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BIOMARKERS AND SURGICAL PREDICTORS IN TEMPOROMANDIBULAR JOINT DISEASE

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Cover: arthroscopic pictures from temporomandibular joints. Top left, adhesion in the posterior recess of the superior joint compartment. Top right, degenerative changes affecting the articular eminence and the disc. Bottom left, posterior attachment with signs of synovitis/capillary hyperaemia. Bottom right, adhesion in the anterior recess released with a coblation probe.

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Biomarkers and surgical predictors in temporomandibular joint disease

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”Bara en enda ros på ett evigt klänge
Så är livet; trist varar länge
Men underbart är kort
Alldeles för kort”
(P. Ramel)

To Monica, Gustav, Albin, Hugo, and Greta

ABSTRACT

Background: Temporomandibular joint (TMJ) disc displacement (DD) is associated with pain and impaired function and has two main sub-diagnoses, DD with or without reduction (DDwR/DDwoR). DD synovial tissue has been sparsely investigated, and further characterisation is needed. Patients with TMJ diseases might need surgery, with arthroscopy being the most common approach. However, no patient-specific surgical predictors have been identified, guiding the clinician in prognosticating the outcome.

Aim: To develop tissue-based diagnostic criteria for patients with DD of the TMJ, and to identify factors that may predict TMJ surgical therapy outcome.

Material and methods: All included studies contain patients operated with arthroscopy or discectomy, due to DDwR, DDwoR, osteoarthritis, or chronic inflammatory arthritis. Surgical outcome in the retrospective study I (n = 224), and the prospective observational cohort studies II (n = 93) and IV (n = 100) were correlated to a predefined set of patient-specific variables. Synovial tissue biopsies were harvested and analysed in studies III (n = 63) and IV. The cytokine profile of patients with DDwR was compared to that of DDwoR in study III, and protein concentrations were correlated to surgical outcome in study IV.

Results: Masticatory muscle palpation pain had a positive association to surgical outcome in studies I, II, and IV. TMJ disability, TMJ psychosocial impact, global pain, and age might be promising predictors, but needs further verification. Significant concentration differences between DDwR and DDwoR were found in 15/28 tested cytokines. Only three cytokines, interferon gamma-induced protein 10 (IP-10) (P = 0.024), osteoprotegerin (OPG) (P = 0.046), and regulated on activation, normal T cell expressed and secreted (RANTES) (P = 0.001) had lower concentrations in DDwoR compared to DDwR. In study IV, higher concentrations of interleukin 8 (IL-8) (P = 0.049), lumican (P = 0.037), matrix metalloproteinase 7 (MMP-7) (P = 0.038), and tissue inhibitor of metalloproteinase 2 (TIMP-2) (P = 0.015) were associated with an inferior surgical outcome.

Conclusions: Bilateral muscle palpation pain seems to be a predictor of unfavourable outcome indicating the surgeon to reconsider further non-invasive treatment. Furthermore, IL-8, lumican, MMP-7, and TIMP-2 were positively correlated to outcome and may serve as predictors for surgical outcome. The inflammatory profile was found to differ between DDwoR and DDwR, further establishing the difference between the two states. Other studies indicate that higher concentrations of IP-10, OPG, and RANTES could have been expected in DDwoR, which is why this contradictory result might indicate biomarker potential.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the listed scientific papers and referred to in the text by their roman numerals.

I. Ulmner M, Kruger-Weiner C, Lund B. Patient-specific factors predicting outcome of temporomandibular joint arthroscopy: a 6-year retrospective study. J Oral Maxillofac Surg. 2017;75(8):1643.e1-1643.e7.

II. Ulmner M, Kruger-Weiner C, Lund B. Predictive factors in temporomandibular joint arthroscopy: a prospective cohort short-term outcome study. Int J Oral Maxillofac Surg. 2020;49(5):614-620.

III. Ulmner M, Sugars R, Naimi-Akbar A, Suslu S, Reseland JE, Kruger-Weiner C, Lund B. Synovial tissue cytokine profile in disc displacement of the temporomandibular joint. J Oral Rehabil. 2020 Jul 8. Online ahead of print.

IV. Ulmner M, Sugars R, Naimi-Akbar A, Tudzarovski N, Kruger-Weiner C, Lund B. Synovial tissue proteins and patient-specific variables as predictive factors for temporomandibular joint surgery. *Submitted*

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LIST OF ABBREVIATIONS

ADAMTS 13	a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13
AIC	Akaike´s information criterion
ASA	American Society of Anaesthesiologists
BMP	bone morphogenic protein
CBCT	cone-beam computed tomography
CCL	chronic closed lock
CIA	chronic inflammatory arthritis
CT	computed tomography
DDwoR	disc displacement without reduction
DDwR	disc displacement with reduction
DF	dilution factor
ECM	extracellular matrix
EGF	epithelial growth factor
G-CSF	granulocyte colony-stimulating factor
GJH	general joint hypermobility
GM-CSF	granulocyte-macrophage colony-stimulating factor
ID	internal derangement
IFN-γ	interferon γ
IL	interleukin
IL-1Ra	IL-1 receptor antagonist
IL-1sR-II	IL-1 soluble receptor type II
IL-6sR	IL-6 soluble receptor
IP-10	interferon gamma-induced protein 10
MCP-1	monocyte chemoattractant protein 1

MIO	maximal interincisal opening
MIP	macrophage inflammatory protein
MMP	matrix metalloproteinases
MRI	magnetic resonance imaging
NRS	numeric rating scale
OA	osteoarthritis
OPG	osteoprotegerin
sTNFR	soluble TNF receptor
TGF-α	transforming growth factor-α
TIMP	tissue inhibitor of metalloproteinase
TMD	temporomandibular disorder
TMJ	temporomandibular joint
TNF	tumour necrosis factor
VAS	visual analog scale

1 INTRODUCTION

The aetiology of disc displacement (DD) and osteoarthritis (OA) of the temporomandibular joint (TMJ) still in the 21st century is not determined. The reason for this might be because the TMJ is the only joint in the human body that is not only pressurised during voluntary function, but also during involuntary actions due to oral parafunction, such as bruxism. Parafunction of the stomatognathic system leads to loading of the TMJ, which can be hard to both measure and detect in a clinical setting. Anamnestic information can be inconsistent and clinically only indirect signs can be seen, such as attrition and buccal and lingual impressions of the teeth. Moreover, parafunction is possibly influenced by stress, physical and psychiatric diseases [1]. These circumstances make it harder for the clinician to evaluate the individual impact of parafunction, but it also challenges the researcher. With identifying the disease aetiology, treatment planning, medical and surgical treatment, and outcomes can possibly benefit. Possible causative factors like parafunction, should be a goal for researchers to uncover.

Arthroscopy and disc operations are the most common TMJ surgical interventions and have reported success rates of 60-88 % [2-6]. A way to improve outcome is to identify patients who will benefit from a specific treatment or, those who will not. Either way, the success rate would improve in favour of the patient group and lowering the cost for treatment when performing surgery on patients who will benefit. The latter would be of importance for society from a health-economy aspect. Unveiling predictive factors might as well lead to answers or clues about the aetiology of TMJ DD and OA.

Synovial tissue proteins have the potential to be used as biomarkers in diagnostic issues, screening disease progress, and reflecting local tissue response to treatment [7]. This has not been a focus in TMJ disease research. Synovial fluid has gained a lot of interest, possibly because harvesting is easier compared to synovial tissue. Cytokines in the synovial fluid might be locally produced, for example from the synovial tissue, but could also result from extravasation due to inflammatory oedema [7-9]. Thereby the concentration of proteins in synovial tissue biopsies might describe the local status more exactly.

This project was focused on the characterisation of synovial tissue in DD and patient-specific factors that might influence TMJ surgery outcome. The patient-specific factors include demographic factors, anamnestic information, clinical measurements, and biomarkers in synovial tissue and synovial fluid.

1.1 ANATOMY AND FUNCTION OF THE TEMPOROMANDIBULAR JOINT

The TMJ is a bilateral synovial joint that creates an articulation between the mandible and the temporal bone [10, 11]. In similarity to other joints in the human body the TMJ is comprised of a convex joint head, the condylar process, which articulates towards a concave articulating fossa, known as the mandibular fossa. Both articulating surfaces are covered by fibrocartilage. Between the two bony components of the joint, an oval, dense fibrocartilage disc is positioned separating the joint into the upper and lower joint compartment. The centre of the disc is the thinnest part and serves as the discs articulating surface, sometimes called the intermediate zone. Towards the periphery of the disc, it becomes thicker with the most prominent part posteriorly, giving it a biconcave shape. It has been speculated that the discs shape might function to fill out incongruences in the joint as it is recognized that joint surfaces of the TMJ do not fit well together without the disc in place. The disc is firmly attached to the condylar process via ligamentous tissue to both the lateral and medial pole. In the anterior aspect the disc is attached to the anterior slope of the articular eminence and to the condylar head. The posterior ligamentous tissue can also be termed the posterior band or bilaminar zone. The ligament is bilaminar where the upper part/lamina attaches to the petrotympanic fissure and the lower lamina attaches to the condylar head [10, 11].

During mouth opening, the joint first executes a rotatory movement in the condyle which creates an articulation in the lower joint compartment between the condylar head and the disc [10, 11]. When the mouth is opened to a distance of about 20 mm between the incisors, the rotatory movement is replaced by a translational movement in the upper joint compartment and the disc articulates against the mandibular fossa and the articular eminence [10, 11].

The disc has been proposed to reduce friction and evidence suggests that a significant increase in friction occurs between joint surfaces after discectomy [12]. Moreover, joint fibrocartilage, including the disc, functions as a shock absorber. When pressurized, cartilage has elastic properties and the ability to excrete interstitial fluid, a phenomenon called weeping lubrication [13, 14]. An example of the importance of this mechanism occurs during tooth grinding/clenching, when the joint is under sustained pressure, the normal lubrication film from the synovial fluid is squeezed from the articulating surface resulting in cartilage to cartilage contact. The TMJ can counteract increased friction over longer periods because of the fibrocartilage and the weeping lubrication. However, during hard, prolonged pressure on the joint surfaces, eventually lubrication diminishes and the risk of joint wear is apparent [12-15]. Studies have shown that a joint without a disc, apart from higher friction at the start of loading, also rapidly increases the amount of friction compared to a joint with an intact disc

[12, 15]. The TMJ disc is composed of 75% collagen type 1, which is arranged in an anteroposterior manner at the intermediate zone in the same orientation as the translational movement of the condylar head [16]. This is thought to be important for wear properties of the disc.

Synovium outlines the intra-articular area except the loadbearing/articulating zones, and consists of a synovial lining cell layer and a sub-lining layer of connective tissue [17]. The synovial lining has a thickness of one to two cell layers, which proliferates during inflammation increasing to five or more cell layers [9]. Two cell types have been identified in the synovial lining; macrophage-like type A cells and fibroblast-like type B cells [17]. Macrophage-like type A cells are predominant in the area closest to the intra-articular space. In addition to phagocytic functions, these cells are also involved in immunological reactions, particularly during inflammation when the number of macrophage-like type A cells increases dramatically forming a thickened synovial cell layer [17]. During joint inflammation, hyper-vascularity can be observed as well as migrating inflammatory cells that infiltrate the synovial lining. The posterior attachment region of the TMJ synovium is most typically affected during inflammation [9, 18]. Fibroblast-like cell type B has a secretory function producing extracellular matrix components such as, collagen type I and type II, fibronectin, proteoglycans and glycosaminoglycans [17, 19]. The synovial fluid has a high viscosity and contains lubricin and hyaluronic acid, which both contribute to the lubrication of the joint surfaces [19]. Since cartilage is neither innervated nor has a blood supply, the synovial fluid supplies nutrition to the cartilage [19].

1.2 TEMPOROMANDIBULAR JOINT CONDITIONS

The TMJ has been recognized with several diseases or disorders, of local or systemic character. Rheumatic diseases, gout, luxation, ankyloses, benign or malignant tumours, and infections might affect the TMJ just like any other joint [10]. Below, the focus is on DD and OA.

DD is defined as the disc improperly positioned in relation to the condylar-fossa complex [20]. A synonymous term to DD is internal derangement. Several epidemiological studies have shown that DD affects up to 30 % of the population although few of those affected develops clinical symptoms that requires health-care attention [21-24]. A female predominance has been identified, which has not yet been properly explained [22, 25]. DD has been described as a sequentially developing disease which starts with DD with reduction (DDwR) that might give rise to clicking or popping from the affected joint [26]. The clicking or popping sound arises from the returning of the displaced disc to its normal position during

mouth opening [20]. The joint sound is reciprocal due to re-dislocation when the patient closes their mouth. On most occasions, displacement occurs in an anterior direction, but the disc can be displaced in all directions. DDwR seldom gives rise to any pain or functional problems, however sometimes the clicking can develop into painful clicking or catching of the disc, known as the intermediate state in the sequential deterioration [20, 26]. Painful DDwR has the potential to severely affect the patients' quality of life due to functional pain, chewing disabilities and psychosocial disturbances [26-29].

If the disc gets fixated in a displaced position and does not reduce during opening, it might work as an obstacle for the translating condyle and restrict joint movement. This is called DD without reduction (DDwoR) or closed lock which is characterized by reduced mouth opening capacity, pain upon opening the mouth and eventually OA on radiographic evaluation [20, 26]. DDwoR was diagnosed in 5 % of individuals in a Swedish and U.S. temporomandibular disorder (TMD) cohort [30]. Currently, no consensus has been defined as to when DDwoR should be categorized as chronic (chronic closed lock [CCL]), as authors use different time-frames, from one to six months [31]. CCL is accounted as late stage DD [26]. Wilkes DD staging criteria has gained wide acceptance, although reports have been made that the described sequence does not seem to be true in all cases [32, 33]. From clinical experience, there seems to be different onsets on DDwoR. Wilkes has described the usual situation, where DDwR with time might be followed by DDwoR [26]. Another situation is when patients experience DDwoR with no previous experience of TMJ clicking or intermittent locking. The latter might be described as a sudden onset (SO) of DDwoR in opposite to the more typical, above described Wilkes characterisation of delayed onset (DO) of DDwoR.

