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Histopathological analysis of the synovium in trapeziometacarpal osteoarthritis

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Abstract

Dorsoradial and anterior oblique ligaments were harvested during surgery in 13 patients with symptomatic trapeziometacarpal osteoarthritis, which had been graded preoperatively by a modified Eaton-Littler radiographic grading. Ligaments, including the periligamentous synovium, were stained with S100 protein, neurotrophic receptor p75, protein gene product 9.5, calcitonin gene related peptide, acetylcholine, substance P, neuropeptide Y, noradrenaline, N-methyl-D-aspartate-receptor and Met/Leu-enkephalin. The synovium was classified as showing no, low-grade or high-grade synovitis. Free nerve endings had higher immunoreactivity for substance P than for N-methyl-D-aspartate-receptor, enkephalin and noradrenaline. The synovial stroma had less immunoreactivity for N-methyl-D-aspartate-receptor than for noradrenaline, substance P and calcitonin gene related peptide. There was no relation between the grade of osteoarthritis and the visual pain analogue scale, synovitis score, immunoreactivity of all antibodies and quantity of free nerve endings or blood vessels. Synovium in trapeziometacarpal joint osteoarthritis produces several neuromediators causing a polymodal neurogenic inflammation and which may serve as biomarkers for osteoarthritis or therapeutic targets.

Keywords

Basal thumb joint, immunohistochemistry, nociception, osteoarthritis, synovium

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Introduction

Trapeziometacarpal joint osteoarthritis (TMJOA) affects the joint cartilage as well as the supporting soft tissues of the joint. The presence of synovitis in osteoarthritis (OA) is associated with more severe pain and joint dysfunction. The thin layer of synovial cells and the underlying vascularized connective tissue stroma provide structures and fluid essential for normal cartilage and joint function (Scanzello and Goldring, 2012). Synovial lining cells, lubricin and hyaluronic acid help to protect and maintain the integrity of articular cartilage surfaces in joints. Synovitis in OA is deemed to be a secondary reaction to cartilage fragments and debris.

The nervous system plays an active role in regulating pain and inflammation (Yam et al., 2018). Neurogenic inflammation refers to the inflammation that is produced through the release of mediators, such as substance P (SP) and calcitonin gene-related reptide (CGRP), from the nervous system. It involves a change in function of sensory neurons due to inflammatory mediators, inducing an enhanced release of neuropeptides from the sensory nerve endings. Polymodal nociceptors – encoding and processing harmful stimuli – serve to protect the body from potential harm, reacting to noxious thermal, mechanical or chemical stimuli, tissue damage and

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inflammation. Three nociceptive pathways consisting of autonomic, sensory and glutamatergic neuromediators have been established (Ackermann et al., 2009). The autonomic nervous system plays a critical role in regulating processes required for maintaining physiological homeostasis and responding to acute stressors. The sympathetic nervous system regulates inflammation at local and systemic levels through the release of noradrenaline together with neuropeptide Y. The parasympathetic nervous system regulates the inflammatory reflex at local and systemic levels through release of acetycholine (Ach) and vasoactive intestinal polypeptide. The neuropeptides SP and CGRP are mediators of the sensory nervous system. Both of them exert pro-inflammatory effects, for example vasodilation and protein extravasation (Brain and Williams, 1985). Anti-inflammatory opioid peptides, such as enkephalins, which inhibit inflammation and nociception, counteract the effects of SP and CGRP (Stein et al., 1990). Activation of glutamatergic receptors, for example the ionotropic N-methyl-D-aspartate (NMDA)-receptor, have been implicated in pain processing (Yan et al., 2013). Each of these nociceptive neuromediators contributes to the perception of pain by altering different neuroceptive pathways.

We have investigated the presence of nociceptive neuromediators by analysing the autonomic, sensory and glutamatergic neuromediators of the dorsoradial ligament (DRL) and anterior oblique ligament (AOL) synovium in patients undergoing surgery for TMJOA.

Methods

Samples

All protocols in this study were approved by the local ethics institutional review board. Thirteen surgical specimens from one male and 12 female patients with a median age of 67 years (range 51–83) were included in this study. Eight right and five left thumbs underwent complete trapeziectomy and suspension arthroplasty for advanced TMJOA. Patients with infectious and post-traumatic arthritis were excluded. Pain was perceived as severe with functional impairment, warranting surgical intervention. A preoperative pain visual analogue scale (VAS) was used to grade the subjective level of pain (Freyd, 1923; Hayes and Patterson, 1921). The scale ranged from 0 to 10 points, where 0 points meant no pain and 10 points extreme pain. The degree of OA in the TMJ was determined preoperatively using a modified Eaton–Littler radiographic staging (Eaton and Glickel, 1987; Eaton and Littler, 1973; Ladd, 2014) from posteroanterior, lateral, Robert (Ladd, 2014) and stress views (Wolf et al., 2009) of the thumb in a blinded fashion by the senior author (A.L.) and another surgeon not involved in the patients' care.

