surfaces, as well as different reasons for articular laxity and hypermobility. All of them lead to deviations in the regular distribution of the load over the articular surfaces and to a secondary OA.

OA PATHOGENESIS

A modern conception for the development of HOA is its staging into 3 (4) stages [45] – Fig. 1.

Fig. 1. Stages of OA according to Kraus et al. [45]

During the asymptomatic and no radiographic (molecular) stage – occur changes in the quality of

hyaluronic acid (HA), the aggrecan, and later in collagen type II, which are the basis for the further damages of the articular cartilage, subchondral bone, articular capsule, ligaments and tendons. While the changes in HA and the loss of aggrecan are considered reversible, the degeneration of collagen type II is an irreversible process and a key step towards the loss of the structural and functional complexity of cartilage.

The molecular stage is shifted to the histological stage, where the preceding cytokine interactions, altering the anabolism, catabolism and the cellular predisposition to proliferation/differentiation/dedifferentiation change the histologic image of the articular cartilage (AC), and make possible the establishment, deduction and the reproduction of these findings to be applied on animal models.

The next preradiographic stage typically reflects advanced histologic changes, that can be found on macroscopic level, using the modern MRI, methods of ultrasonography (US) and bone scintigraphy (BS).

Radiographic stage visualizes the advanced changes in OA – cartilage loss with narrowing of the joint space, bone remodelling with subchondral sclerosis, cysts', osteophytes' formation, as well as manifested complications – subluxations/luxation's and articular deformities.

Histologically the transition between molecular to preradiographic stage is explained in the following way – Fig. 3.

The articular cartilage (AC) is a connective tissue, consisting of resident cells – chondrocytes and extracellular matrix (ECM). ECM consists of collagen fibrils (basically by collagen type II/Coll-II/ and associated with it Coll-IX и Coll-XI) and proteoglycans. Collagen fibrils build a dense stable network, called triple helix, that “packages” the proteoglycans.

The basic proteoglycan in AC is aggrecan – built by core-protein, carrying plenty of glycosaminoglycan's chains (GAG), structured basically by chondroitin sulphate and keratin sulphate. These lateral GAG chains have a marked negative electric charge and thus attract and bind polarized water molecules. Aggrecan has a noncovalent bond with hyaluronic acid (HA) – i.e. glycos-

aminoglycan, the bond is stabilized by a special bonding protein. HA binds more than 100 aggrecan complexes, so in this way is formed a unique aggregate molecular complex (hyaluronan). The latter

Fig. 2. Early molecular basic facts in OA pathogenesis according to Krasnokutsky et al. [2]

Fig. 3. Early histologic changes in articular cartilage according to Karen Boehme/Bernd Rolauffs [31]

is building the basis of AC and determines its properties elasticity and compressibility, while the fibril helix that packages the HA aggregates, determines the resistance of AC to rupture. Histologically AC is presented in 3 zones: superficial zone – (SZ), middle zone – (MZ), and deep zone – (DZ), separated by the subchondral bone (SB) by a zone of calcified cartilage by a histologic structure, known as tide-mark – interface between the AC and calcified cartilage. These zones are formed by a modulation of the phenotypes of epiphyseal chondrocytes during the skeleton maturation. In each zone is observed subdifferentiation of chondrocytes and ECM. Thus, 3 subzones are formed – pericellular, territorial and

interterritorial matrix zone, each of them is presented by a specific distance from the chondrocytes. Under fluorescent electron microscopy the chondrocytes from SZ are typically arranged – in one fiber along their long axis, parallel to the articular surface, built in the pericellular matrix (PCM), territorial (TECM) and interterritorial (ITECM) – ECM – image, termed as SCSO model (Superficial Cell Spatial Organization) – characteristic for the healthy AC [33, 34]. These zones have different characteristics, when analyzed immunohistochemically – PCM and TECM are not luminescent for the Cartilage oligo metric matrix protein (COMP), while ITECM is positive (contains COMP). The earliest histologic changes in AC are

