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VEGF and CD31 expression in arthritic synovium and cartilage of human knee joints

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Abstract

Purpose: To determine the histological differences and the particular aspects of local angiogenesis in knee joint of the patients with osteoarthritis (OA) and rheumatoid arthritis (RA). *Materials and Methods*: In 10 cases with RA and five OA, immunohistochemical stains were performed with CD31 and VEGF-A (Vascular Endothelial Growth Factor). All surgical samples provided from total knee joint arthroplasty. Angiogenesis was quantified in both synovial membrane and cartilage. *Results*: In patients with OA, villous proliferation of the synovial membrane was more prominent that in RA. In the last, invasion of the cartilage by the proliferated synovial tissue was more characteristic. The neovascularization was more intense in RA than in OA, in both synovium and degenerated cartilage. In RA, the vessels were immature in the superficial areas and became larger in the deep synovium. The local angiogenesis was characterized by sprouting and splitting (intussusceptions) mechanisms. In OA, the mature vessels predominated in the subintimal zones. Sprouting or non-sprouting mechanisms of local angiogenesis, which can indicate vascular formation from the resident mature vessels, were not identified in OA. *Conclusions*: Angiogenesis seems to have particular behavior in RA and OA. In RA, local active angiogenesis seems to predominate but in OA up taking of the circulating precursors may be more intensely involved. Intra-articular inhibition of local angiogenesis could have therapeutically impact in RA but not in OA. Finally, we can conclude that there probably are many different pathways leading to the same joint damage having certain therapeutic consequences.

Keywords: CD31, angiogenesis, rheumatoid arthritis, osteoarthritis.

Introduction

One of the roles of synovial membrane is to allow the proper transfer of nutritive substances from blood into synovial tissue [1].

The synovial fluid is composed by several substances including hyaluronan (HA), which plays important roles in the normal joint function. The HA is either degraded in situ or it is absorbed into the lymph vessels being transported in the lymph nodes [1, 2]. Based on the pro-inflammatory activity of the HA, it was supposed that the level of its degradation is associated with the level of intra-articular inflammation and, probably, with the intensity of synovial hyperplasia and/or cartilage erosions. This is the reason why the intra-articular administration of HA seems to improve the clinical outcome of patients with osteoarthritis (OA). At the same time, villous proliferation of the synovial tissue might be due to the failure of lymphatic uptake and drainage of HA [1, 3].

Despite several researches about mechanisms of bone destruction and remodeling in OA and rheumatoid arthritis (RA), this complex pathogenesis that involves cell proliferation, activation of proinflammatory factors, and angiogenesis [4, 5] still remains non-elucidated. The aim of our paper was to analyze the histological differences between the synovial tissue and cartilage in specimens provided from patients with RA and OA. At the same time, the local angiogenesis was quantified with VEGF-A (Vascular Endothelial Growth Factor) and CD31. Platelet endothelial cell adhesion molecule (PECAM-1) also known as cluster of differentiation 31 (CD31) is a protein found on the surface of platelets, monocytes, neutrophils, and some types of T-cells, and makes up a large portion of endothelial cell intercellular junctions. The encoded protein is a member of the immunoglobulin superfamily and is likely to be involved in leukocyte migration, angiogenesis, and integrin activation.

Our preliminary observations were correlated with the feasible data from literature.

Materials and Methods

In 10 cases with rheumatoid arthritis (RA) and five with osteoarthritis (OA), we analyzed the VEGF and CD31 expression in the synovium and cartilage to determine the differences between these two lesions. All samples were obtained at the time of total knee joint arthroplasty. Informed consent was obtained from patients included in study. To perform the immunohisto-chemical stains we used the UltraVision system by D-Line, LabVision in formalin-fixed, decalcified, paraffin-embedded tissues. The VEGF-A (clone VG1) provided by D-Line, LabVision and CD31 (clone JC70A) by Dako. After deparaffinizing, all samples were incubated at 100^0C with pepsin, followed by hydrogen peroxide incubation. The incubation with the primary antibodies was performed for 60 minutes. The

Streptavidin Peroxidase Solution and Biotinylated Goat Anti-Polyvalent Solution (D-Line, LabVision, Fremont, CA, USA) were used for the next steps. The slides were developed using 3,3'-diaminobenzidine (DAB, Dako) and counterstained with Mayer's Hematoxylin.