The senior surgeon (A.L.) carried out a dissection of the AOL and DRL of the TMJ with 3.5 loupe magnification according to the techniques of Bettinger et al. (1999) and Ladd et al. (2012). A strip of each ligament approximately 5 mm in width was harvested, tagging the distal insertion at the thumb metacarpal with a 6-0 nylon suture for orientation. The orientation and identification of each ligament was determined after locating the TMJ with an elevator through the capsular window beneath the abductor pollicis longus (APL) (Bettinger et al., 1999; Jónsson et al., 1996). The AOL was identified anterior and ulnar to the APL and dissected free from the overlying opponens muscle; the DRL was identified dorsal and ulnar to the APL (Bettinger et al., 1999). The presence of ossicles and osteophytes beneath the APL distorted ligament orientation but did not preclude harvesting. The DRL and AOL were harvested with their periligamentous synovium.

Antibodies

The following antibodies were used to investigate the nociception of the synovium: the sympathetic pro-inflammatory antibodies noradrenaline, neuropeptide Y and the parasympathetic anti-inflammatory antibody Ach from the autonomic pathway; SP and CGRP, as well as the opioid anti-inflammatory antibody Met/Leu-enkephalin to investigate the peripheral sensory regulation, as subdivided into sensory pro-inflammatory antibodies; and the NMDA-receptor antibody from the cell-proliferative glutamatergic excitatory pathway. S100 protein (S100), protein gene product 9.5 (PGP 9.5) and nerve growth factor receptor p75 (p75) were used to identify free nerve endings, as previously described (Rein et al., 2012) (Table 1).

Stainings

Specimens were immediately fixed in 4% buffered formaldehyde solution (pH = 7.4) for 24 h at 4°C, dehydrated and embedded in paraffin. Sections of 4 μm were cut and mounted onto silane-coated slides for conventional staining and immunohistochemistry. Two slices were stained for each antibody and staining.

The mounted sections were dehydrated beginning with xylol in decreasing concentrations. Sections were then rehydrated with distilled water. Some antibodies were pretreated and then rinsed in phosphate-buffered saline (PBS) (pH 7.4) 3×5 min. Slides were incubated in 1% H_2O_2 blocking

Table 1. Immunohistochemical antibodies.

			Pretreatment			Incubation	
Antibody	Source	Characteristics	Agent	Time	Dilution	Time (min)	Temperature (°C)
Noradrenaline	Code: HPA004057; Sigma, St. Louis, USA	Polyclonal rabbit antibody against noradrenaline	EDTA	5′/120°C	1:15	60	37
Neuropeptide Y	Code: NBP2-33423; Novus Biologicals, Cambridge, UK	Polyclonal rabbit antibody against neuropeptide Y	P25	10′	1:50	60	37
Nicotinic Acetylcholine Receptor alpha 4 (N1C1)	Code: GTX113653-100; Biozol, Eching, Germany	Polyclonal rabbit antibody against nicotinic acetylcholine receptor alpha 4	P25	10′	1:10	60	37
Substance P	Code: GTX62656-100; Biozol, Eching, Germany	Monoclonal rabbit antibody against substance P	P25	10′	1:25	60	37
CGRP	Code: LS-B10778; Biozol, Eching, Germany	Monoclonal mouse antibody against CGRP alpha	—	—	1:2000	60	RT
Met/Leu-enkephalin (NOC1/35)	Code: sc-47705; Santa Cruz Biotechnology, Heidelberg, Germany	Monoclonal mouse antibody against Leu^5 enkephalin	Trypsin	10′/37°C	1:10	60	37
NMDA-Receptor	Code: MA1-2014; Fisher Scientific, Schwerte, Germany	Monoclonal mouse antibody against NMDA Receptor 2B	P25	10′	1:75	60	37
Sm-actin	Code: M 0851; DakoCytomation, Glostrup, Denmark	Monoclonal mouse antibody against smooth muscle-actin 1A4	—	—	1:750	60	37
S-100	Code: Z 0311; DakoCytomation, Glostrup, Denmark	Polyclonal rabbit antibody against S100	—	—	1:750	60	RT
PGP 9.5	Code: 7863-0504; AbD Serotec, Düsseldorf, Germany	Polyclonal rabbit antibody against PGP 9.5	Citrate buffer	10′	1:250	60	RT
p75	Code: N-3908; Sigma, St. Louis, MO, USA	Polyclonal rabbit antisera against p75	—	—	1:300	60	RT

CGRP: calcitonin gene related peptide; EDTA : diaminoethanetetraacetic acid (code: AP-9004-050, Thermo Scientific, Braunschweig, Germany); NMDA-receptor: N-methyl-D-aspartate – receptor; PGP 9.5: protein gene product 9.5; p75: nerve growth factor receptor p75; P25: Protease 25 (code: AP-9006-005, Thermo Scientific, Braunschweig, Germany); RT: room temperature; S100: S100 protein.

endogenous peroxidase activity for 5 min at room temperature and rinsed for 3 × 5 min in PBS. Subsequently, the slides were treated with an ultra-vision blocking reagent (horseradish) peroxidase-polymer kit (HRP-polymer kit, code: TL-060 - HL; Thermo Scientific, Schwerte, Germany) for 5 min at 37°C, followed by incubation with primary antibodies. After rinsing with PBS 3 × 5 min, the secondary antibody with enhancer of the ultra-vision HRP-polymer kit was applied for 10 min at room temperature. The sections were washed in PBS 3 × 5 min again before the HRP-polymer kit was used for 15 min at room temperature. Afterwards the sections were rinsed in PBS 3 × 5 min once again and detected with 3,3′ diaminobezidine (DAB; code: BS04-110; medac, Wedel, Germany) for 8 min at room temperature.