changes in SCSO model – appearance of a double fibril, structured of longitudinal orientated according to the articular surface cells, along with an increase of the cellular density in SZ, evident for a cellular proliferation [32-34]. Thus, the OA debut is characterized by a cellular proliferation. During this process appear cell cluster formations, deriving from the double fibril – along the periphery of ECM, which is accompanied by a progressive degradation and disorganization of PCM, followed by degradation and disorganization of TECM and ITECM, loss of COMP luminescence, typical for ITECM, accompanied by a new synthesis and deposition (luminescence) of COMP – basically in PCM. In addition to the overproduction of ECM (collagen type II, aggrecan), part of the chondrocytes changes their phenotype, expressing markers and molecules, associated with the so called “hypertrophic chondrocytes”, which are not normally met in the healthy AC in adults, except of the transitional zone of calcified AC (around tide-mark), situated between the deep layers of AC (DZ) and the subchondral bone. These hypertrophic chondrocytes are presenting a state of a terminal differentiation of cells during the development of the growth plate and typically produced molecules of collagen type X, VEGF и MMP-13. All these lead to changes in the quality of ECM (reduction in the synthesis of Coll-II, that is partially replaced by Coll-I, reduction in proteoglycans synthesis, parallel with a shortening of their chains, greater production of keratan sulfate type 6 (on account of the depletion of type 4), resulting in – compromised ability of ECM to bind water molecules, on the background of increased hydration, tissue edema and laxity of collagen fibril structure. The cosequences are disturbed compressibility and elasticity of AC and pathologically changed loading. The chondrocytes reaction to the cyclic overloading is as it has been already above mentioned – increased expression of catabolic enzymes, such as metalloproteinases (MMP-1, 3, 9, 13) and aggrecans of the family ADAMTs (A Disintegrin and Metalloproteinase with Thrombospondin motifs) – ADAMTs-4; ADAMTs-5, associated with the proteoglycan and matrix degeneration, along with inhibition of proteoglycan and collagen synthesis. Additionally, exposed to overloading chondrocytes have an increased expression of proinflammatory cytokines as IL-1 β ; TNF- α and COX2-inductor of PGE2 [19-22]. At this stage is hard to differentiate between OA-AC and ageing AC. A basic marker of the “ageing AC” is the cumulation of end products from the intensified, non enzyme glycation (AGE – Advanced Glycation End products), which are binding to the chondro-

cytes surface via a specific receptor (RAGE-Receptor of Advanced Glycation End products). AGE/RAGE system changes the anabolic and catabolic activity of the chondrocytes. In this way the biomechanic properties of AC are also changed in direction, that predispose the proteoglycans and collagen structures to degradation from MMP, aggrecans and collagenases. Typical for the “ageing AC”, different from OA – AC, is the loss of cellular proliferation and the very low turnover of Coll-II. Through time by the means apoptosis and necrosis the number of the lost chondrocytes is growing, as well as the intensity of the local inflammatory reaction is increased. The ageing of chondrocytes is manifested by shortening of telomeres, increased levels of β -galactosidase and reduced ATP production due to mitochondrial dysfunction. The major reasons for chondrocytes ageing and apoptosis are associated with oxidative stress and the connected increase of the reactive oxygen species – ROS (Reactive Oxygen Species) – immunohistochemic studies establish in apoptotic zones a deposition of ions’ protein, which is a result of a greater production of NO by the chondrocytes and expression of nitric oxide synthase (NOS). These findings give evidence that OA is a process, that is age-dependent, biomechanically triggered and chemically (ROS)-mediated [2, 36, 38].

The chondrocytes are post mitotic cells with actually missing proliferative activity in the healthy and mature AC, that is why they use autophagy as a very effective protective (house-keeping) programme in order to keep their cellular integrity and homeostasis. This is realised by elimination of the exogenous cellular aggressors plus supplementary providing with energy sources in cases of stress, hypoxia, starving etc. Three pathways, associated with autophages are present in the cells of mammals and humans: Atg5; Atg7 – in microautophages (MA), Hsc-70 и LAMP-2A – in Chaperone Mediated Autophagy-CMA and Hsc-70; Vps4 и Tsg101 in endosomal micro autophagy (eCi). In all of them LC3 (C3-type lectin of the aggrecan C3 domain) is included in the “embryonic” phagosome, splitted by fusion between auto phagosome and lysosomes or endosomes. This naturally is giving the floor for a debate – role of the complement in the activation of inflammation, with the help of the congenital or the adaptive immunity. The loss of autophages in AC under the impact of a repeated (cyclic) micro trauma, abnormal loading or proinflammatory cytokines (IC) is the other reason for chondrocytes ageing, age – dependent by the apoptosis cellular death and progression of OA changes. In this way are closed only self-supporting