OA is a low-grade degenerative joint disease that affects the cartilage and bone of the joint [34]. OA can affect the TMJ as well as other joints, with DDwoR patient's typically showing signs of OA [26]. Patients with painful DDwR are rarely affected. This may be attributed to the reduced inflammatory state compared to DDwoR, and the lower levels of cytokines that drive the inflammatory process leading to cartilage and subchondral bone degradation [35]. Of course OA can affect the TMJ without any prior DD and no evidence has been presented showing that one must lead to the other [33]. In some cases, the aetiology behind OA in the TMJ could be DD as mentioned above, but other causes could exist although sometimes obscure. Evidence exists of post-trauma OA, ageing OA, crystal OA and metabolic syndrome OA [34].

1.3 AETIOLOGY OF DISC DISPLACEMENT

Several thoughts and opinions on the aetiology of TMJ DD have been reported or discussed, including trauma, bruxism, general joint hypermobility (GJH), hormonal, and infectious causes either individually or together [36-49]. Trauma may be the single most accepted reason that leads to the development of DD [37, 41, 45]. The trauma itself can, for example, inflict immediate injury to the disc and ligamentous tissues causing displacement of the disc, capsular tear, hemarthrosis, cartilage lacerations. Reports demonstrate that the more violent the trauma, the worse the damage to the joint soft tissue [50].

GJH has been suggested as another possible causative factor for DD, although divergent conclusions are reported [39, 46, 49]. The extended range of motion resulting from GJH is proposed to induce mechanical overload, which might result in DD. An additional explanation has been linked to alterations in collagen composition allowing for faster deterioration [51].

Despite some knowledge concerning DD aetiology, the conclusion drawn by Kiehn and Desprez (1962), was that few patients could define trauma as a cause, whereas the instigating factor could not be identified in the remainder of patients [52]. This conclusion remains true to this date.

1.4 DIAGNOSTIC EVALUATION OF TEMPOROMANDIBULAR JOINT DISC DISPLACEMENT

Patients with DD often have musculoskeletal symptoms in the orofacial area and a thorough examination has to be conducted in order to identify patients with strict muscular diagnosis or when other differential diagnoses are suspected. Firstly, the patients' anamnesis must be surveyed with respect to onset, duration, severity of symptoms, medical history, including past and present medication, heredity, social situation, general joint problems for example. As to self-graded evaluation, for example with pain and functional impairment, a visual analogue scale (VAS) or numeric rating scale (NRS) has often been used [10, 20, 53].

The clinical evaluation must include palpation of masticatory muscles on both sides, in particular the masseter and the temporal muscles. Palpation of the TMJ from the lateral aspect at rest and during opening and closing movements, as well as protrusion and lateral excursions allows assessment of pain, clicking, crepitus, restricted or asymmetric mobility. Visual inspection of the mandibular movements during both opening and closing is of importance. Maximal interincisal opening (MIO), maximal protrusion and lateral excursion to both sides have to be measured with a millimetre-graded ruler. Examination of teeth,

periodontal status and occlusion are also mandatory and should be performed with probes, palpation, percussion, and sensibility tests if necessary. Signs of parafunctions, such as excessive attrition, tongue and buccal impressions, should be documented [10, 20, 53].

If the anamneses and clinical examination indicates that further investigation is necessary imaging often starts with an orthopantomogram. This is a useful diagnostic and cost-effective entity that often can rule out other possible causes for the present symptoms, such as odontogenic infections, cysts, and coronoid hyperplasia. However, it is not an acceptable tool for diagnosing TMJ diseases, and other options have to be considered [54]. Computed tomography (CT) or cone beam CT (CBCT) are the radiographic examinations of choice when possible hard tissue changes need evaluation [55, 56]. Magnetic resonance imaging (MRI) is often regarded as the gold standard for the evaluation of TMJ with medical imaging [57]. It is superior to CT/CBCT in diagnosing soft tissue changes and in depicting the disc status. MRI is also superior in diagnosing TMJ inflammatory states where joint effusion and synovial proliferation might be present, which is an advantage over CT where only indirect changes, like hard tissue erosions can be shown. A putative draw-back to MRI is the less accurate hard tissue diagnostics, such as fossa integrity and early local erosive changes. Ultrasound of the TMJ has been increasingly used, especially in children with juvenile idiopathic arthritis [58]. TMJ ultrasound findings might be difficult to interpret, partly due to that hard tissue anatomy does not allow visualisation of the entire joint compartment, such as the medial and the most superior part of the fossa [59, 60].

In summary, the required imaging modality needs to be determined on an individual basis considering the possibilities and limitations of each method at hand in relation to the clinical findings.

1.5 TREATMENT OF TEMPOROMANDIBULAR JOINT DISC DISPLACEMENT

A consensus for non-surgical intervention has been established as the primary choice to treat TMJ disorders/DD whenever applicable [31, 61]. Approaches consist of information, occlusal splints, physiotherapy and pharmaceutical treatments as examples. If non-surgical treatments fail, there are surgical options to consider in the context of diagnosis and disease severity.

Arthroscopy and arthrocentesis are both minimally invasive surgical options [62, 63]. Initially, arthroscopic intervention was described as a diagnostic tool, which soon developed into the commonly used arthroscopic lysis and lavage [64]. In this procedure apparent adhesions and fibrous bands are released in a semi-blind manner with a blunt obturator and the joint thoroughly flushed concurrently with saline solution or Ringer-Acetate [64].

DDwoR is the main indication for this treatment, but any condition with a suspected inflammatory cause can be considered [64-67]. Arthroscopic surgery is a further development of arthroscopic lysis and lavage where the so-called triangulation technique is used. Two or more ports of entry into the upper joint compartment are used and the operator is able to introduce devices, such as coblator probes, biopsy forceps, and shavers, to instrument the joint under direct visualisation [68].

Open TMJ surgery has a longer history of practice in treating DD [69, 70]. The most prevalent open surgical method in Sweden is discectomy, used primarily to treat handicapping DDwR with reported success rates of 80-85% [5, 67, 71]. Long-term follow-up shows good function and pain free motion even after 30 years [71-73]. The downfall of this method is that a small percentage clearly and quite rapidly deteriorate, resulting in more pain and sometimes in fibrous or even bony ankyloses [74].

Modified vertical ramus osteotomy or condylotomy is an extra-articular method to treat DD [75, 76]. With this method the condylar process is osteotomised and consequently drops creating a larger space between the joint surfaces. In this manner, handicapping DDwoR often resolves, although occlusion is always altered but can be compensated for using temporary guiding elastics [75, 76]. This latter method is judged to be slightly inferior compared to discectomy by the Swedish Board of Health and Welfare because of less scientific support [67].

Several different methods to surgically reposition the disc have been evaluated, sometimes with impressive results, but generalisability to a larger population of surgeons have not been shown [77-80].

1.6 PREOPERATIVE PREDICTORS FOR TEMPOROMANDIBULAR JOINT ARTHROSCOPY

Arthroscopy is the most frequently used TMJ surgery method in Sweden according to the Swedish Board of Health and Welfare (http://www.socialstyrelsen.se/statistik/statistikdatabas). Since no TMJ surgical method has a 100% success rate, the ability to predict treatment outcome on an individual basis is desirable. Unfortunately, data on positive or negative predictors or prognostic factors influencing the outcome of arthroscopic surgery is limited.

TMJ surgical predictors found in the literature are reviewed below and highlighted in bold italic in the introduction, results and the discussion section, to simplify reading.

Comorbidity as a predictor for TMJ surgical outcome has been investigated. A reported comorbidity is fibromyalgia, where no differences in outcome has been detected between fibromyalgia-, and non-fibromyalgia patients in the DD patient group investigated [81]. Other variables, such as gender, age at surgery, and duration of symptoms have also not been found to be predictive of outcome [81]. Psychological evaluation before TMJ surgery has been proposed because TMD patients seem to have a more pronounced psychological burden compared to a non-TMD population [1, 29]. Stress as a factor for parafunction has been shown, suggesting that mental distress might counteract the beneficial effects of surgery. This is supported in a study on patients with high chronic anxiety score, who were shown to have lower pain reduction after arthroscopy than other patients in the study [82]. The use of benzodiazepines, which might be an indirect measure of psychiatric disease, has been found to be correlated to an unsuccessful outcome of arthroscopic surgery [83].

MIO as a preoperative variable has been evaluated in relation to surgical outcome with divergent conclusions [84, 85]. Findings indicate that preoperative MIO cannot be used as a predictor since a study including 167 patients showed no significant difference [84]. Although, 14 patients included in another study showed a difference in preoperative MIO when comparing outcome [85]. However, these latter results should be interpreted with caution because of the limited patient cohort [85].

Disease grading systems have been suggested as predictors for surgical outcome. Wilkes classification for DD was evaluated as a preoperative predictor of outcome but no differences between the different stages of DD were detected [86, 87]. Dimitroulis TMJ surgery classification, an alternative to Wilkes criteria, defined five different categories from normal TMJ as first stage and thereafter in descending order to stage five, denoted as catastrophic TMJ changes [88]. Stage 1-3 arthroscopy success rates were found to be 90-100 % and stages 4 and 5 all had unsuccessful outcomes with the same surgical procedure [84]. The Dimitroulis TMJ surgery classification remains to be evaluated by independent researchers.

Patients age has been investigated as a predictor for outcome with conflicting results. No statistical differences have been found in two studies, although a trend towards younger age in the successful groups were noted [89, 90]. On the contrary, in another study significant failure-rates and progression to open surgery in younger patients groups (21-30 years old) compared to a 60 years and higher age groups were found [84]. In the same study, using mean-age instead of age-group, comparison did not reveal any differences in surgical outcome [84]. A dichotomous age variable, older or younger than 40 years, was used when evaluating arthroscopy and both groups showed a significantly better outcome when

measuring MIO and pain [91]. However, postoperative pain was significantly lower in the older age group [91]. In summary, no clear evidence on age affecting surgical outcome could be found.

Gender has been proposed as a predictor for surgical outcome, particularly due to the strong female predominance [22, 25]. Myofascial pain, which is a TMD diagnosis, has been reported to be more prevalent among women in a Swedish population [92]. Although, TMD incidence in a large population study revealed that there was no significant difference between genders [93]. A six-month follow-up revealed that 54% of women and 41% of men had persistent problems, which might indicate a prevalence/incidence problem when measuring disease. Women might experience persistent TMD to a higher degree than men indicating higher prevalence amongst women [93]. No association between surgical outcome and gender were found in the limited amount of published papers on the topic, calling for further investigations [84, 90].

A systematic review about timing of intervention when diagnosed with DDwoR, either acute or chronic, both non-surgical and surgical action were reviewed, but no definitive conclusion could be made [31]. The authors recommended that surgery should be postponed for six months or more from ***TMJ symptom duration*** onset of DDwoR [31]. Late versus early surgical intervention has been investigated where mean symptom onset were 33 months and 5.4 months respectively [94]. Pain score decreased significantly in the early intervention group compared to late. No group differences were recorded regarding increase of MIO [94]. Divergent findings were declared where duration of TMJ symptoms did not affect outcome in a statistical significant way [89, 90].

A 0 to 10-graded VAS or NRS has been frequently used for patient self-assessment of ***TMJ pain*** when evaluating outcome of arthroscopy [66, 83-85, 94, 95]. TMJ pain severity prior to surgery has been suggested as a predictor of outcome, where higher self-assessed pre-operative pain has been found to be correlated to a worse surgical outcome [83, 90], opposed by the findings of Breik et al. who found no impact of preoperative VAS on surgical outcome [84].

As highlighted above, several demographic and clinical variables have been proposed and evaluated as predictive factors for surgical outcome, although few if any could be stated as definitive at the moment.

1.7 SYNOVIAL FLUID AND SYNOVIAL TISSUE CHARACTERISATION

Synovial fluid, in relation to synovial tissue, can be more easily harvested and has often been the choice for sampling and analysis. The TMJ has a negative intra-articular pressure and the synovial fluid has a high viscosity so it is not possible to extract synovial fluid directly from the joint under normal circumstances. Synovitis increases the volume of synovial fluid but even with substantial TMJ inflammation present it is hard or impossible to get an exchange of synovial fluid from simple cannulation and aspiration [96]. The methods used to sample synovial fluid are basically the same but with a few deviations. The upper TMJ compartment is cannulated and saline solution injected to "wash" the joint. The saline solution is then extirpated or repeatedly washed and thereafter extirpated [97-100]. The main drawback of these methods is that the volume of synovial fluid extracted is unknown and subsequent evaluations are limited. The addition of vitamin B12 to the saline washing solution is one method that has been developed to determine the volume of synovial fluid in the aspirated washing solution, which can be subsequently analysed in a spectrophotometer [101-103]. In this way representative concentrations can be determined for the sought-after components and furthermore allow comparison between similar studies [101-103]. In contrast, synovial tissue can only be sampled during open surgery or via an arthroscopic procedure, either semi-blind or under direct visualisation/triangulation.

In a healthy joint the cells constituting the synovial membrane are mostly fibroblast-like type B, and macrophage-like type A cells [10, 104]. When synovial macrophages are activated due to inflammation they produce a number of pro-inflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6 [19, 104]. Several articles have been published that have focused on the cytokines present in the TMJ synovial fluid in relation to different TMJ diagnoses, such as DD and rheumatoid arthritis [35, 103]. In addition, a few articles have characterised cytokines profile in normal joints [8, 105]. As a result, the evidence suggests that cytokines are immunomodulating molecules that act in the TMJ during DD mediating ligamentous degradation, pain, chondral-, and bone-degeneration [106, 107].