This was followed by rinsing in distilled water and counterstaining with haematoxylin. Finally, sections were dehydrated and covered with Entellan (Merck, Darmstadt, Germany). Positive and negative controls are performed as previously described (Hewitt et al., 2014).

Histomorphologic analysis and cell counting

Histological examination of the stained tissue sections was done using a Zeiss light microscope (Observer.Z1; Carl Zeiss MicroImaging, Thornwood, NY, USA) with a Zeiss camera (Zeiss Axiocam MRm, Thornwood, NY, USA). Haematoxylin-eosin (HE) stained slices were used to examine tissue morphology and to classify synovitis as none, low-grade or high-grade, according to the classification of Krenn et al. (2006). This includes three features of chronic synovitis: the enlargement of the lining cell layer; the cellular density of synovial stroma; and the leukocytic infiltrate (Figure 1). The immunoreactivity of antibodies was analysed in the transmission mode at original magnifications of 25×, 100×, 200×, 400× at the lining cell layer, the synovial stroma and the free nerve endings, respectively. Immunoreactivity analysis was descriptive and noted as positive, if the total lining cell layer, the synovial stroma or the free nerve endings of the analysed specimen showed immunoreactivity. Blood vessels were counted in the smooth muscle-actin stained tissue and identified by specific immunoreactivity of smooth muscle-actin of the smooth muscle cells in the wall of the vessels. Free nerve endings were analysed in the S100, p75 and PGP 9.5 stainings according to the classification of Freeman and Wyke (1967) and counted in the S100 stained sections. The area of the analysed tissue was measured with ImageJ software (Schindelin et al., 2015) and reported in millimetres squared. The number of blood vessels and free nerve endings were adjusted to the size of the analysed tissue to determinine the density of blood vessels or free nerve endings per millimetre squared. All specimens were blinded for histological analysis.

Statistical analysis

The results of the modified Eaton–Littler score, the synovitis score and the immunoreactivity of the different antibodies are reported as absolute values. Medians with minimum and maximum and 95% confidence interval (CI) of the mean are used for descriptive statistics of free nerve endings, blood vessels and the VAS.

The first purpose was to analyse the immunoreactivity of the stainings in the synovium and free nerve endings between the different grades of synovitis, the VAS and modified Eaton–Littler score in all 26 ligaments, for which the two-tailed Fisher's exact test was used.

The second purpose was to compare the immunoreactivity of the different stainings to each other in the synovial lining cells, the synovial stroma and free nerve endings in all 26 ligaments, respectively. The Friedman test, followed by the Wilcoxon test with posthoc Bonferroni adjustment, were used.

The third purpose was to compare the amount of free nerve endings and blood vessels between the DRL and AOL. The Kolmogorov–Smirnov test found that the data did not have a normal distribution. The Mann–Whitney test was used.

The fourth purpose was to test correlations with regard to synovitis score, radiographic arthritis score, preoperative VAS, vascularity and free nerve endings. Correlation analysis was done using

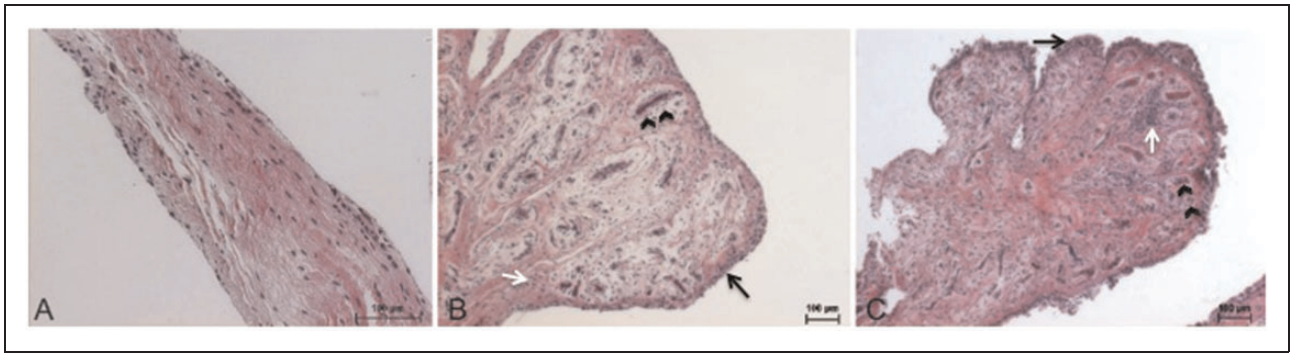

Figure 1. Synovitis score according to Krenn et al. (2006). Anterior oblique ligaments are stained with haematoxylin-eosin. (a) The synovial lining cells form one layer, the synovial stroma shows normal cellularity and no inflammatory infiltrates are observed in a specimen with no synovitis. (b) Low grade synovitis. Slight enlargement with two to three layers of the synovial lining cells (black arrow), slight inflammatory infiltration with small perivascular aggregates of lymphocytes and/or plasma cells (arrowheads) and slight synovial stroma activation, seen as low cellularity with slight oedema and slight fibrosis with some fibroblasts (white arrow). (c) High-grade synovitis. There are more than three layers of synovial lining cells (black arrow), a moderately increased cellularity of the synovial stroma with occasional multinucleated cells (arrowheads) and numerous lymphocytes or plasma cells, sometimes forming follicle-like aggregates (white arrow). Original magnification ×50.