Immunohistochemical staining scoring

VEGF and CD31 immunoreactivity was evaluated in the synovial membrane in both RA and OA. The chondral vascular density, in the vascularized areas proximal to the cartilage, was also assessed with CD31. To determine the vessel density in the synovial membrane, with CD31, we counted the number of vessels, at 200× magnifications, in the most highly vascularized ('hot-spot') areas. At the same time, in superficial and deep zones, we analyzed the vessels' type: mature resident vessels, with large lumen and thick wall and neoformed vessels, smaller in size, thinner than the other ones.

Results

Clinico-pathological data

The median age of patients with RA was 53.66± 17.04 years ranging from the age of 34 to 64 years. In OA, the median age was 67.5±9.19 years (minimum 61, maximum 74-year-old). The male:female ratio was 1:9 in RA and 4:1 in OA.

Histological features

From the five specimens with OA, three presented villous proliferation of the synovial cells, in the other two cases cartilage degeneration being observed. The proliferated villous membrane was covered by synovial cells with stratified arrangement. The inflammatory infiltrate was predominantly composed by mononuclear cells and was more prominent peri-vascular located, surrounding the resident mature vessels, in the sub-intimal zones.

In cases with RA, the villous synovial proliferation, although present, was not so well expressed as in the other cases. One of the constant features, in these cases, was the intense vascularization around the degenerated cartilage. On the other hand, we noticed invasion of cartilage by the proliferated synovial tissue. Thickening and fibrosis of the synovia was also more intense than in OA. Three of the cases with RA presented asbestos-like degeneration of the cartilage and in one of them proliferation of osteoclasts was noticed inside the cartilage.

These preliminary observations show that in the OA the inflamed synovium presented mostly a villous hypertrophy but in RA this proliferation was more intensely observed in the deep areas and had an invasive pattern, invading the cartilage (Figure 1).