1.7.1 Cytokines in synovial fluid from healthy temporomandibular joints

As mentioned, few studies have analysed cytokine content in TMJ synovial fluid in healthy individuals. A study using the vitamin B12 method identified TNF-α to be present in 33 sampled joints, with the presence of interferon-γ (IFN-γ) and IL-2 in some [8]. IL-1β, IL-6 and IL-10 were only detected in two, one and one joint, respectively [8]. In contradiction, no detection of TNF-α but low concentrations of IL-1β were found among TMJ healthy controls [35]. No cytokines were detected in an investigation of healthy joints where ten different

cytokines were studied [105]. Furthermore, this study highlights the difficulties in synovial fluid sampling, as only seven samples from 33 were not contaminated with blood and thus eligible for evaluation [105].

1.7.2 Cytokines in synovial fluid from temporomandibular joint affected by disc displacement and osteoarthritis

Elevated TNF-α levels correlated positively to pain and/or DD/OA in the joint compared to controls [35, 98, 108]. TNF-α concentration has been shown to correlate positively to concentrations of IL-6 and soluble TNF receptors I and II (sTNFR-I, sTNFR-II) [98]. In rheumatoid arthritis patients, TNF-α has also been shown to mediate cartilage and bone destruction in the TMJ [109]. Magnitude of TNF-α synovial fluid levels was crudely proportional to the severity of TMJ destruction that was assessed radiographically, suggesting it to be an important pro-inflammatory TMJ cytokine [35, 98, 108, 109]. However, partly contradictory results were found measuring TNF-α and IL-6 in DD and TMJ healthy subjects, where no significant differences could be detected [110]. Other studies have found correlations between diseases in the TMJ and levels of TNF-α, IL-6, and IL-1β [107]. IL-6, IL-1β, IL-8, IL-11 have been found in higher concentrations in DD/OA related to radiographic detection of hard tissue degeneration, although no correlation to clinical symptoms as pain and mouth opening capacity were seen [35, 98, 111, 112]. Elevated levels of IL-10 were shown to correlate to good outcome of arthrocentesis due to DDwoR, which might be coupled to IL-10's anti-inflammatory properties [111]. IL-6, on the contrary, had significant correlation to unsuccessful results, regarding both detection rate and concentration. The unsuccessful results from arthrocentesis might also be related to a deficient IL-10 response in the inflammatory cytokine cascade [111]. Osteoprotegerin (OPG), a member of the TNF-receptor family, showed significantly lower concentrations in OA patients compared to both controls and DD [113]. The osteoclastogenesis inhibitory function of OPG indicates a bone protective function that diminished with OA. TNF-α and IL-1β were not related to the OPG levels in this study [113]. Cytokine concentrations of TNF-α, IL-6, IL-1β, sTNFR-I, sTNFR-II, IL-6 soluble receptor (IL-6sR), IL-1 soluble receptor type II (IL-1sR-II), and IL-1 receptor antagonist (IL-1Ra) did not correlate with clinical symptoms, such as pain from TMJ and mouth opening, and a weak negative correlation was observed between all cytokine receptors and VAS pain [98].

Contradictions occur, but cytokines seem to mediate and modulate inflammation in the TMJ and therefore have a probable role in the eventual chondral- and bone-deterioration. Weak associations between cytokine receptors sTNFR-I, sTNFR-II, IL-6sR, IL-1sR-II and VAS

pain have been shown, but otherwise no cytokines seem to correlate with clinical symptoms suggesting an intricate cytokine interaction in inflammation [35, 98, 111, 112]. Radiographic deterioration or inflammation seems to positively correlate with cytokines TNF-α, IL-1β, IL-6, IL-8, and IL-11 and negatively to OPG [35, 98, 108, 111, 113].

Some studies reviewed are of pioneer status in this context and each report adds knowledge to the community dedicated to TMJ conditions. It is worth mentioning that many studies did not find cytokines of interest in more than 20-40% of the samples, but it is reasonable to assume that the methods of analyses were not sensitive enough given the very small retrievable and in washing solution diluted volumes of synovial fluid [8, 35, 98, 105, 111, 112]. Few of the above listed studies quantify the amount of synovial fluid obtained. This is a flaw and should be considered.

1.7.3 Synovial tissue cytokines in disc displacement and osteoarthritis

Compared to synovial fluid, few studies have analysed cytokine content in synovial tissue. TNF-α and IL-1β have been localized to the synovial lining and endothelial blood vessel cells in synovial tissue, and seems to relate to the histological grading of synovitis [114]. Immunohistochemical staining of synovial tissue biopsies from the posterior attachment of DD and OA patients, found OA patients to have significantly increased localization of IFN-γ, IL-1ra and CD68 stained cells [115]. IL-1α, IL-1β, and CD45RO stained cells were most frequently localized in both patient groups. Observed less frequently were TNF-α, IL-1ra, and IL-2. Both patient groups had similar VAS self-assessment of TMJ pain and similar mandibular function impairment questionnaire scores, which was perhaps why this could not be related to any differences in cytokine or cell localization [115]. Analysis of IL-8 in synovial tissue and synovial fluid of DDwoR patients were compared to healthy controls, for example non-inflamed habitual luxating joints [116]. Synovial fluid IL-8 concentration and the number of IL-8 positive synovial tissue cells in DDwoR patients were significantly higher compared to controls. IL-8 positive cells could be neither correlated to preoperative VAS functional pain nor to infiltrating cells around blood vessels [116].

Synovial cells were isolated from synovial tissue biopsies, cultured and then exposed to pro-inflammatory cytokines in two *in vitro* studies [117, 118]. IL-1β was used to treat the cultured synoviocytes, which increased cellular production of monocyte chemoattractant protein-1 (MCP-1) compared to controls [118]. IL-1β enhanced MCP-1 production in synovial tissue of rat and induced synovitis. MCP-1 positive cells were demonstrated in the synovial tissue, indicating migrating monocytes or synoviocytes producing MCP-1 or both. This suggests MCP-1 as a part of TMJ inflammation [118]. Both TNF-α and IL-1β were used to treat

cultured synovial cells and the gene expressing macrophage inflammatory protein (MIP) 3α was found to be significantly upregulated [117]. A positive concentration-dependent relation between excretion of MIP-3α and concentration of IL-1β and TNF-α were found, implicating MIP-3α as a part of the inflammation process in TMJ.

To summarise, analysis of synovial tissue and cytokines or other signalling-peptides has mainly employed immunohistochemical analysis and *in vitro* experiments with cultured synovial cells. Antibody staining of paraffin-formalin fixed biopsies aimed at localizing TMJ production of cytokines, including IL-1β, TNF-α, IL-8 and IFNγ has been demonstrated [114-116]. Synovial cell response to IL-1β and TNF-α has been shown both *in vivo* and *in vitro* with sequential excretion of chemoattractants, like MIP-3α and MCP-1 [117, 118]. However, the drawback of *in vitro* studies is that they do not recapitulate the *in vivo* situation, where a vast variety of different signalling-peptides interacts in an extremely complex setting. On the other hand, these are the best approaches at hand, as it is impossible to embrace the complexity of immunological responses without investigating single cytokines in the inflammatory interactivity of the TMJ.

2 AIMS

2.1 GENERAL AIM

The general aim with this project was to develop tissue-based diagnostic criteria for patients with DD of the TMJ, and to identify factors that may predict the outcome of surgical therapy of the TMJ.

2.2 SPECIFIC AIMS

- To analyse patient-specific variables in relation to surgical outcome by studying a patient cohort previously subjected to TMJ arthroscopic lysis and lavage.
- Prospective evaluation of potential predictors of surgical outcome in relation to the diagnoses DDwoR, OA/arthralgia, and chronic inflammatory arthritis (CIA).
- To evaluate synovial tissue characteristics and quantify cytokine concentrations in relation to the diagnoses DDwR and DDwoR. A secondary aim was to scrutinise the sub-diagnosis DDwoR-DO and DDwoR-SO according to cytokine concentrations and eventual differences.
- Relate synovial tissue cytokine concentration to outcome of surgery. The secondary aim was to investigate eventual correlations between surgical outcome to different subjective and objective patient-specific variables.

3 MATERIAL AND METHODS

3.1 STUDY DESIGN

All four studies were performed at the Department of Craniofacial Diseases (formerly Department of Oral and Maxillofacial Surgery), Karolinska University Hospital, Stockholm, Sweden, in accordance with the Declaration of Helsinki.

3.1.1 Study I

The regional ethics committee approved a retrospective study (reference number: 2014/1087-31). The study population consisted of patients who had arthroscopic lysis and lavage within the period of the 1st of January 2008 to the 31st of December 2013. No informed consent was needed to access the medical chart.

3.1.2 Study II

A prospective observational clinical trial with four different diagnostic groups was performed after an ethical approval from the local ethical committee (reference number: 2013/1575-31/3 and amendment 2014/764-31/2). Patients' written informed consent was mandatory before inclusion. A power calculation was made, based on the assumption that an above all mechanical aetiology (delayed onset) had a surgical success rate of 40% and that a primarily inflammatory DDwoR (sudden onset) had a success rate of 80%. A power of 80% and $P = 0.05$ should be reached with 23 patients in each group. A calculated drop-out rate of 10% would give 25 patients in each group. The four diagnostic groups were: DDwoR with sudden onset, DDwoR with delayed onset, OA, CIA. All patients were planned for arthroscopy and anamnestic-, clinical-, as well as perioperative variables were collected. Outcome of surgery were assessed at the control six months postoperative.

3.1.3 Study III

A prospective observational clinical study comparing cytokine concentrations between DDwR and DDwoR had an approval from the ethical committee (reference number: 2014/622-31/1). Written informed consent was mandatory before inclusion. A power calculation based on earlier findings of differences in IL-6 concentration between DDwR and DDwoR patients were made [35]. A power of 80% and $P = 0.05$ should be reached with 23 patients in the DDwR group and 46 in the DDwoR group. A calculated drop-out rate of 10% gave 25 and 51 patients in each group respectively. Patients with handicapping DDwR had discectomy and DDwoR patients had arthroscopy.

3.1.4 Study IV

Patients with the diagnoses DDwR, DDwoR, OA, and CIA were consecutively included in a prospective observational cohort study (reference number: 2014/622-31/1). The inclusion period was from December 2014 to January 2017 and a written informed consent was mandatory before inclusion.

3.2 INCLUSION AND EXCLUSION CRITERIA

In studies II-IV all subjects had to have tried non-invasive therapy for at least three to six months before surgery was considered. The non-invasive treatment could be an orthotic splint, physiotherapy, anti-inflammatory medication, etc. In study I, this was not a pre-requisite since the surgical unit did not have these guidelines at the time.

3.2.1 Study I

All patients that had arthroscopic lysis and lavage because of the diagnosis DDwoR, OA/arthralgia, and CIA were included. If the outcome of surgery was not possible to determine from the medical record the patient were excluded.

3.2.2 Study II

Consecutive adult patients referred to the surgical unit with a diagnosis of DDwoR (sudden or delayed onset), OA/arthralgia, or CIA were eligible. All patients had their diagnosis after clinical and radiographic examination in accordance with DC/TMD [20]. In addition, DDwoR patients had to have a MIO of ≤ 35 mm and all patients had to have functional pain and/or functional impairment of VAS ≥ 4. Exclusion criteria were patients younger than 18 years, patients who previously had discectomy, and patients unable to give informed consent.

3.2.3 Study III

Consecutive patients, 18 years or older, referred to the surgical unit with DD (DDwR or DDwoR) were included. Diagnoses were set after clinical and radiological examination [20]. All patients had to have functional pain and/or functional impairment of VAS ≥ 4. A restricted MIO of ≤ 35 mm was required for patients with DDwoR. Patients younger than 18 years were excluded as were patients medicating with any disease modifying anti-rheumatic drugs, patients with CIA, and patients previously treated with discectomy.

3.2.4 Study IV

The same inclusion and exclusion criteria as in study II, except that patients with DDwR were also included. The CIA diagnosis had to be confirmed by a rheumatologist.

3.3 CLINICAL INVESTIGATION

3.3.1 Study I

The medical charts were searched for variables recorded preoperatively and at the postoperative six months follow up. Pre- and perioperative clinical variables searched for were: age, gender, TMJ diagnosis, physical and psychiatric diagnosis, duration of TMJ problem, trauma to the head or neck region prior to TMD, joint hypermobility, tinnitus and/or ear fullness, American Society of Anaesthesiologists (ASA) scoring (ASA 1-4), previous TMJ surgery, operated joint, lavage volume, arthroscopic grading of synovitis, fibrosis and degenerative changes [18, 119]. Subjective VAS-grading of TMJ functional pain, TMJ functional impairment, TMJ psychosocial influence, and global pain were collected both pre- and postoperatively, as well as objective measures of MIO, pain on lateral palpation of TMJ, pain on palpation of masticatory muscles. Successful surgery was appraised when MIO were ≥ 35 mm and TMJ functional pain were ≤ VAS 3.

3.3.2 Studies II and IV

Clinical examinations were performed preoperatively, one week postoperative and one, and six months after surgery. Demographics, patient history, and clinical variables were recorded for each patient as in study I. In addition: protrusive and lateral excursions to both sides were recorded, Wilkes criteria were assessed by two of the authors (M.U. and B.L), GJH were registered in agreement with the Beighton score [120]. All objective measures were investigated and measured in accordance with DC/TMD [20]. TMJ-, and masticatory muscle palpation pain were recorded in three categories: no palpation pain, unilateral pain, or bilateral pain. Cut-off for successful treatment was MIO ≥ 35 mm, and subjective scoring of TMJ pain, TMJ disability and TMJ psychosocial influence showing ≥ 40% improvement or a value of ≤ 3. Good treatment outcome was deemed with a MIO of ≥ 35 mm and one or two of the subjective scoring of TMJ pain, TMJ disability, and TMJ psychosocial influence showing ≥ 40% improvement or a value of ≤ 3. An intermediate outcome was noticed when MIO and subjective scoring did not show any improvement, and lastly, a deteriorated outcome showed MIO and subjective grading worse than before treatment.