Spearman's rho coefficient. All 26 ligaments were examined together for the correlation analysis. The level of significance was set at $p \leq 0.05$.

Results

Visual pain analogue scale

A preoperative VAS of 7 (range 4–9; 95% CI: 5.8 to 7.1) was obtained. The values of the VAS did not have a significant influence on positive immunoreactivity stainings for free nerve endings, synovial lining cells and synovial stroma.

Radiographs

Radiographic examination revealed OA Grade II in four, Grade III in three and Grade IV in six patients. The immunoreactivity in all the investigated stainings of the synovial lining cells, the synovial stroma and the free nerve endings showed no significant alterations with respect to the modified Eaton–Littler score.

Synovitis score and synovium

There was no synovitis in three AOL and three DRL specimens, low-grade synovitis in six AOL and nine DRL ligaments and high-grade synovitis in four AOL and one DRL (Figures 1 and 2). No significant differences between the synovitis score and all immunoreactivity stainings were seen for free nerve endings, synovial lining cells and the synovial stroma.

When comparing all stainings against each other, positive immunoreactivity of NMDA receptor in specimens ($n = 4$) was significantly less than for CGRP ($n = 20$), SP ($n = 16$) and noradrenaline ($n = 18$; $p < 0.0001$, respectively) in the synovial stroma (Figure 3). In contrast, there were no significant differences between all stainings for synovial lining cells (Figure 3).

Free nerve endings and blood vessels

Free nerve endings were mainly found in the vicinity of blood vessels and in the interstitium of the densely packed collagen fibres (Figure 4). Positive immunoreactivity of enkephalin ($n = 4$), noradrenaline ($n = 3$) and NMDA-receptor ($n = 2$) was significantly less in free nerve endings than for S100 ($n = 26$; $p < 0.0001$, respectively). Furthermore, the positive immunoreactivity of PGP 9.5 ($n = 20$) was significantly higher in free nerve endings than for noradrenaline ($n = 3$; $p = 0.00069$) and NMDA-receptor ($n = 2$; $p = 0.00034$). In addition, the positive immunoreactivity of SP ($n = 19$) was significantly higher than for enkephalin ($n = 4$; $p = 0.00034$), noradrenaline ($n = 3$; $p = 0.00017$) and NMDA-receptor ($n = 2$; $p < 0.0001$) (Figure 5). No significant differences in the density of free nerve endings were found between the DRL ((1.5; range 0.04–3.7; 95% CI: 1 to 2.3)/mm^2) and AOL ((2.6; range 0.13–6.2; 95% CI: 1.9 to 4)/mm^2). Furthermore, no significant differences in the density of blood vessels were seen between the DRL ((18.4; range 8–88.9; 95% CI: 12.6 to