vicious circles, leading to progressive destruction of AC. All of these features of early and late changes in OA can be induced in a model of human AC explants, stimulated by FGF-2 [34, 37-42].

The described changes in AC are not an isolated phenomenon. AC and the subchondral bone (SB) act as an integral functional unit according to the changed biomechanical loading. SB is a general term, including the subchondral bone plate (cortical bone), the underlying trabecular bone and the bone marrow space. SB is bordering to the calcified cartilage zone, by which is separated by a histologic structure, known as tidemark, already above mentioned. In an answer to the changed biomechanics SB reacts with an accelerated turnover, which leads to a cumulation of osteoid substance (sclerosis), along with lowered mineralization – linked with the production of abnormal trimeric type I collagen, that has low affinity to calcium. Thus, the process of SB thickening, known as eburnation, is a result of an increased material density in reduced mineral density, increased porosity and thinning of the cortical plate and the underlying trabecular bone. The process of SB remodelling consists of the eburnation (subchondral osteosclerosis), the appearance of subchondral bone cysts (influx of synovial fluid (SF) under pressure in the zones of the destructed trabeculae or local necrosis), formation of a new bone – osteophytes, as proliferation along the joint margins and bone marrow lesions (zones of bone marrow edema), visualized by MRI [43]. Parallel with the SB changes the initial loss of cartilage smoothness, accompanied by the occurrence of fissures are deepened, the fissures are transformed into splits, penetrating in MZ and deeper – to SB. These

fissures, erosions, ulcerations and lacunae facilitate the vascularization and the reciprocal transport of debris from AC/SB, cytokines and growth factors through the osteochondral junction, precipitating the interactions AC/SB (AC and SB act as an integral biomechanical structure in states of bone health and disease). These interactions are capable of joint self amplification and respectively to determine the disease progression.

In the basis of neoangiogenesis and vascularization stays TGF- β mediation and the onset of the process is in the very beginning – the early phase of OA. The zones of vascularization are associated with a local replacement of the bone marrow (BM) by a fibro vascular tissue, containing cells, expressing VEGF, accompanied by an accelerated osteoclast activity, infiltration of the bone marrow spaces by inflammatory cells, increased endothelial proliferation and vascular density [42, 43, 46]. The invasive microcirculatory vascular elements in the calcified cartilage and synovia can play the role of vectors or pipes for the nerve fibers and additional stimuli for the nerve growth factor (NGF – Nerve Growth Factor), acting synergistically with the basic factor-TGF- β via ALK5-Smad2/3 pathways [44].

The third “player” in OA pathogenesis is the synovitis with low graded local inflammation – Fig.4. The synovial tissue (ST) is a highly specialized connective tissue, covering the diarthrodial joints, encircling the tendons and forming the synovial bursae and the fatty cushions. ST is responsible for the production of SF, its volume and quality. Normal ST consists of 2 layers – outer (subintima), which is approximately 5 mm thick, denser, and consisting of different types connective tissue – fibrous, fatty and

Fig. 4. Involvement of synovium in OA pathogenesis, A. Mathiessen, P. Conaghan [36]

areolar. This layer is rich in Coll I, microcirculatory vessels-presented by its three parts (arterial, venous and lymphatic), but is relatively poor of cells. The inner layer (intima) is 20-40 μm thick, presented 1-4 cell layers – synoviocytes, that cytochemically and immunohistochemically are referred to 2 cell types (macrophages and fibroblasts). The histologic model of ST in OA includes superficial cellular hyperplasia, stromal vascularization, underlying fibrosis and increased cellular infiltration by mononuclear and macrophages [2-4, 35-37].