3.3.3 Study III

Clinical examination was performed preoperatively and demographic-, patient history-, and clinical variables were registered as per study II.

3.4 SURGICAL INTERVENTION

3.4.1 Study I

All patients had arthroscopic lysis and lavage together with either general anaesthesia or with sedation [64]. Registration of lavage volume (mL), and grading of synovitis (0-3), fibrosis (0-2), and degenerative changes (0-3) were extracted from the medical charts [18, 119].

3.4.2 Study II

The participants had TMJ arthroscopy under general anaesthesia [64]. During surgery, lavage volume (mL) and arthroscopically graded synovitis (0-3), fibrosis (0-2), and degenerative changes (0-3) were recorded [18, 119].

3.4.3 Study III

Patients diagnosed with DDwoR had TMJ arthroscopy and those diagnosed with handicapping DDwR discectomy. All included patients had the interventions under general anaesthesia [64, 71].

3.4.4 Study IV

Patients with DDwoR, OA, and CIA had TMJ arthroscopy and those diagnosed with handicapping DDwR had discectomy [64, 71]. All interventions were performed under general anaesthesia.

3.5 SAMPLE COLLECTION

Synovial tissue and synovial fluid samples were taken in studies III and IV, but not in studies I and II.

3.5.1 Collection of synovial fluid

Harvesting synovial fluid in a predictable manner from small joints like the TMJ is not possible without using some sort of washing solution. All included patients had synovial fluid harvested with a push-and-pull method according to a specified protocol [102].

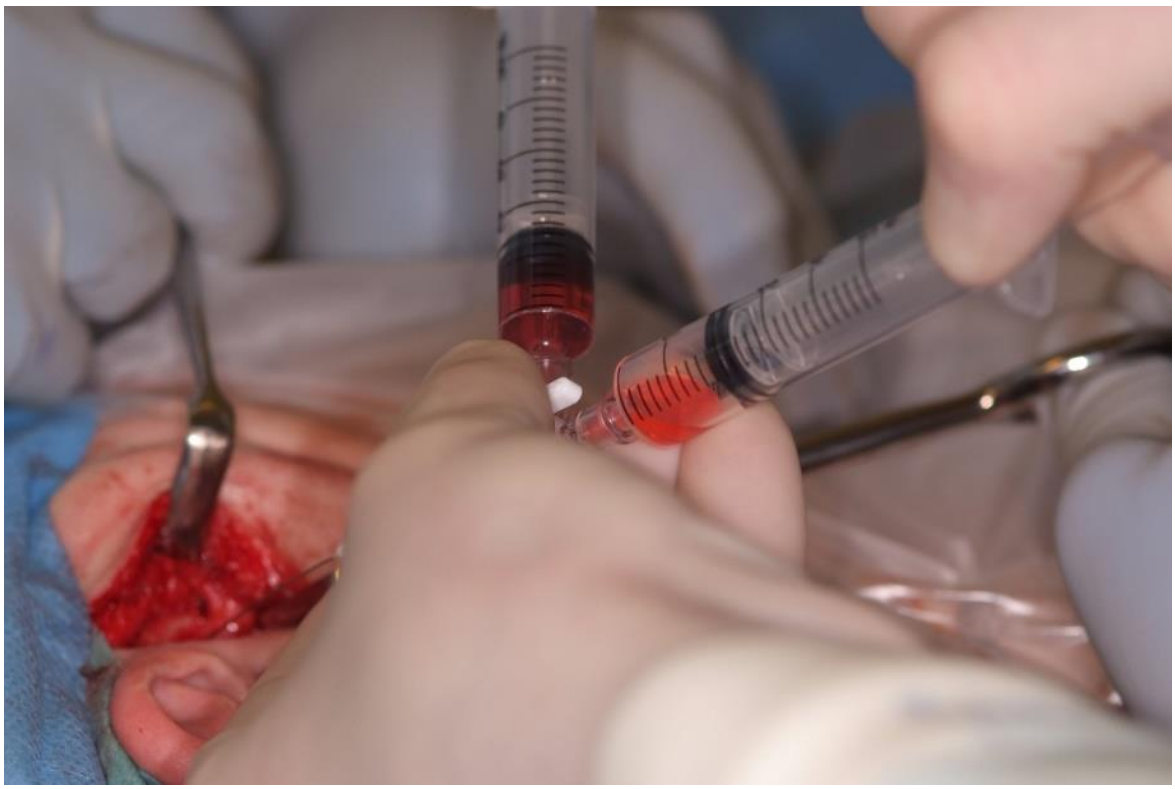

Figure 1. Sampling of synovial fluid with the push-and-pull method during discectomy, before incision of the lateral joint capsule.

Briefly, a mixture of saline solution (9mg/mL) and vitamin B12 (Behepan [hydroxocobolamin 1 mg/mL], Pfizer Inc, New York, NY, USA) was mixed and used as a washing solution with a total volume of 4.0 mL. The push-and-pull method included two syringes of 5 mL mounted together on a stopcock valve. One of the syringes should be empty and the other contain the washing solution. After penetrating into the superior joint compartment with a needle, 1.0 mL of the washing solution was injected into the TMJ, after which the empty syringe was used to aspirate. This procedure was repeated four times, 1.0 mL at a time, so that all the washing solution has been injected and stepwise aspirated. The synovial fluid samples were harvested under general anaesthesia but before introduction of any fluids in the TMJ. In the case of arthroscopy, the sample was taken before actual arthroscopy, and in the case of discectomy synovial fluid was harvested before incising the joint capsule (Figure 1). The retrieved volume of synovial fluid was then possible to evaluate by analysing the dilution of the washing solution [102].

3.5.2 Collection of synovial tissue

Synovial tissue biopsies were sampled during surgery and always from the superior aspect of the posterior bilaminar zone (i.e. the area facing the upper joint compartment).

Triangulation technique was used to harvest synovial tissue biopsies of approximately 4 mm^2 during arthroscopy (Figure 2) [68].

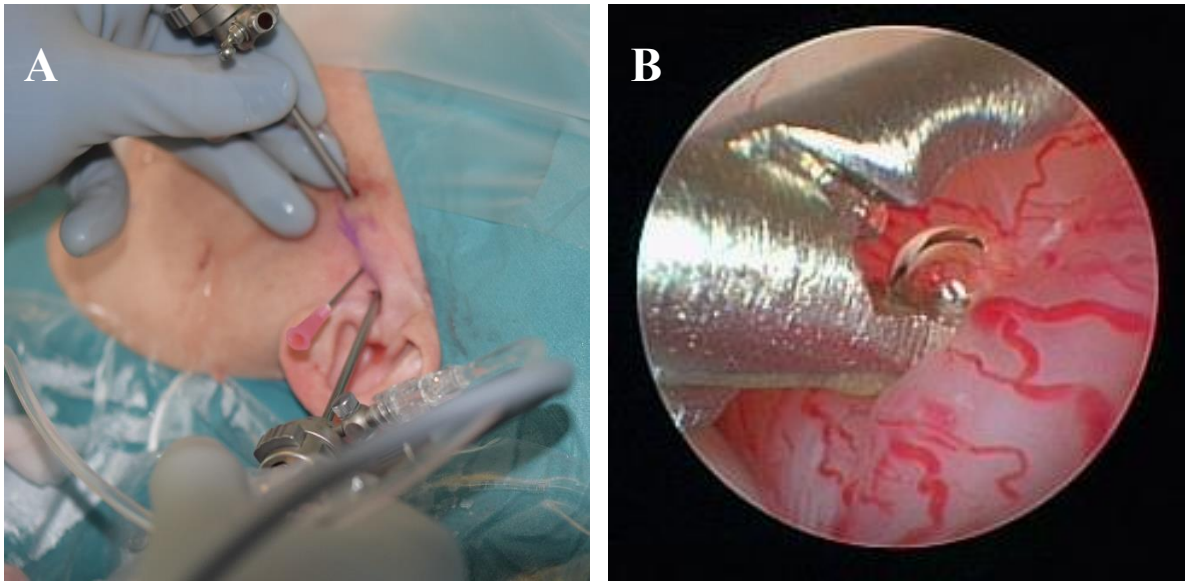

Figure 2. A) The triangulation technique with two instrument canals and one out-flow needle. The instrument canal closest to the patients' ear containing the optics, and the other containing the biopsy forceps. B) A photograph from the posterior recess of the superior TMJ compartment showing the working end of the biopsy forceps taking a synovial tissue sample from the posterior bilaminar zone.

When performing discectomy, the synovial tissue samples were taken as the disc was removed. The samples were then put in RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) and refrigerated for 24 hours followed by storage in -80 degrees freezer after RNAlater had been removed. One synovial tissue sample was put in 4% paraformaldehyde for fixation and then subjected to routine histology assessment after being paraffin embedded, sectioned, and stained with haematoxylin and eosin (H&E, Histolab Products AB, Gothenburg, Sweden).

3.6 SYNOVIAL FLUID PRE-ANALYSIS, DILUTION FACTOR

The dilution and quality of the synovial fluid aspirate was determined before storage. The weight of the aspirated sample had to exceeded 0.5 mg to be considered as reliable [103]. Since blood contains cytokines like the synovial fluid, blood contamination could skew the analyses. Blood contamination was evaluated visually according to a four-graded scale where grade 0-1 (no or hardly visible blood contamination) was appraised as functional [103]. The synovial fluid sample was then centrifuged and the resulting supernatant analysed in a spectrophotometer (Hitachi U-2000, Hitachi Ltd, Tokyo, Japan) [103]. The resulting absorbance value from the synovial fluid sample and an undiluted reference washing solution was used to determine the dilution factor (DF) according to the formula:

$DF = Abs_{Asp}/Abs_{Wash}$.

Abs_{Asp} refers to the absorption value of the aspirate and Abs_{Wash} refers to the absorption value of the washing solution. The DF had to be ≤ 0.985, otherwise a too small portion of the aspirate contained synovial fluid, making analysis of fluid contents unreliable or unfeasible [103].

3.7 SAMPLE ANALYSES

Only applicable to studies III and IV.

3.7.1 Assessment of total protein content

The total protein concentration for all fluid and tissue samples were determined with the Qubit Fluorometer together with Qubit protein assay (Thermo Fisher Scientific). The normalised ratio, i.e. the quota of the specific protein concentration and the total protein concentration, was then calculable.

3.7.2 Specific proteins in synovial fluid

Synovial fluid was analysed with Quantikine ELISA High Sensitivity Kits (R&D systems, Bio-Techne Corp., Minneapolis, MN, USA) with respect to cytokines IL-1β (HSLB00D), IL-6 (HS600B), IL-8 (HS800), IL-10 (HS100C), and TNF-α (HSTA00D). Due to the dilution of the samples according to the above described method, all results were adjusted with the DF in order to obtain the real concentration of the synovial fluid cytokines.

3.7.3 Specific proteins in synovial tissue

The synovial tissue samples were treated to extract the protein contents. The method used was designed to isolate small samples of tissue obtained from arthroscopy [121]. The extracted proteins were analysed with the multi-analytic profiling system Luminex 200 (Luminex Corp., Austin, TX, U.S.) using the immunoassays:

- Human Cytokine/Chemokine Magnetic Bead Panel (HCYTOMAG-60K [Merck Millipore, Burlington, MA, U.S.]) which was custom produced for epithelial growth factor (EGF), fibroblast growth factor 2, eotaxin, transforming growth factor-α (TGF-α), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-10, platelet derived growth factor (PDGF) AA, PDGF-AB/BB, IL-17A, IL-1Ra, IL-1β, IL-6, IL-7, IL-8, interferon gamma-induced protein 10 (IP-10), MCP-1, MIP-1α, MIP-1β, RANTES, TNF-α, TNF-β, and vascular endothelial growth factor.

- Human MMP Magnetic Bead Panel 2 (HMMP2MAG-55K [Merck Millipore]) for matrix metalloproteinases (MMP) 1, 2 ,7, 9, and 10.
- Human TIMP Magnetic Bead Panel 2 (HTMP2MAG-54K [Merck Millipore]) for tissue inhibitors of metalloproteinase (TIMP) 1, 2, 3, and 4.
- Human Magnetic Luminex Assay 20 plex (LXSAHM-20 [R&D systems]) which was custom produced for lumican, neural cell adhesion molecule-1, fibroblast activation protein α, OPG, osteonectin, tenascin C, a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 (ADAMTS13), collagen type I α1 and IV α1, fibronectin, intercellular adhesion molecule 1, hepatocyte growth factor receptor, triggering receptor expressed on myeloid cells 1, bone morphogenic protein (BMP) 2, 4, and 9, aggrecan, and syndecan-1, and 4.

3.8 STATISTICAL ANALYSES

Stata version 15 (StataCorp, Collage Station, TX, USA) and IBM SPSS version 25.0 (IBM Corp, Armonk, NY, USA) were used to analyse the data. Some of the statistics were performed in an equivalent way in all the studies. The descriptive statistics for patient demographic-, objective-, and subjective variables, as well as for diagnoses were calculated as mean ± standard deviation for all continuous data, and as number and percentage for categorical data. Fisher's exact test and chi-square were used for bivariate data and Student's t-test for mean values. The level for statistical significance was set at $P \leq 0.05$.

Below are specific statistical considerations and methods listed.

3.8.1 Study I

A multivariate logistic regression model was used to analyse differences in outcome between performing surgeons. The dependent variable was successful outcome. The variable surgeon was modelled as dichotomous, and the most experienced surgeon was used as the reference category. Gender, diagnosis, and age were included in the model to compensate for potential confounders.

3.8.2 Study II

A bivariate linear regression model was used to correlate patient-specific variables to the individual outcome variables MIO, TMJ pain, TMJ disability and TMJ psychosocial impact.