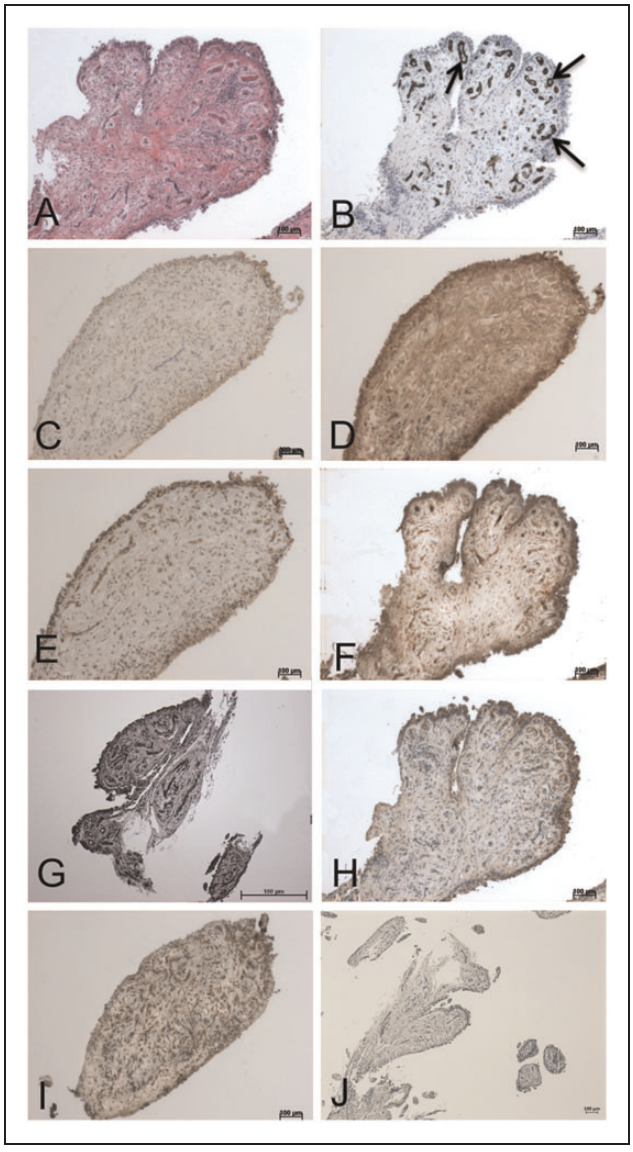

Figure 2. High-grade synovitis. An anterior oblique ligament is stained with (a) haematoxylin-eosin, (b) smooth muscle-actin, (c) N-methyl-D-aspartate (NMDA)-receptor, (d) noradrenaline, (e) neuropeptide Y, (f) acetylcholine, (g) substance P, (h) calcitonin gene-related peptide, (i) enkephalin, (j) a control. A pronounced vascularity is visible in the smooth muscle actin staining (arrows in B). Original magnification $\times 50$.

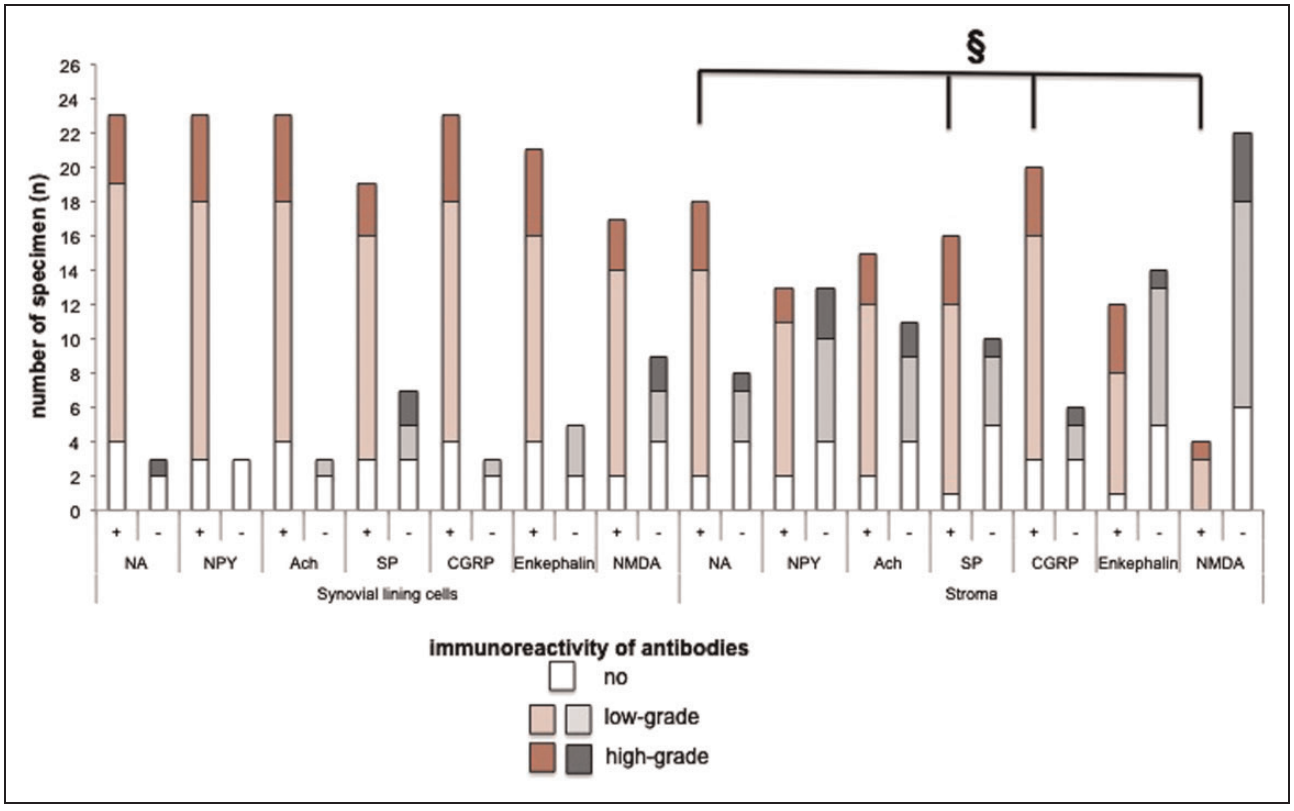

Figure 3. Distribution of the mediators in the synovium. The immunoreactivity of the different antibodies of the synovial lining cells and the synovial stroma are shown as absolute values differentiated into specimens with no, low-grade or high-grade synovitis, respectively. The immunoreactivity of NMDA receptor was significant less positive than for CGRP, SP and noradrenaline in the synovial stroma.
§: $p < 0.0001$;+: positive immunoreactivity; – : no immunoreactivity; NA: noradrenaline; NPY: neuropeptide Y; Ach: acetylcholine; SP: substance P; CGRP: calcitonin gene-related peptide; NMDA: N-methyl-D-aspartate-receptor.

38.02)/mm^2) and AOL ((24.2; range 12.4–34.4; 95% CI: 20.5 to 27.50/mm^2).