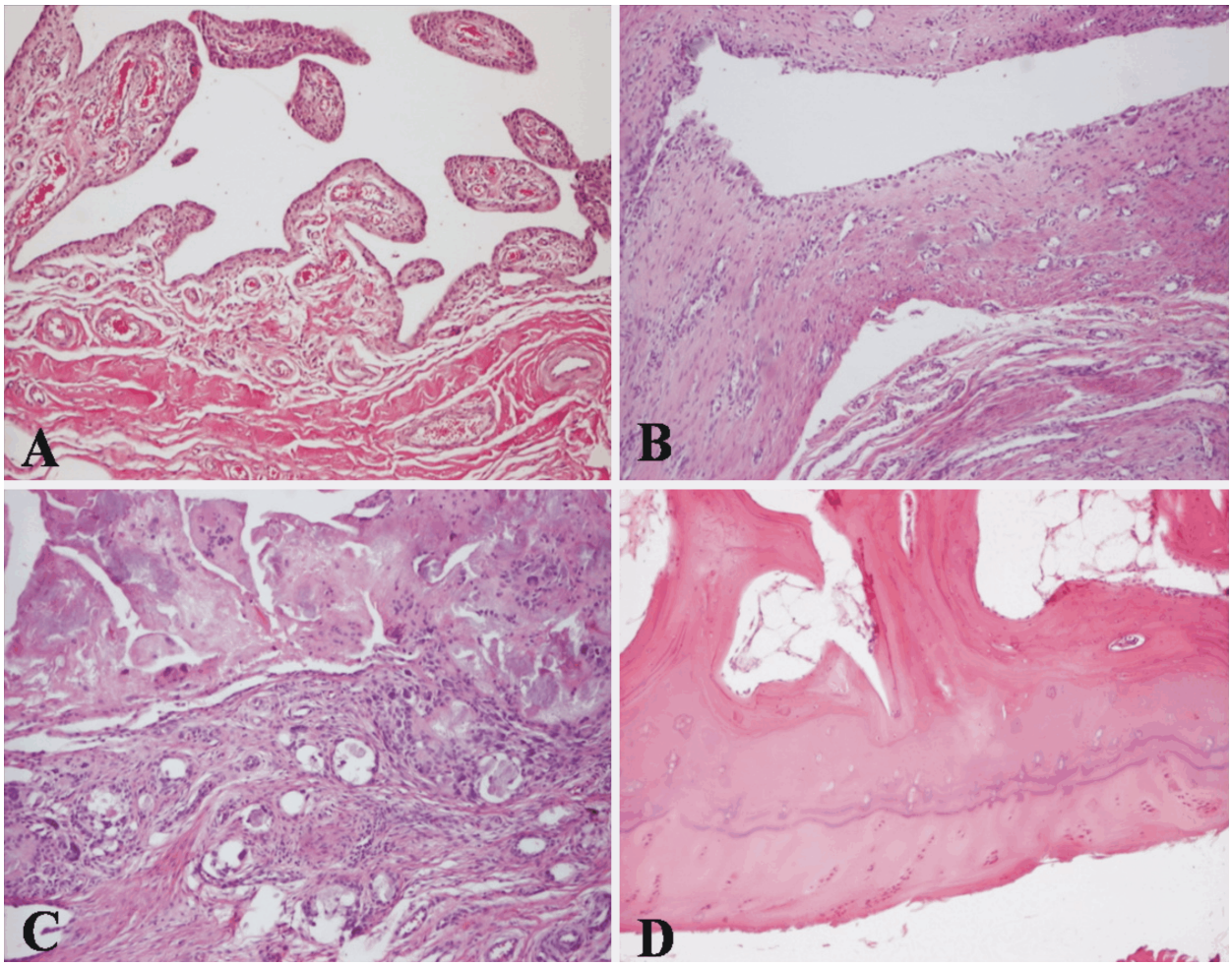

Figure 1 – ***Histological findings (HE stain, ob. 4×): (A) Villous proliferation of the synovial cells in a case with osteoarthritis; (B–D) In rheumatoid arthritis, the villous proliferation is not well expressed (B) but the proliferated synovial tissue invades the cartilage (C) and asbestos-like degeneration of the cartilage can be observed (D).***

The inflammatory infiltrate was more intense in RA than in OA, especially in the subintimal areas, and was predominantly composed by lymphocytes, plasma cells and macrophages.

CD31 expression

In patients with RA, the neovascularization, characterized by increased number of small vessels, with thin wall, marked with CD31, was more intense than in OA. The average value of the vessel density (VD) was 9.03 vessels/field in RA and 4.75 vessels/field in OA. The peri-chondral vascular density was 4.23 in RA and 2.45 vessels/field in OA. In RA, the vessels were smaller in the subintimal areas and tended to be mature, with large lumen, in the deep areas (Figure 2). The sprouting and splitting (intussusceptive) active angiogenesis were the two identified patterns of angiogenesis. In contrast, in OA the superficial vessels were mostly mature than in the deep layers. The neoformed vessels were leakier, with small lumen (Figure 2). We did not identify either sprouting or other non-sprouting active local mechanisms, which should indicate their formation from the resident mature vessels. In RA, one of the constant features was the intense vascularization around the degenerated cartilage, especially in the invasion areas (Figure 2). In both RA and OA, the superficial synovial lining cells showed focal CD31 positivity.