Ordinal regression was used associating surgical outcome to patient-specific variables. Palpatory pain of the masticatory muscles were modelled as a dichotomous dummy-variable

in the ordinal calculation. No palpation pain was set as the reference. All the factors that showed significant impact in the unadjusted analyses were used in a multivariate ordinal regression model, where the confounders age and gender also were included.

A forward logistic regression model with the four postoperative outcome variables as the dependent variable, one at the time, were made. The preoperative patient-specific variables, which were found to be significant in relation to respective postoperative variable, were all used as independent variables in the corresponding model.

3.8.3 **Study III**

Normalised ratio, i.e. the specific protein concentration divided by the total protein concentration, as well as the specific protein concentrations were used in the statistical analysis. Quantile regression was used analysing the median value since none of the specified protein concentrations were normally distributed (Figure 3).

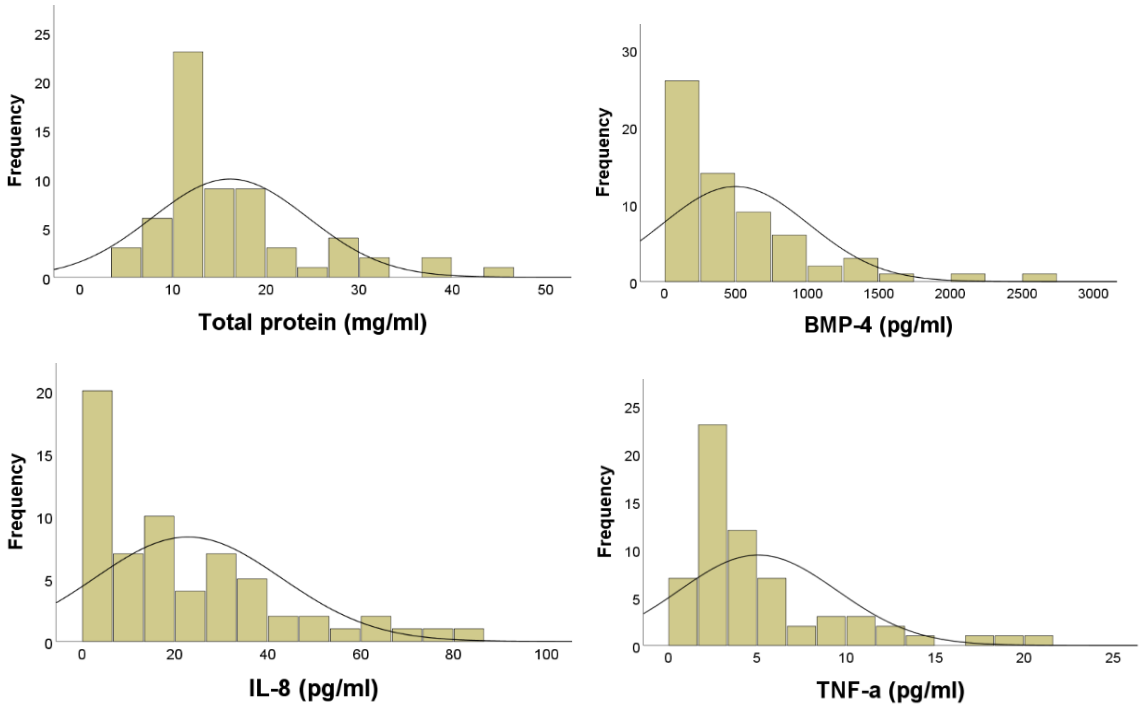

Figure 3. Histograms illustrating the skewed distribution of concentrations in all the proteins analysed, here represented by synovial tissue total protein, BMP-4, IL-8, and TNF-α.
BMP, bone morphogenic protein; IL, interleukin; mL, millilitre; pg, picogram; TNF, tumor necrosis factor.

The model to predict the median protein concentration included the TMJ diagnoses (DDwoR/DDwR or DDwoR-DO/DDwoR-SO) in the unadjusted analyses. Previous TMJ trauma, sex, age, and duration of symptoms were accounted as potential confounders why they were included in the adjusted analyses. The *P*-values were based on 20 bootstrap samples, and a *P*-value of ≤ 0.05 was regarded as significant.

3.8.4 **Study IV**

Ordered logistic regression was used for both univariate and multivariate computations. There was only one patient in the surgical outcome group “deteriorated”, which resulted in the merger of the outcome groups “deteriorated” and “intermediate”. The concentration of the specified proteins was used as the dependent factor in the statistical analyses.

To estimate the quality of the multivariate regression model Akaike’s information criterion (AIC) was used. The best quality was reached when the potential confounders CIA, TMJ disability, masticatory muscle palpation and the interaction of CIA and positive finding of masticatory muscle palpation tenderness, were included. Masticatory muscle palpation was dichotomised, no finding of palpation tenderness was merged with unilateral positive sign, since this manoeuvre also made the model stronger.

4 RESULTS

4.1 STUDY I

This retrospective medical chart study identified 224 TMJ arthroscopic lysis and lavage interventions from 1/1 2008 until 31/12 2013, where it was also possible to interpret the result of surgery. Patients had one of three potential diagnoses, DDwoR, OA, or CIA. In the study, the three included diagnoses were named CCL, OA, and systemic arthritis, but in this text CCL has been changed to DDwoR and systemic arthritis to CIA. Two subjectively VAS-graded variables were called TMJ functional impairment and psychosocial influence because of TMJ disorder, but here changed to TMJ disability and TMJ psychosocial impact, respectively. These changes are made to add conformity in this text, otherwise they are synonymous.

4.1.1 Surgical outcome

166 out of 224 patients (74%) had an outcome that was deemed as successful or good. Three patients (1%) deteriorated and 55 patients (25%) had an intermediate outcome. The different surgeons (surgeon categories) had success rates between 60.9-80.0%. No significant differences were found between the reference category surgeon and the others in the multivariate analysis.

4.1.2 Patient-specific predictive factors

Several patient-specific variables were investigated and related to outcome. Four variables were found to be statistically significant.

Preoperative ***MIO*** was analysed and in the successful group, the mean MIO was 32.9 mm and in the unsuccessful group 29.7 mm. This difference was statistically significant (P = 0.002). When dividing the patients into diagnostic subgroups, CIA patients showed a significant difference in preop MIO (P = 0.003), while DDwoR did not (P = 0.114). Patients in the OA group were not investigated in terms of MIO, because limited MIO was not a clinical diagnostic criterion.

Comorbidities were investigated and 39 patients in this study had an on-going psychiatric disorder, such as anxiety or depression. Of these, twenty had a successful outcome (20/39, 51%) compared to patients without a psychiatric disorder (130/185, 70%). This difference was significant (P = 0.033). When grouping the material into separate diagnoses, only DDwoR had sufficient number of patients with psychiatric disorder to allow for an analysis. Successful outcome was registered for 38% of the DDwoR patients (9/34) with a psychiatric

disorder compared to 72% without (71/98), which indicated a statistically significant difference ($P = 0.002$).

Patients with preoperative bilateral ***masticatory muscle palpation pain*** were found to have a significantly worse outcome from arthroscopy (successful 55%, 26/47) compared to patients with no findings of palpation pain (successful 75%, 56/75) ($P = 0.031$). Unilateral masticatory muscle palpation pain did not affect outcome in a significant way.

The subjective variable ***global pain*** was classified by the patients on a VAS scale graded 0-10. A total of 40 patients had medical chart recordings before arthroscopy. In this cohort, 27 patients had a successful outcome (67%, mean VAS 3.5 ± 3.3). The unsuccessful group (33%, mean VAS 5.9 ± 3.1) had a significantly higher global pain assessment ($P = 0.032$).

Three other VAS graded subjective variables were also evaluated: TMJ pain, TMJ disability, and TMJ psychosocial impact. None of them showed any significant difference between the two outcome groups. These preoperative recorded variables were neither found to significantly influence outcome: TMJ palpation pain, ASA-score, trauma affecting the TMJ region, duration of symptoms before arthroscopy, inflammatory diseases not otherwise specified, medications. Lavage volume during arthroscopy could not be related to altered outcome. Age was not found to differ between outcome in the whole cohort. When performing diagnosis sub-grouping, age was significantly higher in the CIA successful group ($P = 0.032$). In the other two diagnosis-groups there were no age differences.

4.2 STUDY II

This prospective observational study was based on a cohort of patients scheduled for arthroscopy due to one of the diagnoses DDwoR, OA/arthralgia, and CIA. The diagnosis DDwoR was further divided into patients having sudden onset (SO) of DDwoR, i.e. without prior TMJ symptoms, or having delayed onset (DO) which implies a history of long-term clicking or intermittent catching of the TMJ disc preceding DDwoR. Written informed consent was a prerequisite and a total of 93 patients were included and followed the protocol.

4.2.1 Surgical outcome

Forty-nine patients out of 93 (53%) had a successful outcome of surgery, according to the stipulated outcome measures. 23/93 (25%) had a good outcome, 19/93 (20%) intermediate, and 2/93 (2%) a deteriorated outcome (Figure 4). When comparing the preoperative measurements to the postoperative of both subjective outcome measures (TMJ pain, TMJ dysfunction, TMJ psychosocial impact, global pain) and objective (MIO, protrusion, lateral

excursion right and left) for the whole cohort, all variables were significantly improved ($P \leq 0.005$).

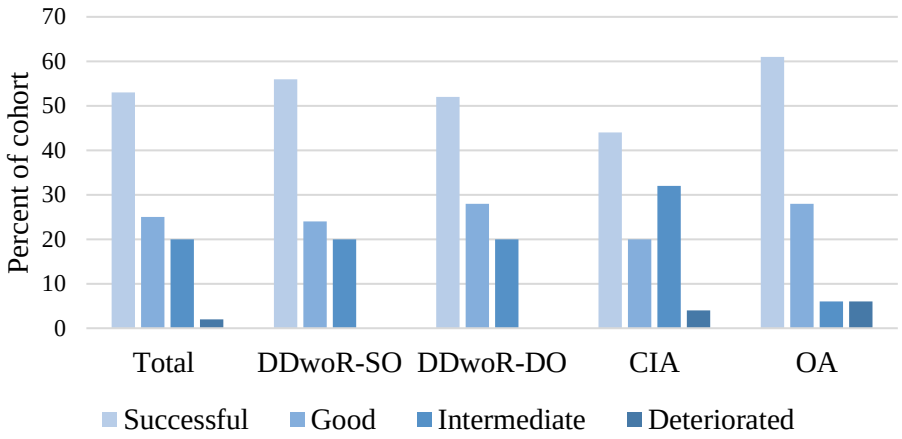

Figure 4. Outcome of arthroscopy at the final control, six months postoperative, for the total patient cohort and the diagnostic sub-groups. OA had a better outcome compared to the other diagnoses, and CIA had the worst. CIA, chronic inflammatory arthritis; DDwoR, disc displacement without reduction; DO, delayed onset; OA, osteoarthritis; SO, sudden onset.

4.2.2 Patient-specific predictive factors

Increased ***masticatory muscle palpation pain*** was found to be significantly associated with a diminished outcome in both the adjusted (OR 2.56; $P = 0.048$) and unadjusted analysis (OR 0.52; $P = 0.008$). No other variables showed significance in the adjusted analysis (incorporated variables were age, operated side, and gender). The distribution of muscle palpation findings can be seen in Table 1. Positive signs of bilateral palpation pain indicated a less favorable outcome (32% with a successful outcome) compared to unilateral (53%) and no palpation tenderness (63%).

Table 1. Preoperative registration of masticatory muscle palpation tenderness related to outcome of arthroscopy. Medical records of muscle palpation were missing in six patients.

	Palpation pain of masticatory muscles, *n (%)*			
Outcome	None	Unilateral	Bilateral	*Total*
Successful	27 (63)	10 (53)	8 (32)	*45 (52)*
Improved	10 (23)	5 (26)	7 (28)	*22 (25)*
Intermediate	5 (12)	4 (21)	9 (36)	*18 (21)*
Deteriorated	1 (2)	0 (0)	1 (4)	*2 (2)*
Total	*43 (100)*	*19 (100)*	*25 (100)*	*87 (100)*

The variable ***operated side*** showed significant association to outcome in a negative manner (OR 0.24, *P* = 0.012), i.e. bilateral joint arthroscopy indicated a worse outcome, when analysed in an unadjusted ordinal logistic regression model.

Mean ***patient age*** in the total cohort was 43.8 years (± 16.6) and patients with a higher age at operation showed a better outcome in unadjusted analyses (OR 1.03, *P* = 0.032). No statistically significant age differences were found in the diagnostic sub-groups. Apart from patient age, operated side and masticatory muscle palpation pain, none of the other investigated patient-specific variables had any significant impact on outcome.

To evaluate possible impact of preoperative variables on the outcome variables postoperative MIO, TMJ pain, TMJ disability, and TMJ psychosocial impact, unadjusted linear regression was performed (Study II, Table 3). All preoperative variables significantly associated with a postoperative outcome variable in the unadjusted analyses were then included in a forward-conditional logistic regression model, with the preoperative variables characterised as independent. Postoperative TMJ pain showed a significant relation to the preoperative variables TMJ pain (β 0.36, *P* = 0.002) and masticatory muscle palpation tenderness (β 0.80, *P* = 0.008). TMJ disability, ASA, age, and TMJ psychosocial impact had no association with postoperative TMJ pain in the forward-conditional logistic regression analysis. Postoperative TMJ disability had a positive association to preoperative masticatory muscle palpation tenderness (β 0.78, *P* = 0.016) but not towards preoperative TMJ psychosocial impact. The outcome factor TMJ psychosocial impact was analysed in relation to the preoperative factors age (β -0.06, *P* = 0.003), TMJ psychosocial impact (β 0.31, *P* = 0.005), and masticatory muscle palpation tenderness (β 0.71, *P* = 0.042). The pre- and perioperative factors TMJ pain, ASA score, fibrosis score, and Wilkes criteria were found to be non-significantly related to postop TMJ psychosocial impact. Finally, postoperative MIO were significantly associated to preoperative MIO (β 0.291, *P* = 0.000), but not to preoperative lateral excursion to the right or left, protrusion, or Wilkes criteria.