Correlation analysis

There was no correlation between the grade of radiographic arthritis, the synovitis, and the VAS and the quantity of free nerve endings or blood vessels (Table 2).

Discussion

Articular synovitis in OA is deemed to be a secondary reaction to cartilage damage and debris (Saito, 2003). Synovitis may contribute to the pathogenesis of OA (Goldenberg et al., 1982; Haywood et al., 2003; Scanzello and Goldring, 2012). In the current study, we examined the potential correlation of known autonomic, sensory and glutamatergic nociceptive markers to clinically relevant disease. We used the classification of Krenn et al. (2006), a general score applied to the spectrum of arthritides (Krenn et al., 2012; Molligan et al., 2016; Prieto-Potin et al., 2015). The Krenn scheme distinguishes OA synovitis as being relatively low-grade compared with high-grade rheumatoid arthritis synovitis, but still distinguishable from normal synovium. Our study, with six of 26 OA specimens with no and 15 of 26 OA specimens with low-grade synovitis, supports this finding.

Synovial inflammation drives synovial angiogenesis, which is linked to pain from OA (Haywood et al., 2003; Mapp and Walsh, 2012; Molligan et al., 2016). However, in the present study there was no correlation between the synovitis score and the quantity of blood vessels. Synovitis is generally associated with pain and joint dysfunction (Scanzello and Goldring, 2012), a finding supported by the fact that nociceptive mediators of the autonomous, sensory and excitatory pathways were all upregulated in the synovial lining cell layer. In addition, high amounts of the neuromediators CGRP, noradrenaline and SP were found in the synovial stroma. This is in accordance with previous reports. Increased concentrations of SP and CGRP have been found in the synovial fluid

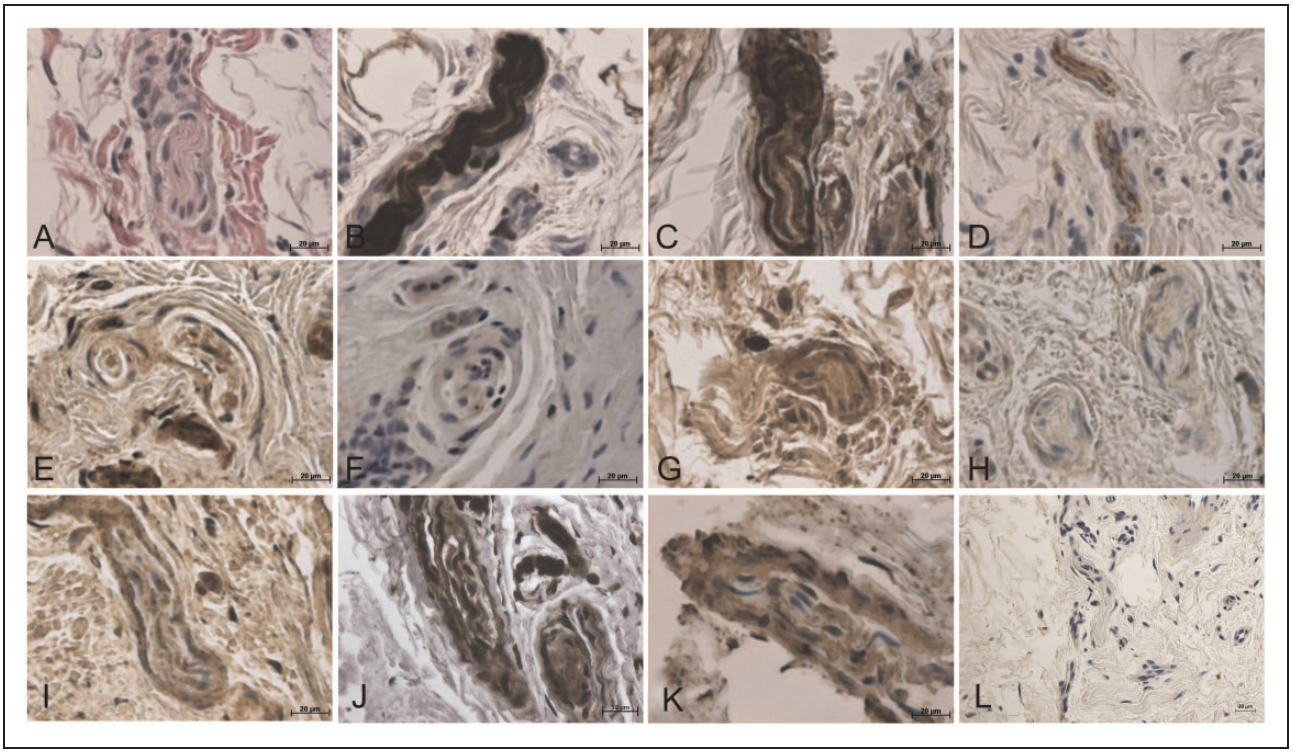

Figure 4. Free nerve endings. A free nerve ending from the dorsoradial ligament with no synovitis is stained for (a) haematoxylin-eosin, (b) S100 protein, (c) low-affinity nerve growth factor receptor p75, (d) protein gene product 9.5, (e) noradrenaline, (f) neuropeptide Y, (g) acetylcholine, (h) N-methyl-D-aspartate (NMDA)-receptor, (i) substance P, (j) calcitonin gene-related peptid, (k) enkephalin, (l) and a control. Original magnification ×400.