The fundamental question in OA pathogenesis is which of the three pathologic processes (AC-breakdown; SB-remodeling; ST- smoldering synovitis with episodes of activity) is the triggering? Which cytokine/growth factor plays the triggering role and respectively is the promotor of the disease process?

Having in mind the temporal course of the processes on histologic basis and our knowledge of their mutual dependence, we can say the following:

1. The OA debut is characterized by a cellular proliferation, accompanied by changes in ECM.

2. The alterations in SB (findings of accelerated turnover, cumulation of an osteoid substance and zones of bone marrow edema) – are found either in an advanced OA (along with the other signs of remodeling – subchondral cysts, osteophytes), or in the earliest phases – directly after the evidence-based cellular proliferation in AC (double strings pattern).

3. The earliest histologic proofs for synovitis (hyperplasia with the growing number of the surface synoviocytes and higher cellular density (T cells and macrophages infiltration) are detected early, but never before the cellular proliferation (double string-pattern) and the initial changes in ECM, along with these are observed and the initial changes, typical for SB remodeling.

Thus, the OA debut is characterized by a cellular proliferation, accompanied by changes in ECM, followed by the development of synovitis of low intensity.

Role of the cytokines

The cellular proliferation cannot be amplified by the inflammatory cytokines (IC) (IL-1 β ; TNF- α ; IL-6; IL-17; IL-8; IL-1; IL-18), on the contrary, they suppress the proliferation. Despite IC to be able to induce the changes in ECM via the pathways of activation of MMP and ADAMTs, as well as to self-support the inflammatory reaction by the changes in ECM and the cellular membranes (COX-2-activation, activation of microsomal PGE2 synthase-1, $\uparrow$ liberation of NO by iNOS), they are not able to start the OA process [31]. Thus, despite synovitis is a part of OA pathogenesis

and plays an active role in the disease progression, it is not the primary cause for idiopathic OA. The rivals of this theory put on the first place the idea of synovitis as a starting point via the pathway of activation of congenital immune response by triggers – matrix molecules, complement and crystals, as well as the promoting role of low graded systemic inflammation – e.g. “meta-inflammation” in metabolic syndrome and obesity. However, this does not explain the chronology and the inducibility of the histologic findings [34].

Starting cytokines

1. *TGF- β* – in a healthy human AC TGF- β signaling induces proliferation and expression of the anabolic genes by the activin like receptor kinase 5 (Activin Like Kinase 5-ALK5). In the ageing process and in OA is happening a switch to a dominant activation of activin like receptor kinase 1 (ALK1) and to activating A receptor 1 (Aktivin A Receptor Like type 1/ACVRL1), SMAD 1;5;8 (SMA and MAD-associated protein) signal pathways. This switch to a predominant activation of ALK1; ACVRL1 и SMAD1;5;8 signal pathways transforms TGF- β from an anabolic cytokine into a catabolic factor, promoting chondrocyte hypertrophy, ECM degradation, synovial fibrosis and osteophyte formation. However at present no proinflammatory functions of TGF- β signaling are established in AC [31, 43].

2. *FGF-2* – in human AC, FGF-2 is detected, associated with perlecan-heparan sulfate proteoglycan in PMC, takes part in many signaling pathways, regulating:

- ✓ Proliferation and cellular differentiation (marker of the mesenchyme stem and progenitor cells – MSPC, responsible for their proliferation and the chondrogenesis),

- ✓ Migration (promotes the expression of MCP-1 = monocyte chemotactic protein-1, chemoattractant for the monocytes),

- ✓ Inflammation (promotes the expression of proinflammatory cytokines TNF- α ; IL-1 β ; IL-6; IL-8),

- ✓ Angiogenesis (via VEGDF),

- ✓ ECM degeneration – via MAPK (Mitogen Activated Protein Kinase). FGF-2 – MAPK interaction is basic for the activation of MMP-1 and MMP-13. In this way FGF-2 mediates the processes of proliferation, antianabolism, catabolism and inflammation on the level of AC [31, 47].