VEGF expression

In those cases with villous proliferation of the synovium, especially in OA, the synoviocytes intensely expressed VEGF. Pannus was also diffuse stained by VEGF. In contrast, focal positivity was revealed by the synoviocytes and also in pannus of patients with RA (Figure 2).

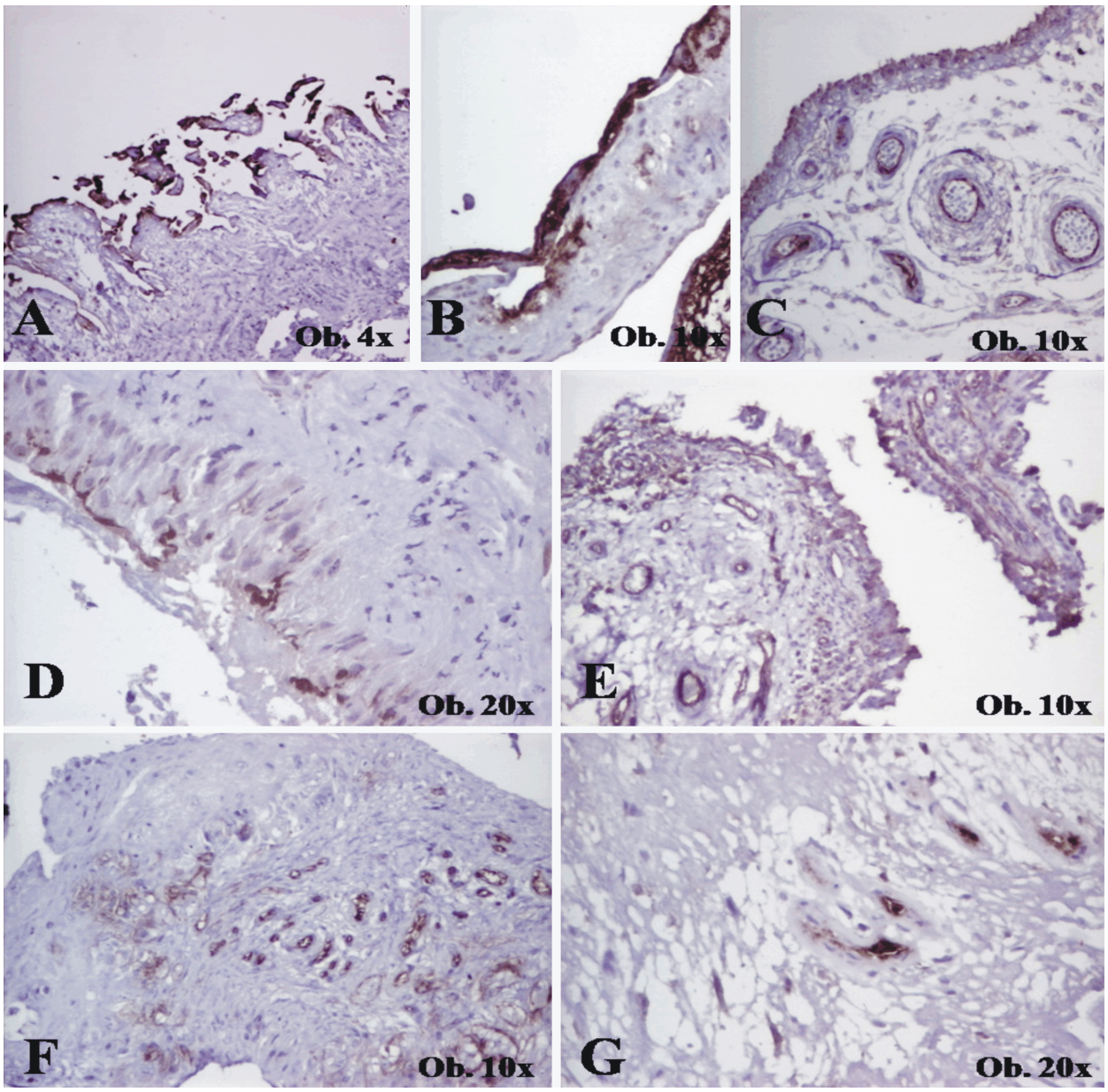

Figure 2 – ***Immunohistochemical findings: (A–C) In osteoarthritis, the synoviocytes (A) and pannus (B) are diffusely marked by VEGF and resident mature vessels can be noticed with CD31 (C); (D–G) In rheumatoid arthritis, the VEGF is focally expressed in the proliferated synoviocytes (D) and CD31 shows an intense vascular proliferation (E) with invasion of the cartilage (F) and neo-vascularization of the degenerated cartilage (G).***

In both RA and OA inconstant positivity for VEGF in the endothelial cells of large vessels was observed, in superficial and also in the deep area and adipose tissue. In RA, we also noticed that VEGF marked the endothelial cells from the vascularized areas around the degenerated cartilage.

Discussion

In most of the studies published in the last years, the authors agreed that different molecular mechanisms are implicated in the pathogenesis of bone destruction and remodeling in RA and OA [6, 7]. In both processes, the synovial inflammation plays an important role in degradation of cartilage and hypertrophy of the synovial tissue [5, 8]. At the same time, some resident fibroblasts of the synovial membrane are either activated or dye during the process of arthritis [7]. In RA, failure of apoptotic response of synovial cells leads to their aberrant proliferation and activation of specific fibroblast-like cells, which have an invasive behavior [6]. This invasivity, also observed in our cases, seems to be more specific for RA than OA.

Although it was proved that intense angiogenesis is characteristic for RA-related arthritis [4, 5, 9] it is unclear if this process is in relationship to level of inflammation and hypoxia or other independent mechanisms are implicated. The precursor cells of the neoformed vessels might be formed *in situ* but migration of circulating progenitors was also hypothesized [10]. Other researchers proved that inflammation could be responsible by neoangiogenesis but, at the same time, the