MODERN MARKER PROTEINS IN SERUM AND SYNOVIAL FLUID IN PATIENTS WITH DIFFERENT ONSET OF KNEE OSTEOARTHRITIS: CAN WE IDENTIFY A “HIGH RISK” PATIENT PROFILE FOR INCIDENCE AND RAPID PROGRESSION OF KNEE OSTEOARTHRITIS?

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Introduction: Osteoarthritis (OA) of the knee joint often begins long before middle age, but cannot be diagnosed until it becomes symptomatic decades later, at which point structural alterations are already quite advanced. Some data suggest that the onset and rate of progression of OA is variable; however, insight on the natural history of OA remains sparse. We asked whether we could identify on early on either those patients who will experience rapid progression of disease or those who will not by a particular selective marker or a combination of markers of cartilage degradation and synthesis in the synovial fluid (SF) in context with the histopathology of the synovial tissue. Furthermore, we correlated these markers with their content in serum and associated their appearance with different onsets of OA and with the patients’ opinion about their knee and associated problems.

Methods: 68 patients with advanced OA of the knee joint, who required an artificial knee joint replacement, were randomized in patients with early onset and rapid progression of OA (<60 years of age; early OA group), in patients with the average age for advanced OA (60-70 years; average OA group) and in patients with late onset of advanced OA (>70 years of age; late OA group). With the patients’ consent preoperative serum samples, intraoperative samples of the knee joint fluid and of the synovial tissue were obtained. Aliquots of the fluids were centrifuged and the clarified supernatants were frozen and stored at -80°C until analyzed by Enzyme-linked Immunosorbent Assay (ELISA).

Clinical Parameters: Individual Body Mass Index (BMI) and gender were factored in. Laboratory Parameters: Test results for inflammation (leucocytes/nl; C-reactive protein [CrP] mg/dl) were assessed. Knee injury and Osteoarthritis Outcome Score (KOOS): The KOOS was used to assess the patients’ opinion about their knee and associated symptoms and function and ranged from 0 (“extreme problems”) to 100 (“no problems”).

Enzyme Immunoassay: Matrix Metalloproteinases (MMP-1, MMP-3, MMP-13, degradative enzymes), Tissue Inhibitor of MMPs (TIMP-1, TIMP-2), promoters of cartilage degradation (Interleukin-1beta, Tumor Necrosis Factor alpha), PGE2 (inflammatory mediator, signals pain production) were measured using ELISA kits from Ibex Technologies (Montreal, Quebec, Canada).

Results: Clinical Parameters: Gender was evenly distributed in the early OA group, in the late OA group women were in the majority (men-to-women ratio in the early group, average and late OA group: 1:1.0; 1:1.1; 1:1.8). The average age in the early, average and late OA groups was 52.8±6.1 years (n=16), 65.2±3.2 years (n=25) and 77.4±6.2 years (n=25). The lowest BMI had patients in the late OA group (27.69±4.69; early OA group: 30.47±5.69; average OA group: 31.79±6.51). Routine Laboratory Parameters: C-reactive protein (CRP) and leucocytes were highest in the early OA group, but not significant (74.1±10.7;86±2.40; average OA group: 56.6±8.17;90.2±3.43; late OA group: 50.6±9.6;6±1.53). KOOS: In the category “symptoms” the early OA group declared the most problems (41.4±15.74; average OA group: 43.69±18.49; late OA group: 50.39±28.59). In the categories “pain” and “activities of daily living” there was an increase in problems from the early OA group to the late OA group. In total there was no significant difference between the categories in the early, the average and the late OA group (Fig. 1).

Enzyme Immunoassay: Synovial fluid (SF) concentrations of MMP-1 were highest in the early OA group and average OA group (71,88±102,23 ng/ml; 70,50±173,09 ng/ml; late OA group: 37,64±47,19 ng/ml) and revealed similar tendencies with serum levels (10,72±6,82 ng/ml; 6,47±2,92 ng/ml; 5,55±2,97 ng/ml). MMP-3 exhibited highest SF levels in the early OA group (510,80±907,81 ng/ml; average OA: 2758,62±6928,32 ng/ml; late OA: 1360,95±1707,99 ng/ml) but showed no correlation with serum levels. MMP-13 reached highest SF concentrations in patients with late OA, similarly TNFalpha, TIMP-1 was not statistically different between the OA groups in SF and revealed increasing levels from the early OA to the late OA group in serum (197,95±41,44 ng/ml; 215,41±40,06 ng/ml; 242,28±42,19 ng/ml). TIMP-2 level in SF and serum were lowest in the early OA group and revealed a ratio of 1.7:1 to MMP-1 and 1:40,3 to MMP-3. This ratio improved in the late OA groups (TIMP-2:MMP-1: average OA group: 2.86:1; late OA group: 5:16,1; TIMP-2:MMP-3: average OA group: 1:13,99; late OA group: 1:14,21). SF PGE2-concentrations were highest in the early OA group. The CPII:C2C ratio was in the early and the average OA group around the half of that in the late OA group (4,75:1; 3,47:1; 3,24:1); this differences were statistically significant (p > .001). SF levels of C2C and CPII did not correlate with serum levels (r = -0.349, p = 0.81; r = -0.330, p = 108).

Histopathology: The early OA group revealed the most intensive inflammatory infiltration, activation of resident cells and activation of synovial stroma (Fig. 2). The values of all parameters summarized, the early OA group demonstrated an inflammatory grade of 2 by Krenn et al. (moderate synovitis), whereas the synovial tissue in the average OA and the late OA group exhibited slight synovitis (grade 1; Krenn et al.).

Discussion: This study revealed a significant association of the CPII-to-C2C ratio in the synovial fluid with an earlier onset and progression of OA in the knee joint. Furthermore, increased MMP-1 in serum and in the synovial fluid seemed to show a trend to a higher risk of early OA as well as lower ratios of TIMP-2 to MMP-1 and MMP-3. Inflammatory parameters to synovial fluid and synovial tissue appeared increased in the early OA group, indicating a potential contribution of inflammatory mediators to an earlier onset of OA. Clearly, patients at risk for a rapid progression should be at focus of our current and future efforts to modify, i.e., slow or stop, disease progression in OA by more precise diagnostic tools that afford to start treatment when structural alterations are not advanced.

Figures:

- Fig. 1: Knee injury and Osteoarthritis Outcome Score (KOOS) in patients with early (<60 years), average (60-70 years) and late onset (>70 years) of knee OA (100 indicates no problems, 0 indicates extreme problems).
- Fig. 2a: Example for slight synovitis (400X, HE).
- Fig. 2b: Example for strong synovitis (400X, Masson-Trichrome).

Association of the histopathological synovitis score in patient with different onset of OA of the knee joint.

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References:
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