from OA joints (Dong et al., 2015; Wang et al., 2015). CGRP has been described as a biomarker for monitoring disease severity and playing a predictive role on prognosis and progression of knee OA (Dong et al., 2015) by promoting the release of inflammatory cytokines, and inducing a chain reaction of inflammation, which is associated with pain transmission and modulation. Furthermore, the expression of CGRP-positive and SP-positive neurons in the joints of OA patients is increased, which reveals the potential function of neuropeptides in painful degenerative joint disease (Saito and Koshino, 2000; Saxler et al., 2007). This is in accordance to the present results, where higher amounts of SP-positive free nerve endings were observed. Substance P exerts a trophic influence on neural tissue caused by inflammation through vasodilatation, plasma extravasation, the stimulation of synoviocytes to proliferate and produce inflammatory mediators (Lotz et al., 1987) and activation of macrophages, neutrophils or endothelial cells to induce phagocytosis and chemotaxis (Saito and Koshino, 2000).

Met - and Leu-enkephalins have a high affinity for the δ opioid receptor; both of them have been found in human synovium (Spetea, 2013). This is in accordance with the present study, in which enkephalin was detected in synovial lining cells in a high amount, with a moderate amount in the synovial stroma. In contrast, enkephalin was rarely found in free nerve endings. The opioid system is a key player in inhibition and modulation of pain (Stein et al., 1993). Enkephalin locally produced by synovial tissues maintains a certain level of intrinsic pain control due to inhibition of neurogenic inflammation by decreasing the release of SP from peripheral terminals of primary afferent neurons (Mousa et al., 2007).

Previous studies have established a correlation between the progression of cartilage damage and the presence of a reactive or inflammatory synovial membrane (Ayral et al., 2005; Sokolove and Lepus, 2013). This is in contrast to our investigation, in which there was no correlation between the radiological grading of OA, VAS and histological synovitis score. This indicates that the development of TMJOA is attributable to several factors, including hypermobility and permanent mechanical stress acting on the joint (Brandt et al., 2009; Jónsson et al., 1996; Ladd et al., 2013).

This descriptive analysis is of a small sample of patients with TMJOA rather than of a spectrum of patients. The predominantly female population