new-formed vessels maintain the chronic inflammatory state by transporting of nutrients and oxygen [11]. Our preliminary results showed, in RA, sprouting of new vessels from the preexisting ones and splitting of the resident mature vessels, which can prove that RA did not required significant uptake of the circulating progenitors for angiogenesis but increased local angiogenesis might favorize the active infiltration of synovium into cartilage and its vascularization. In OA, the angiogenesis was less expressed than in RA and active local angiogenesis was not observed. Further studies in a large number of cases are necessary to elucidate this observation.

The real significance of local formation of new vessels and the differences between RA and OA remain a challenging subject. On the one hand, a proper vascularization is mandatory for local self-repair [10], and remodeling, and lack of CD34 expression associates vascular leakage, increased vascular permeability in the joints and exacerbated arthritic disease [12]. On the other hand, intense vascularization is indispensable for pannus formation and synovial proliferation [10, 13] and intra-articular inhibition of angiogenesis seems to suppress them in a dose-dependent manner and may be a new therapeutic target [9, 14]. The perivascular distribution of lymphocytic infiltrate, observed in Hematoxylin–Eosin in our study is in line to literature data. Căpitănescu B *et al.* showed that these mononuclear cells could be either B- or T-lymphocytes and are predominantly arranged surrounding the newly formed vessels [5]. If these observations were proved experimentally, the antiangiogenic drugs could be added to Rituximab, the anti-CD20 agent already used in RA [15]. To find the limit between how much is enough and how too much performing of *in vivo* studies are mandatory.

Conclusions

The angiogenesis seems to play important roles in both RA and OA. In RA, local active angiogenesis seems to predominate but in OA up taking of the circulating precursors may be more intensely involved. If further experimental researches prove indeed these aspects, intra-articular inhibition of local angiogenesis could have therapeutically impact in RA but not in OA.

Acknowledgments

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