In this study, a sub-division of DDwoR were made: DDwoR-SO and DDwoR-DO. Significantly more patients with DDwoR-SO had experienced trauma to the jaws preceding the TMJ diagnosis (*P* = 0.036). Otherwise, no differences were at hand between the diagnoses.

4.3 STUDY III

A prospective observational study was performed including patients with DDwR and DDwoR. Sixty-three patients were included after written informed consent, 47 diagnosed with DDwoR and 20 diagnosed with DDwR.

Patient characteristics were similar between the two diagnostic groups but differed in four variables, where three of them could be considered as being associated with the different characteristics of the two diagnoses. DDwR had significantly greater MIO compared to DDwoR (P = 0.000) and longer duration of TMJ symptoms (P = 0.008). DDwoR had significantly higher TMJ pain score (P = 0.019) and higher Wilkes criteria (P = 0.000). A subdivision of DDwoR into sudden and delayed onset was also made in this study. Trauma to the jaws prior to diagnosis was the only variable that differed significantly (P = 0.024) between type of onset, affecting more DDwoR-SO than DDwoR-DO patients (41% compared to 11%).

4.3.1 Synovial tissue analysis

The synovial tissue total protein concentration did not differ in a significant way between DDwR patients (11.80 mg/mL, ±2.43) and DDwoR (15.65 mg/mL, ±9.49) with multivariate quantile regression. Prior jaw trauma, unlike sex, age, and duration of TMJ symptoms, were found to significantly correlate to a higher total protein concentration (coef. 2.73; P = 0.021; 95% CI 0.44-5.03).

A total of 28 different cytokines were analysed in a multi-analytic profiling system (Luminex 200, Luminex, Austin, TX, USA). GM-CSF, IL-17, and TGF-α were below detectable concentration values and statistical analyses were therefore not possible. Univariate comparison of cytokine concentrations between DDwoR and DDwR showed significantly higher concentrations of DDwoR cytokines BMP-2 (coef., -331.90; P = 0.000), BMP-4 (coef., -439.79; P = 0.000), EGF (coef., -30.35; P = 0.014), eotaxin (coef., -86.52; P = 0.000), G-CSF (coef., -229.77; P = 0.001), IL-1β (coef., -1.32; P = 0.004), IL-7 (coef., -30.96; P = 0.000), IL-8 (coef., -20.59; P = 0.000), IL-10 (coef., -4.69; P = 0.000), TNF-α (coef., -2.45; P = 0.000), TNF-β (coef., -2.03; P = 0.018), and significantly higher concentrations of DDwR cytokines IP-10 (coef., 172.91; P = 0.000), PDGF-AA (coef., 170.10; P = 0.021), and RANTES (coef., 6677.67; P = 0.021).

Multivariate analyses were performed on the remaining 25 cytokines, including the possible confounders sex, age, duration of TMJ symptoms, and previous trauma to the jaws. The results revealed small changes compared to the unadjusted analyses. All cytokines with

significantly higher DDwoR concentrations in the univariate analyses also had significantly higher concentrations in the multivariate analyses, in addition to the cytokine MIP-1β (coef., -14.11; *P* = 0.007) which was also significantly higher. Proteins with significantly higher concentrations in the multivariate analyses in patients with DDwR are illustrated in Figure 5. PDGF-AA showed no significant difference in the multivariate analysis, as in the univariate analysis.

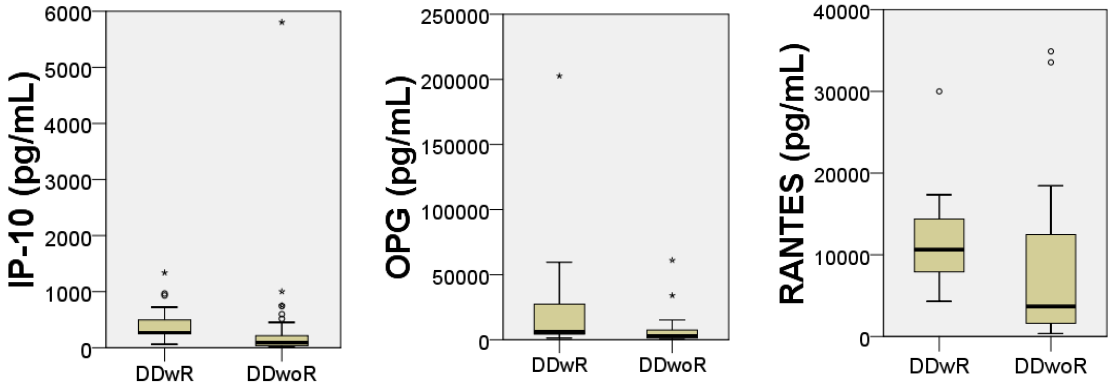

Figure 5. IP-10, OPG, and RANTES were detected at significantly higher levels in patients with DDwR compared to patients with DDwoR in multivariate analyses. IP-10 (coef., 178.9; *P* = 0.024; 95% CI, 24.7-333.1), OPG (coef., 2230.1; *P* = 0.046; 95% CI, 38.3-4421.9), RANTES (coef., 6548.3; *P* = 0.001; 95% CI, 2876.0-10620.6).
CI, confidence interval; DDwoR, disc displacement without reduction; DDwR, disc displacement with reduction; IP-10, interferon gamma-induced protein 10; mL, millilitre; OPG, osteoprotegerin; pg, picogram; RANTES, regulated on activation, normal T cell expressed and secreted.

Sex had a significant association with EGF (coef., 23.21; *P* = 0.032) and IL-1ra (coef., 30.82; *P* = 0.028) (Figure 6). TMJ symptom duration had a significant association to OPG (coef., 138.97; *P* = 0.014). However, there were no correlations between any of the cytokines and the variables age and TMJ trauma.

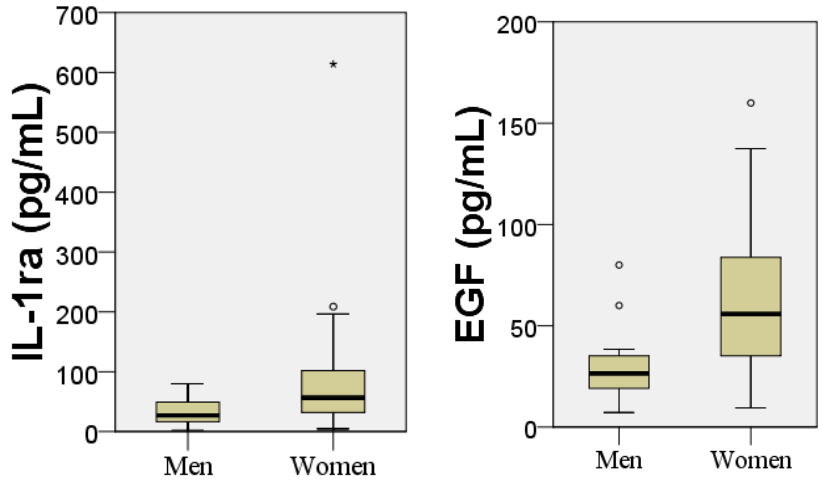

Figure 6. Cytokines IL-1ra and EGF showed gender dimorphism with a median IL-1ra concentration of 26.89 pg/mL (range 1.40-80.00) in men and 56.47 pg/mL (range 4.47-613.96) in women and EGF median concentration 26.45 pg/mL (range 7.15-80.00) in men and 55.84 pg/mL (range 9.50- 160.00) in women.
EGF, epidermal growth factor; IL-1ra, interleukin1 receptor antagonist; mL, millilitre; pg, picogram.

When performing a subgroup analysis on concentration differences between the diagnoses DDwoR-SO and DDwoR-DO, DDwoR-SO had significantly higher concentrations of BMP-2 (coef., 253.92; *P* = 0.016), BMP-4 (coef., 342.24; *P* = 0.026), TNF-α (coef., 4.62; *P* = 0.000), and TNF-β (coef., 2.72; *P* = 0.047). Multivariate analyses were also performed, and DDwoR-SO were found to have higher concentrations regarding BMP-4 (coef., 401.19; *P* = 0.004), eotaxin (coef., 137.18; *P* = 0.032), and IL-8 (coef., 14.38; *P* = 0.014). The included potential confounders age, sex, and duration of TMJ duration all were non-significant, but the variable TMJ trauma had one significant relationship with lower concentrations of PDGF-AA (coef., -131.09; *P* = 0.015).

4.4 STUDY IV

Patients with the diagnoses DDwR, DDwoR, OA, and CIA were included in this prospective cohort study. At study closure 100 patients had given their written informed consent and followed the protocol.

4.4.1 Surgical outcome

In the successful outcome group (*n* = 56, 56%) MIO increased with a mean of 8.4 ±7.2 mm (*P* = 0.000) and TMJ pain decreased with VAS 4.1 ±2.4 (*P* = 0.000). The other outcome measures were also significantly decreased, TMJ disability VAS 4.2 ±1.8 (*P* = 0.000), and TMJ psychosocial impact VAS 3.5 ±2.6 (*P* = 0.000). Figure 7 illustrates the outcome of surgery.

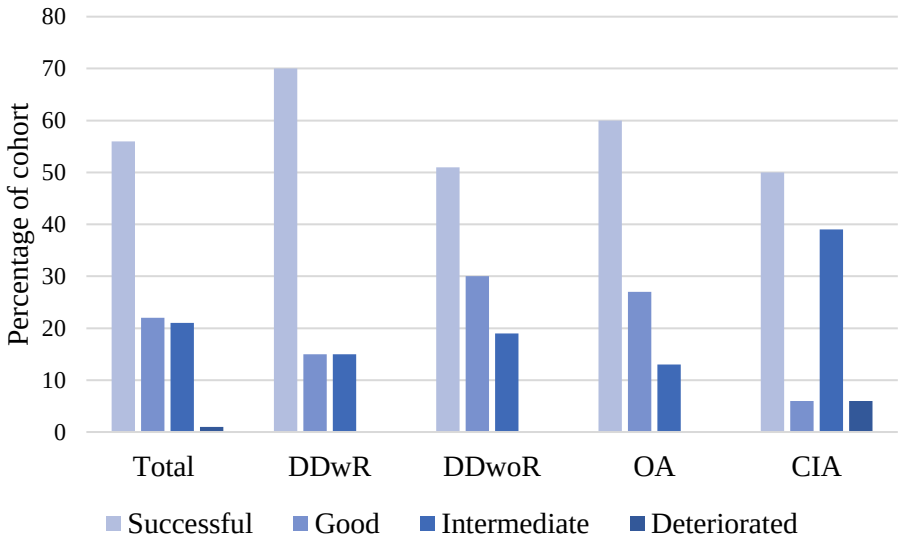

Figure 7. Outcome of TMJ surgery at the final control six months postoperative, divided into the diagnostic sub-groups. DDwR has the best surgical outcome and thereafter in descending order, OA, DDwoR, and CIA.
CIA, chronic inflammatory arthritis; DDwoR, disc displacement without reduction; DDwR, disc displacement with reduction; OA, osteoarthritis.

Comparing the best and worst outcome groups, DDwR and CIA respectively, to the rest of the total cohort high-lighted some preoperative differences. The patients with DDwR were represented with the lowest TMJ pain (P = 0.008) and with least palpation tenderness of both the lateral aspect of the TMJ (P = 0.000) and the masticatory muscles (P = 0.000). The patients with CIA, on the other hand, showed more palpation tenderness of the TMJ (P = 0.000) and the masticatory muscles (P = 0.006) related to the rest of the cohort.

4.4.2 Patient-specific predictive factors including synovial tissue proteins

A variety of clinical preoperatively registered variables were analysed in a univariate ordered logistic fashion. TMJ palpation tenderness (coef., 0.89; P = 0.044), masticatory muscle palpation tenderness (coef., 1.97; P = 0.000), TMJ disability (coef., 0.29; P = 0.011), TMJ psychosocial impact (coef., 0.15; P = 0.032), and global pain (coef., 0.13; P = 0.043) were all associated with a more negative surgical outcome the higher the value. Regarding the other clinical parameters, the univariate analyses were non-significant. All the significant preoperative variables were included in a multivariate ordered logistic regression model and ***masticatory muscle palpation tenderness*** was the only variable showing significance (coef., 1.69; P = 0.001).

The synovial tissue samples were analysed for concentrations of 51 different cytokines, chemokines, glycoproteins and proteoglycans. There were too few observations of GM-CSF, IL-17, and TGF-α, which was why calculations could not be performed on them. The specific proteins concentration was related to outcome of surgery and two proteins, eotaxin (coef., 2.89×10^{-3}; P = 0.038) and syndecan-1 (coef., 1.11×10^{-4}; P = 0.024), had higher concentrations associated with a worse outcome (Figure 8).

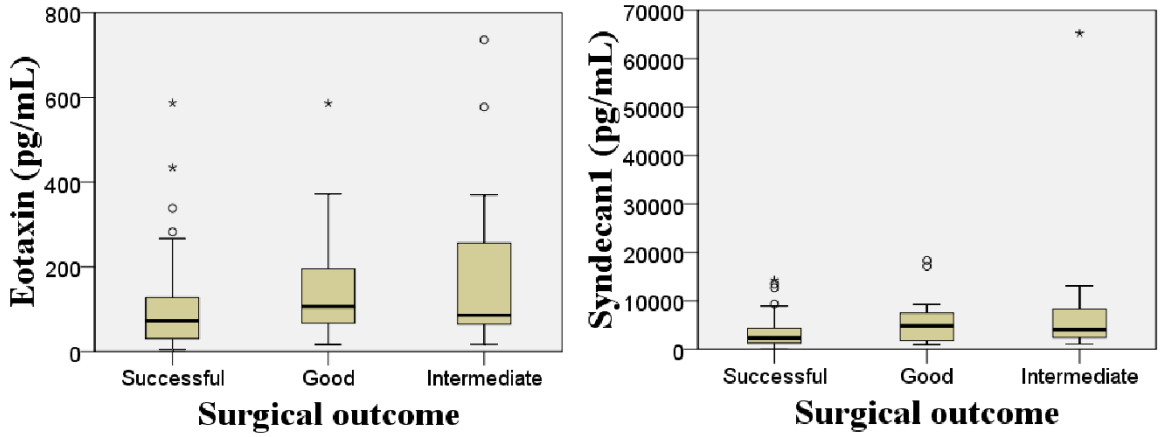

Figure 8. Box plot illustrating higher concentrations (pg/mL) of eotaxin and syndecan-1 in relation to diminished surgical outcome. Eotaxin mean concentration for successful outcome, 107.7 ±114.4; good, 156.8 ±148.0; intermediate, 181.8 ±201.2. Syndecan-1 mean concentration for successful outcome, 3512.3 ±3250.9; good, 5682.4 ±4861.8; intermediate, 8665.4 ±14551.3.