Additional signal pathways, mediated by growth factors and their effects:

- ✓ WNT signal pathway – has a complex influence on AC. The canonic signal pathway-WNT/ β -catenin determines the processes of the

proliferation induction and the dominating anti-catabolic effects over healthy AC and the early changes in OA. The noncanonic Ca^{2+} /calmodulin-dependent protein kinase 2 (CaMK2) pathway induces dedifferentiation and expression of catabolic genes. The OA progression depends on the balance inhibition/activation of WNT signal pathways with stopping of proliferation and accelerated catabolism. At present there are no proofs of a proinflammatory activity of WNT pathways in humans [31, 48].

✓ Hedgehog signalling pathway – a key signal pathway during the development of the chondrocyte growth plate and the bone elongation, determining the processes of proliferation and differentiation. Consequently, the pathway is responsible for an induction of a catabolic gene expression. Till present there are no proofs of a proinflammatory activity in humans [31, 49, 50].

✓ NOTCH signal pathway – it can induce and accelerate the proliferation in AC, besides it suppresses the BMP-2 expression, which leads to anabolic gene expression and suppression of the expression of proinflammatory and catabolic genes, including IL-8 and MMP-13 [31, 51].

✓ ILGF-1 signal pathway – possesses proproliferative (promitotic) and anabolic function (amplifying the synthesis of proteoglycans and the expression of Coll-II). Till present there are no data of a proinflammatory activity of the pathway in humans [31].

✓ VEGF signal pathway – possesses procatabolic function (promotes the expression of MMP1 and MMP-3 mRNA), does not affect the proliferation in human AC, does not induce the expression of proinflammatory genes, despite the fact that in other tissues plays a similar role [31, 54].

✓ Inflammatory cytokines (IL-1 β ; TNF- α ; IL-6; IL-17; IL-8; IL-1; IL-18) – play an important role in the processes of accelerated catabolism (can induce and accelerate the changes in ECM by the activation of MMP and ADAMTs) and inflammation – support the inflammatory reaction by the change of ECM and cellular membranes (COX-2-activation, activation of the microsomal PGE2 synthase-1, $\uparrow$ liberation of nitric oxide by iNOS). They cannot induce or stimulate the proliferation, on the contrary, they suppress the cellular proliferation [31, 42, 52].

✓ miRNAs – regulating the signal pathways of the growth factors and inflammatory cytokines. They are demonstrated on Fig. 1.8. Summarized they determine the stimulation of IL-6 signalling, global

suppression of TGF- β signalling, especially of the anabolic SMAD3-regulated pathway, suppression of MMP-13 on account of the stimulation of the rest of MMP [31, 52, 53].

CONCLUSION

The proliferative process in AC depends on FGF-2; TGF- β ; ILGF-1; WNT- and NOTCH – signal pathways. Catabolic changes in ECM can be triggered by: expression of catabolic genes, induced by FGF-2; switch of TGF- β signalling from anabolism to catabolism (MMP-13 expression) with a predominant activation of ALK1; inflammatory cytokines (IL-6); activation of the noncanonical CaMK2/WNT pathway, interaction between BMP and WNT noncanonical (CaMK2) pathway, despite the clear, indirect from WNT effect of BM is anabolic, hedgehog signal pathway; VEGF.

Inflammation may be triggered by: FGF-2; IC (IL-1 β ; TNF- α ; IL-6; IL-17).

The only cytokine, capable on itself to induce proliferation, degeneration of ECM and synovitis on the basis of different receptor utilisation and signal pathways is FGF-2 [54].

FGF-2 is identified as a unique cytokine, capable at one time to induce all the 3 key processes in the pathogenesis of OA. It is responsible not only for the proliferative and catabolic gene expression, as well as for RNA expression of inflammatory cytokines (TNF- α ; IL-1 β ; IL-6; IL-8; MCP-1). Besides, its role for the promotion of MAPK and NF- κ B signal pathways are capable to induce a self-amplified inflammation – typical for the late OA. Thus, FGF-2 is the only cytokine, that takes part either in the proliferation, characteristic of the early OA, or in the destructive and inflammatory progression, typical for the late OA.

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