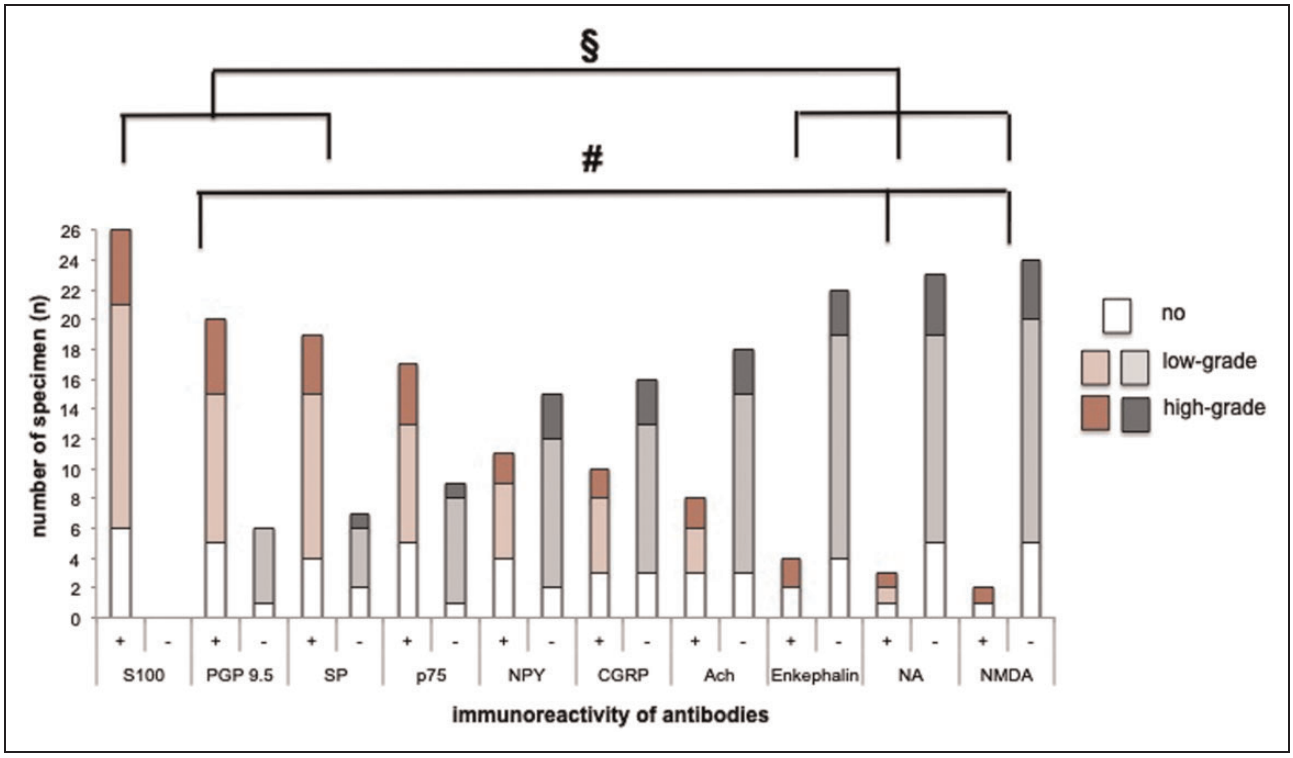

Figure 5. Immunoreactivity of free nerve endings. The immunoreactivity of the different antibodies of free nerve endings is shown as the number of the specimen differentiated into specimens with no, low-grade or high-grade synovitis. The positive immunoreactivity of enkephalin, noradrenaline and NMDA-receptor was significantly less than for S100 (§: $p < 0.0001$) and SP (§: $p < 0.001$). Furthermore, positive immunoreactivity of PGP 9.5 was significantly higher than for noradrenaline, and NMDA-receptor (#: $p < 0.001$).
S100: S-100 protein; PGP 9.5: protein gene product; SP: substance P; p75: low-affinity nerve growth factor receptor p75; NPY: neuropeptide Y; CGRP: calcitonin gene-related peptide; Ach: acetylcholine; NA: noradrenaline; NMDA: N-methyl-D-aspartate-receptor.

Table 2. Results of correlation analysis. There were no significant results.

	Eaton score	Synovitis score	VAS
Free nerve endings	$r = -0.07$; $p = 0.22$	$r = -0.27$; $p = 0.4$	$r = -0.15$; $p = 0.45$
Blood vessels	$r = -0.31$; $p = 0.32$	$r = 0.2$; $p = 0.32$	$r = -0.18$; $p = 0.4$
Eaton score	—	$r = 0.06$; $p = 0.77$	$r = 0.047$; $p = 0.82$
Synovitis score	—	—	$r = -0.06$; $p = 0.76$

VAS: visual pain analogue score.

reflects a typical treatment cohort and the synovial characteristics of male patients with OA remains to be examined. Furthermore, no data about the level of synovitis and its correlation to radiographic severity or VAS are available from an asymptomatic but arthritic population or healthy controls. A high preoperative VAS and radiographically confirmed OA warranted treatment by surgery. This is one explanation why no correlation was found between the synovitis score and VAS, as no patients with low VAS or mild arthritic changes would be treated surgically.

Symptomatic arthritis may be variably treated with nonoperative means, such as analgesia, physical therapy and activity modification (Bernstein, 2015). Intra-articular corticosteroid injections relieve pain in TMJOA for a short time; they act as anti-inflammatory and immunosuppressive agents, reducing prostaglandins, leukotrienes, bradykininins and histamines, interfering with inflammatory cell adhesion and migration, inhibiting synthesis of neutrophil superoxide, decreasing immunoglobulin production and stabilizing neural membranes (Hameed and Ihm, 2012). The relationship of nociceptors, synovitis

and severity of radiographic disease is currently unknown in patients treated without surgery. The present study has shown that clinically relevant nociceptive mediators are distributed in the synovium of TMJOA, which diversely represents the autonomic, sensory and glutamatergic systems, which implies that the many mechanisms of these nociceptor mediators are responsible for the pain caused by OA. These different nociceptive pathways may explain why anti-inflammatory and analgesic medication or intra-articular corticosteroid injections are often ineffective in reducing OA-related pain over time (Conaghan et al., 2015; Hameed and Ihm, 2012; Sale et al., 2006).

Denervation of the TMJ has been shown to reduce pain in patients with TMJOA (Tuffaha et al., 2019). One may hypothesize that this is, in part, due to the removal of innervation to the synovial membrane, which stops the neurogenic inflammatory pattern found in our study. Denervation and synovial excision without a traditional trapezectomy is a current form of surgical treatment (Ehrl et al., 2016). However, it may disrupt the proprioceptive functions of the periarticular tissue, consequently creating a dynamic impairment of TMJ stability (Mobargha, 2015).

In conclusion, synovial tissues from patients treated surgically for TMJOA was positively immunoreactive for most of the tested antibodies, demonstrating a polymodal neurogenic inflammation. The innervation of the synovial membrane is complex, with nerve fibres containing a host of neuroactive substances produced in the synovium. The degree of synovitis, however, did not correlate to the degree of radiographic arthritis or VAS. Our findings may serve as a foundation to understand the pain symptoms so frequently encountered in OA patients, as well as provide guidelines for future therapeutic interventions.

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Authors' contributions All authors made substantive intellectual contributions to this study, in conception and design (SR, JO, EH, SM, AL), acquisition of data (SR, JO, EH, SM, AL), analysis and interpretation of data (SR, JO, EH, AL), drafting and revising the manuscript (SR, JO, EH, SM, AL), as well as final approval of the version to be submitted (SR, JO, EH, SM, AL).

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Ethical approval All protocols in this study were approved by the local ethics committee review board. Dissection of the ligaments was performed in the Department of Orthopaedic Surgery, Chase Hand Center, Stanford University, Stanford, CA, USA. Histological investigations were done at the Center of Orthopaedics and Traumatology, University Medicine 'Carl Gustav Carus', Dresden, Germany.

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