All proteins were separately analysed in a multivariate ordered logistic regression model together with the potential confounders masticatory muscle palpation tenderness, age, TMJ disability, the diagnosis CIA, and the interaction between CIA and masticatory muscle palpation tenderness. IL-8 (coef., 2.17×10^{-2}; $P = 0.049$), lumican (coef., 9.99×10^{-8}; $P = 0.038$), MMP-7 (coef., 3.06×10^{-5}; $P = 0.038$), and TIMP-2 (coef., 3.11×10^{-5}; $P = 0.015$) had higher concentrations associated to an inferior outcome. Bilateral masticatory muscle palpation tenderness was significantly associated with an inferior outcome in all but three of the multivariate analyses on specified proteins, which strengthens the assumption of its predictive potential. The interaction between CIA and bilateral masticatory muscle palpation tenderness had a negative coefficient in all the multivariate analyses, where significance was found in the analysis of ADAMTS13, IL-1β, and TNF-β. This indicates that positive masticatory muscle palpation tenderness in patients with CIA does not predict an inferior outcome from TMJ surgery, as the muscle palpation variable does for the other diagnoses DDwoR, DDwR, and OA. The potential confounders CIA and TMJ disability both had positive coefficients indicating a negative impact on outcome. CIA was found to be significantly associated with a negative surgical outcome in 9/48 multivariate protein analyses, and TMJ disability in 5/48.

4.5 PRELIMINARY RESULTS FROM SYNOVIAL FLUID SAMPLING

The results from the synovial fluid analyses were originally intended to be included in this thesis. Due to different circumstances the results will be presented in more detail later in an appropriate scientific setting. Below is a brief summary of the material collected.

One hundred and one patients were included in the study belonging to the ethical approval 2014/622-31/1. Three patients were included in the study and operated before the synovial fluid sampling technique was mastered, thus a total of 98 patients were eligible for sampling (Figure 9).

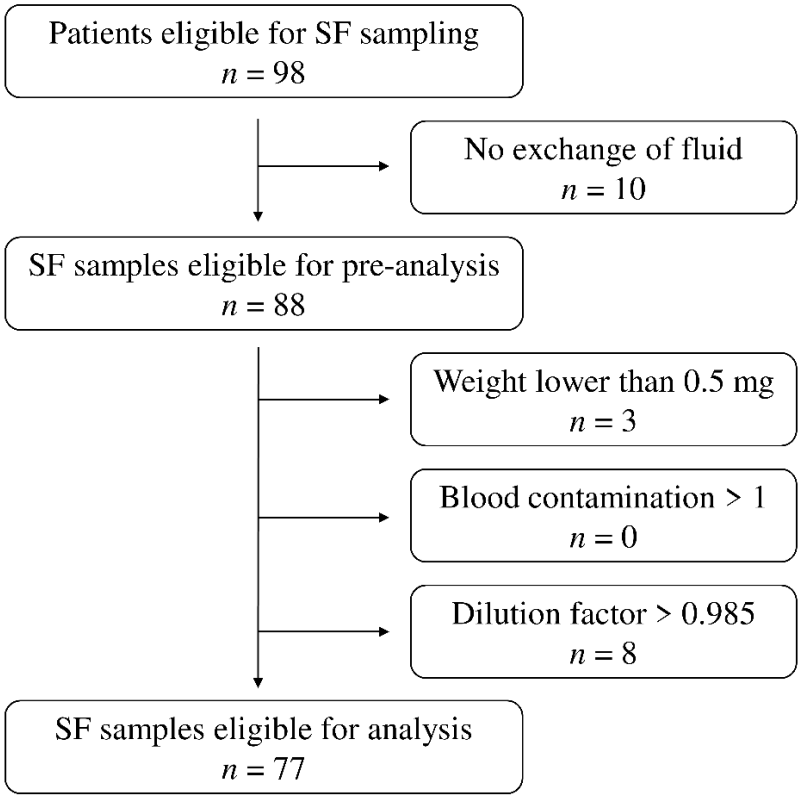

Figure 9. Flow-chart illustrating sampling of synovial fluid. The pre-analysis of the samples incorporates weighing of the sample, ocular determination of blood in the sample (0 = no blood, 1 = hardly visible blood contamination, 2 = visible blood contamination, 3 = blood-like aspirate), and measuring the dilution factor in a spectrophotometer.
SF, synovial fluid.

From the 77 patient-samples, high-sensitive ELISA tests have been done for detection of cytokines IL-1β, IL-6, IL-8, IL-10, and TNF-α. These tests have not yet been analysed in a research context, but the median and mean concentrations are shown in Table 2.

Table 2. Presentation of synovial fluid concentrations of five different cytokines and the total protein concentration

Cytokine	Total protein (mg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)	IL-8 (pg/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)
Samples with detected conc., *n* (%)	77 (100)	34 (44)	70 (91)	70 (91)	58 (75)	47 (61)
Mean conc.	63.36	29.64	105.67	28.27	36.59	11.72
Median conc.	20.68	6.31	9.40	11.92	13.78	6.87
SD	297.24	89.12	348.76	69.19	59.60	17.89
Min	0.77	0.30	0.16	0.16	0.64	0.08
Max	2620.42	515.39	2270.57	564.57	352.43	110.97

Conc., concentration; IL, interleukin; mg, milligram; mL, millilitre; max, maximum; min, minimum; *n*, number; SD, standard deviation; TNF, tumor necrosis factor; pg, picogram

5 DISCUSSION

To systematically characterise a diagnosis from different aspects, such as demography, clinical variables, natural course, aggravating factors, biomarkers etc, is of great importance to understand disease pathology. Without understanding the pathology, strict diagnostic criteria will only occasionally apply, revealing unknown aetio-pathogeneses will hardly take place, and correct and successful treatment will randomly occur. Research is needed since much is still obscured regarding DD of the TMJ. This thesis was an attempt to increase our knowledge of TMJ diseases, particularly DD.

5.1 TISSUE CHARACTERISATION OF TMJ DISC DISPLACEMENT

In study III it was shown that demography and patient characteristics were almost the same comparing DDwR to DDwoR. Although, there were significant differences regarding TMJ pain and Wilkes criteria, higher for DDwoR, which might reflect the sequential development and aggravation earlier reported [26]. The higher VAS-value of the subjective variable TMJ pain in DDwoR might be correlated to significantly higher concentrations of pro-inflammatory cytokines such as IL-1β, TNF-α, but also chemokines as IL-8 and MIP-1β. The higher Wilkes score in DDwoR, reflecting clinical, surgical, and radiographical signs of inflammation and degeneration, would most certainly be attributed to the same concentration dependent action of cytokines and chemokines. Twelve cytokines were shown with higher concentrations in DDwoR compared to DDwR indicating a higher inflammatory activity, but contradictory IP-10, OPG and RANTES had significantly lower concentrations in DDwoR. IP-10 and RANTES are associated with bone resorption in rheumatic arthritis, but also found in higher concentrations in other chronic inflammatory states promoting angiogenesis, leukocyte recruitment, and fibrosis [7, 122, 123]. In relation to these earlier reports, IP-10 and RANTES might be expected to have higher concentrations in DDwoR, but the results of the current studies showed the opposite, which was hard to explain. OPG, the decoy receptor of RANK ligand (RANKL) preventing bone resorption, was also found to have a lower concentration in DDwoR patients [124]. Similar results from TMJ synovial fluid analyses have earlier been presented [125]. In the current study, OPG was also found at a significantly higher concentration correlating to an extended duration of TMJ symptoms in the total cohort. Since DDwR patients were found to have significantly longer periods of TMJ symptoms before surgery and significantly higher concentrations of OPG compared to DDwoR, it might be concluded that TMJ DD symptoms raise OPG concentration in a time-dependent manner. Whether IP-10, RANTES, and OPG interact in DDwoR to regulate the bone homeostasis response remains to be determined, but could be suggested based on the

unexplainable low concentrations compared to the other investigated proteins, and due to earlier understandings of concentration dependent mechanisms demonstrated in rheumatoid arthritis [7, 122, 124]. From the included studies in this thesis we could conclude that bilateral cases of DDwoR were uncommon in relation to patients with CIA. A speculation was that bilateral cases of DDwoR might be attributable to CIA, i.e. the affected patients had been misdiagnosed. Harvesting a small biopsy of synovial tissue during arthroscopy and analysing it with regards to OPG, IP-10 and RANTES might be a future diagnostic regime for these patients.

In TMJ disorder there is a strong female preponderance amongst operated patients, often at a 9:1 ratio, which was also apparent in the studies included in this thesis [2, 65, 83, 84, 91, 126]. Women were shown to have significantly higher concentrations of both EGF and IL-1ra in TMJ DD synovial tissue. EGF has been shown to have higher levels in women's kidneys and urine, and IL-1ra has a proven gene polymorphism which might explain higher female levels of IL-1ra [127, 128]. The difference in concentration of EGF and IL-1ra might be a clue to the skewed gender distribution in DD needing surgical intervention and needs further attention.

The onset of DDwoR often follows a typical pattern, with preceding clicking and catching of the disc, i.e. DDwR precedes DDwoR. This sequential deterioration has earlier been described by Wilkes, although sometimes patients present with DDwoR without earlier symptoms from the TMJ [26]. To differentiate these two different onsets of DDwoR they can be divided into delayed onset, DDwoR-DO, or sudden onset, DDwoR-SO. When investigating these sub-groups of DDwoR similar demographics were found. However, the variable TMJ trauma was found significantly different, with DDwoR-SO more affected. DDwoR-SO patients also had significantly higher concentrations of BMP-4, eotaxin, and IL-8 compared to DDwoR-DO. Eotaxin increases osteoclast activity and is upregulated during inflammation, whilst BMP-4 helps bone and chondral repair [129, 130]. IL-8 stimulates phagocytosis and might be one of the regulators of inflammatory-driven increased bone turnover, which requires increased numbers of scavenger cells [131]. It remains to be determined whether TMJ trauma was an inciting event, initiating higher levels of these cytokines that should be subject of future investigations.

Although DDwoR-SO patients have no earlier history of TMJ problems they still display an equal radiographic and clinical status compared to DDwoR-DO. This might support that TMJ inflammation is higher in DDwoR-SO, which we found some support for, see above. The altered inflammation in DDwoR-SO could negatively compensate for the prodromal TMJ

symptoms and its possible inflammatory and degenerative effects, experienced by DDwoR-DO patients. The time-period experiencing DDwoR before surgery was equivalent between patients with DO and SO. Since patients with DDwR (confluent with DO) could have quite diverging quality of symptoms, and that a multitude of cytokines had significantly lower concentrations compared to DDwoR, the DO-period seems to have had minor effect on DDwoR-DO TMJ status at time of surgery.

5.2 CLINICAL VARIABLES AS PREDICITVE FACTORS

Bilateral ***masticatory muscle palpation pain*** as an indicator for myalgia, was the only variable that was significantly associated with a poorer surgical outcome in studies I, II, and IV. Positive findings of muscle pain should advocate the surgeon to reconsider surgery and thoroughly evaluate the non-invasive interventions already done. Although, the variable being a predictor of a bad outcome should not necessarily lead the surgeon to refrain from surgery. Despite that the outcome has proven to be inferior for patients with bilateral positive findings of muscle pain, far from all patients fail surgery on these premises. For patients with CIA, findings of bilateral masticatory muscle pain do not seem to be a negative predictor, but almost a tendency towards the opposite. Patients with CIA often have bilateral TMJ problems and therefore possibly secondary bilateral myalgia. TMJ DD and TMJ OA patients most often has unilateral TMJ problems, which is why bilateral myalgia might indicate that the primary problem is not the TMJ.

Patients age has been advocated as a predictive variable in TMJ surgery, where higher age (≥ 60 years) had significantly better outcome related to the age group 21-30 years [84]. Mean-age instead of age-group comparison did not reveal any differences in surgical outcome [84]. Other studies found no difference in surgical outcome due to patients age [89, 90]. In study II, older age at time of operation was also found to be a positive predictor for TMJ surgery in the unadjusted analysis. A similar situation was observed in study I, but only in patients with CIA and not the whole patient cohort. In study IV, age was not significant in the unadjusted analysis, even though patients in the successful group had the highest mean age. Patients age might be a valid predictor of surgery according to earlier work and this thesis [84]. Further studies with larger numbers of included patients is suggested.

Preoperative ***MIO*** was positively correlated to outcome in study I and postoperative MIO was strongly associated with preoperative MIO in study II. Other studies have shown diverging results on MIO as a predictor for surgical outcome [84, 85]. The studies in this thesis also includes patients without limited mouth opening as a diagnostic criterion, for example patients diagnosed with OA and CIA. This fact might obscure the real impact of

preoperative MIO on the surgical outcome. Larger studies exclusively including patients with restricted mouth opening capacity are needed to better evaluate MIO as a potential surgical predictor.

Comorbidities might influence outcome of TMJ surgery. There are reports on chronic anxiety patients having lesser pain decrease after surgery compared to non-anxiety patients, and that use of benzodiazepines (anxiolytic medication) was related to an unsuccessful outcome [82, 83]. In study I, the comorbidity psychiatric disorder was found to significantly decrease successful outcome compared to patients with no history of psychiatric disorder. In studies II and IV no findings of outcome-related comorbidities could be found. Study I comprised a larger cohort compared to the other studies, which might explain that a difference was recognisable. Since anxiety and other psychiatric disorders appear to be overrepresented in TMD cohorts, these co-morbidities deserve further attention [132].

With a strong female preponderance reported for patients undergoing TMJ surgery, ***gender*** as a predictor of surgery seems feasible, however earlier reports have not found any gender differences [84, 90]. We found no relation between surgical outcome and gender in any of the performed studies on the matter. Between 83-89 % of the total cohorts in study I, II, and IV were women, which was in accordance with earlier reporting [66, 83, 84, 86, 91].

The severity of DD might influence patient's recovery after surgery. Therefore, ***disease-grading systems*** could guide the clinician in surgical outcome prognosis. In two different studies evaluating the most wide-spread disease grading system, Wilkes criteria, including 23 and 41 patients respectively, showed no such associations [86, 87]. Wilkes criteria was evaluated in studies II and IV, but no statistical significance was found indicating a relation between outcome and grading.

Duration of TMJ symptoms has been investigated, and Israel et al. concluded that an early surgical intervention after onset of TMJ symptoms promoted a better outcome, but others found no difference [89, 90, 94]. Neither one of the studies I, II, and IV showed any difference in outcome due to symptom duration. A systematic review concluded no evidence for timing of non-surgical or surgical interventions on patients with DDwoR in relation to symptom onset, but recommend that surgery should be postponed for at least 6 months [31]. This standpoint seems adequate since the natural time course of symptomatic DDwoR often shows to be self-regulating [133].

Apart from MIO, ***TMJ pain*** subjectively assessed on a VAS is one of the most used parameters for evaluation of TMJ surgery. TMJ pain has also been evaluated as a predictor

for surgical outcome with two reports that found a relationship between higher preoperative TMJ pain and a worse outcome, and one without [83, 84, 90]. In none of our studies preoperative TMJ pain was found to indicate the outcome of surgery.

Patients VAS estimation of preoperative ***global pain*** (i.e., pain affecting other parts of the body, but not the TMJ) have been used on a regular basis at the Department of Craniofacial Diseases, Karolinska University Hospital, Stockholm, for over 10 years. This is a convenient way to assess the patients perceived general pain state. From this simple estimation it is not possible to say anything about pain duration, pain threshold, pain coping ability, etc. None the less, we found a positive association between preoperative global pain VAS value and a surgical outcome in univariate analyses in both studies I and IV. Pain comorbidities have earlier been reported to be positively associated to TMD, which is why this easy manageable variable could be a valid prognostic factor [134, 135].

The subjective measurement ***TMJ disability*** is a local development of the commonly used dietary score [136, 137]. With dietary score, the patients grade what kind of food that they can eat, that ranges from VAS 0 which is equal to "can eat anything", to VAS 10 that equals "can eat liquid food" [136, 137]. TMJ disability is a broader patient-estimate of a reduced function, from VAS 0 "can eat anything, can move the mandible within normal range, not preventing yawning, singing, kissing, etc", to VAS 10 "can eat liquid food, severe problems with mandible movements substantially impairing normal daily activities". In study IV, the univariate analysis of TMJ disability was positively associated with outcome in a significant way, but not in the multivariate analysis. Despite this, the variable was included in the multivariate analyses on synovial tissue proteins since it strengthened the model according to AIC. The TMJ disability variable seems to be promising in predicting surgical outcome, since a worse TMJ disability score tend to indicate a worse outcome. If dietary score or TMJ disability has any correlation to local inflammation or degenerative changes have to be investigated. Larger studies are suggested, probably addressing the question in a better way.

TMJ psychosocial impact was the last of the three subjective outcome variables, besides TMJ pain and TMJ disability, used in studies II and IV. The patients were asked to grade the impact of their TMJ disorder on their psychosocial well-being on a VAS graded 0-10. In study IV there was a positive association between TMJ psychosocial impact and surgical outcome, but that was not shown in study II. Psychosocial factors and their impact on the patients TMJ symptoms as well as the reverse, TMJ symptoms impact on psychosocial factors, seems to be important for the outcome of both non-surgical as well as surgical interventions [1, 20, 29, 82, 132]. There is a report on a weak association between lower

chronic anxiety score and a greater pain decrease in TMJ arthroscopy [82]. The variable TMJ psychosocial impact needs further verification of its function as an outcome predictor in TMJ surgery, but also an evaluation of validity and reliability compared to questionnaires on the same topic.

Different ***TMJ diagnoses*** seems to have different outcomes after surgery, which is why diagnoses might be used as predictors as well [3-6]. In this thesis, CIA had a worse outcome compared to other diagnoses, even though no statistical significances were found when tested in studies II and IV. The follow-up time in both studies II and IV was six months, which was chosen on empirical grounds. Patients with CIA might have the worst prognosis after TMJ surgery in deteriorating over time due to their, often progressive, illness. Patients diagnosed with OA might experience a higher tendency to deteriorate again after successful surgery compared to patients with DD. This is not investigated longitudinally, and a suggestion might be to evaluate surgical outcome in relation to TMJ diagnoses after a more extensive time period

To conclude, the finding of bilateral masticatory muscle palpation tenderness was the only clinical variable uniformly found to be associated with a poor outcome. From this perspective it seems to be a strong predictor for an inferior outcome. Other promising patient-specific variables identified were MIO, age, psychiatric disease, TMJ psychosocial impact, TMJ disability, and global pain. All variables mainly with the same need for investigations in larger cohorts.

5.3 SYNOVIAL TISSUE PROTEINS AS PREDICTIVE FACTORS

In study IV we found an association between higher concentrations of IL-8, lumican, MMP-7, and TIMP-2 and an inferior surgical outcome. The chemokine IL-8, with association to severity of rheumatoid arthritis and TMJ DD, has also been reported to up-regulate MMP-7 in oral squamous cell carcinoma [131, 138, 139]. MMP-7 has a role in joint degeneration with its ability to degrade extracellular matrix (ECM) components such as proteoglycans [140-142]. The small, leucine-rich, proteoglycan lumican has indirectly been linked to MMP-7 in a rhesus monkey model of early pregnancy, showing that relaxin increases MMP-7 levels and decreases lumican levels [143]. Lumican has been related to wound healing and found with increased levels in degenerated TMJ discs [144, 145]. TIMP´s are considered to be the main endogenous inhibitors of MMP´s and TIMP-2 might be a continuous ECM protector in joints due to its mRNA non-responding profile towards different stimuli [146, 147]. Although, other studies have shown TIMP mRNA response to stimuli with osteopontin or

relaxin levels [148, 149]. As IL-8, MMP-7, TIMP-2, and lumican might be linked together, future studies can be directed towards assessing regulation and interaction activity with relaxin as a possible key protein.

In the current study, all four proteins were shown to be associated with surgical outcome in a concentration-dependent negative manner and might be promising indicators for TMJ surgery. Other indications for taking synovial tissue samples would be to confirm or re-evaluate diagnostics, guidance in the use of treatment options, or for research purposes.

5.4 METHODOLOGICAL CONSIDERATIONS

All four studies were observational cohort studies and the eligible patients were referred to the surgical unit. Decision on surgery were made after non-invasive therapy had been tried for at least three months and thereafter evaluated. There were no demands on what kind/-s of non-invasive therapy that had preceded the decision to do surgery. Thus, there were neither any demands on level of care unit that had performed the non-invasive therapy, i.e. it could have been a specialist in orofacial pain and jaw function, a general practitioner, or a physiotherapist. These circumstances might induce a possible selection bias.

The demography of the selected patients seemed to follow other studies on surgical outcome [66, 83, 84, 86, 91]. The women were predominant in a 9:1 ratio and the mean age were just above 40 years of age. From this perspective the patient samples seemed to be representative.

In studies III and IV there were nine patients that did not want to participate, compared to none in study II. This was assumedly due to the additional synovial sampling in these studies. Compared to study II, where no samples were taken, it might be speculated that patients with certain personalities in this way deselected themselves, thereby skewing results. The implications are unknown, though.

In study I, one concern was that the department at the time period had no strict protocol for examination and collection of variables. The medical records are therefore dependent on the individual clinician’s own routine on registration. A certain degree of subjectivity in examination and registration can therefore not be ruled out. In some cases, notes on postoperative TMJ pain and sometimes even MIO were absent, which was why the authors had to evaluate the medical records and determine if the information presented was translatable to the stipulated outcome measures. In unclear cases, the patient was excluded. In the other studies performed, a pre-defined case report form was used, and all the clinical

investigators were educated in how to perform and register variables and diagnoses in a standardised manner according to the DC/TMD, where applicable.

In study II, different surgeons performed the operations, which might enhance the external validity, but probably not the internal. All performing surgeons had been trained by the same senior surgeon, which is why they at least in theory operated similarly. In studies III and IV it was a single surgeon who performed all the operations resulting in the opposite situation.

Throughout all studies, there were no external outcome assessors investigating the patients at the follow-up visits. It was usually the performing surgeon who did the registration, but sometimes a surgeon-colleague. Surgeons performing assessment of their own work might induce a two-way bias [150]. The surgeon might favour hers/his skills and overestimate the outcome of surgery and the patient might feel dependent on, and not want to offend the treating surgeon, being over-optimistic about their view of the outcome. Both scenarios could implicate a bias towards a better outcome [150].

The results on predictive patient-specific factors are based on surgical outcome registered at the six-month postoperative control. The decided time period was based on empirical knowledge and the departments routine, as successful or good surgical outcomes at six-month follow-up only occasionally deteriorate. One might speculate that a longer follow-up period would present a sturdier result, i.e. the effect of placebo on the whole cohort and the suppression of inflammation in CIA patients would vanish and decreasing successful outcome. In that sense, long term follow-up might be an important complement.

The decision to use synovial tissue samples in studies III and IV and not synovial fluid were mainly because inflammation of the TMJ has been shown in the synovia and that synovial fluid possibly contains a mixture of accumulated locally and distantly produced proteins [7-9]. In this sense synovial tissue might present a better snapshot of the current state of inflammation compared to synovial fluid. On the other hand, synovial fluid harvesting is easier and minimally invasive and from that perspective has a more patient- and user-friendly approach.

The synovial tissue protein extraction method used was described to have a quite large intraindividual concentration difference [121]. This was described to depend on rheumatoid arthritis intra-synovial tissue variability in cytokine production. The sampling error could be reduced to 20% if six to nine biopsies from the same patient were used [121]. The study was based on synovial tissue biopsies from hip or knee taken during arthroplasty, which was why the access to synovial tissue was high. No predefined areas of harvesting synovial tissue was

mentioned, which might have decreased the sample error considerably. Taking synovial tissue biopsies from the TMJ, a considerably smaller joint compared to the knee and hip, does not allow these numbers of samples without the risk of injuring the patient. This fact is hard to compensate for due to circumstances. To reduce inter-individual variation the samples were always taken from the posterior bilaminar zone.

In Studies III and IV many proteins were tested for differences between diagnosis and between outcome, respectively. Although multiple analyses were made, Bonferroni correction was not included. Since the method of testing proteins in the TMJ were novel and that we tested several proteins, we rather had type I errors than type II errors. In study III as many as 15/28 proteins were found to be significant. This implies that the result hardly can be by coincidence, for which otherwise Bonferroni correction is made, i.e. to decrease type I errors or incorrectly rejecting the null hypothesis [151]. In study IV only four proteins came out as significant after multivariate analyses. These results might be by chance and have to be verified in other studies.

Since our knowledge remains limited regarding TMJ diseases, further characterisation is motivated. Furthermore, enhanced knowledge of surgical outcome in relation to patients' characteristics might result in better surgical prediction and patient selection. Better tools for prognosticating treatment and describing illness would most certainly improve care givers decision-making, revitalise treatment development, and shorten patients suffering.

6 CONCLUSIONS

The aim of this thesis was to develop tissue-based diagnostic criteria for patients with DD of the TMJ, and to identify patient-specific factors that may predict TMJ surgical outcome. The thesis elucidates some new findings regarding TMJ illness and its treatment in general and for DD in particular.

In the analysis of a historic TMJ cohort treated with arthroscopy, the preoperative variables MIO, global pain, masticatory muscle palpation pain, and psychiatric comorbidity, were shown to predict outcome. Of these, only masticatory muscle palpation pain could be confirmed to predict outcome in subsequent prospective studies. The identified variables might be indicators for pain states of chronic nature, including hyperalgesia, chronic myofascial pain, and reduced coping strategies, related to a poor outcome.

Positive signs of bilateral masticatory muscle palpation pain proved to be a strong clinical indicator of failing TMJ arthroscopy related to the diagnoses DDwoR, DDwR, and OA. Although, the variable did not predict an unsuccessful outcome in patients with CIA. Bilateral masticatory muscle palpation pain might suggest the treating surgeon to re-evaluate non-surgical treatment and to postpone surgery, dependent on case analysis. Patients age and bilateral surgery were also identified with potential as future predictors.

In a majority of tested cytokines, significant concentration differences were found between diagnoses DDwR and DDwoR. The findings indicate an overall higher local inflammatory activity in DDwoR. The DDwoR subgroup SO had significantly higher concentrations of BMP-4, eotaxin, and IL-8 compared to DO. The described cytokine profiles might serve as biomarkers identifying disease, evaluating disease progress and treatment outcome.

Synovial tissue protein extracts were investigated for their potential as concentration-dependent surgical predictors. The proteins IL-8, lumican, MMP-7, and TIMP-2 had higher concentrations associated with worse outcome and might therefore become valuable predictors in selected cases.

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