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# Is neurogenic inflammation involved in tendinopathy? A systematic review

Shimon Vinay Zedeck Wasker,[1] Dimitris Challoumas,[2] Wai Weng,[1] George A C Murrell,[1] Neal L Millar [2]

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[1]Orthopaedic Research Institute, St George Hospital Sydney, University of New South Wales, Sydney, New South Wales, Australia
[2]School of Infection and Immunity, College of Medicine, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

**Correspondence to**
Dr Neal L Millar;
neal.millar@glasgow.ac.uk

## ABSTRACT

Neurogenic pain and inflammation have been hypothesised to play an important role in tendinopathy. This systematic review aimed to present and assess the evidence on neurogenic inflammation in tendinopathy. A systematic search was conducted through multiple databases to identify human case–control studies assessing neurogenic inflammation through the upregulation of relevant cells, receptors, markers and mediators. A newly devised tool was used for the methodological quality assessment of studies. Results were pooled based on the cell/receptor/marker/mediator assessed. A total of 31 case–control studies were eligible for inclusion. The tendinopathic tissue was obtained from Achilles (n=11), patellar (n=8), extensor carpi radialis brevis (n=4), rotator cuff (n=4), distal biceps (n=3) and gluteal (n=1) tendons. Through pooling the results of included studies based on the marker of neurogenic inflammation assessed, we identified possible upregulation of protein gene product 9.5 (PGP 9.5), N-methyl-D-aspartate Receptors, glutamate, glutamate receptors (mGLUT), neuropeptide Y (NPY) and adrenoreceptors in tendinopathic tissue versus control. Calcitonin gene-related peptide (CGRP) was not found to be upregulated, and the evidence was conflicting for several other markers. These findings show the involvement of the glutaminergic and sympathetic nervous systems and the upregulation of nerve ingrowth markers supporting the concept that neurogenic inflammation plays a role in tendinopathy.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The pathophysiology of tendinopathy remains incompletely understood.
⇒ Neurogenic inflammation is assumed to play a role in tendinopathy.
⇒ Better understanding of the implicated pathophysiology mechanisms can help with treatment of this challenging condition.

### WHAT THIS STUDY ADDS

⇒ We confirmed that neurogenic inflammation is present in tendinopathic tissues.
⇒ Our findings demonstrated involvement of the glutaminergic and sympathetic nervous systems in tendinopathy.
⇒ Nerve ingrowth markers were also found to be upregulated in diseased versus healthy tendon tissues.

### HOW THIS STUDY MIGHT AFFECT RESEARCH,PRACTICE OR POLICY

⇒ These findings suggest that further investigation on the role of neurogenic inflammation is warranted, partcilary to trty and address pain mechanisims in tendinopathy.

## INTRODUCTION

Tendinopathy is a common, often disabling condition associated with tendon pain, functional decline and reduced exercise tolerance.[1–3] Physical examination may encompass local swelling, tenderness and decreased range of motion. Some patients experience sudden ruptures without any pre-existing clinical symptoms, suggesting that tendinopathy development may, in some cases, be asymptomatic.[4] Histopathological evaluation of tendinopathic biopsies shows disorganised and calcified collagen fibres, elevated ground substance levels, morphological alterations of mitochondria and nuclei and the presence of mucoid patches, vacuoles and lipid cells.[4–9]

It has been hypothesised that tendinopathy occurs when tendon tissue undergoes chronic overload, which induces a state of hyperthermia, hypoxia and reduced vascularity, such that it cannot recover adequately.[10] Individuals' variations (age, genetics, sports activities, environmental conditions) may account for different repair threshold-associated responses to overload.[4] The contemporary 'biochemical' tendon pain model hypothesised that an unidentified biochemical mediator-driven stimulation of nociceptors in or around the tendon was the cause of pain in tendinopathy.[11] Furthermore, it has been suggested that tendinopathic damage occurs within an asymptomatic and symptomatic phase due to imbalanced protective and regenerative processes that ensue as part of a response to tendon overuse.[2] The continuum model of tendinopathy described chronic tendon disease as three continuous stages[11]: stage 1 is when tenocytes develop a homogeneous, non-inflammatory metaplastic and proliferative cell response to load

bearing; stage 2 comprises a healing response mediated by chondrocytes and myofibroblasts which secrete proteoglycan and collagen; stage 3 includes tenocyte apoptosis, and matrix and collagen breakdown, with no scope of reversibility.[11] In light of growing evidence, the 'biochemical' hypothesis has been revived in recent literature, suggesting that locally produced substances might drive vascular regulation, tissue modulation and/or pain mediation.[12]

Neurogenic inflammation is a subtype of inflammation that occurs when peripheral terminals of primary sensory neurons are triggered by local depolarisation, axonal reflexes or dorsal root reflexes, such as in the event of mechanical stress or injury.[13] These peripheral terminals release bioactive substances, such as substance P and calcitonin gene-related peptide (CGRP), which trigger the 'classical/chemical' inflammatory pathway upstream.[13] Neuromediators play an essential role in maintaining tendon homoeostasis.[14] It has been reported that tendinopathic pain is accompanied by neovascularisation, neoinnervation and elevated 'algogenic' substances (CGRP, glutamate, substance P), which have been hypothesised to cause neurogenic inflammation.[14–19] Finally, among others, the involvement of catecholamines, neurokinin-1 receptors (NK-1R) and N-methyl-D-aspartate receptors (NMDR-1) has been reported in tendinopathy.[19–26]

Our study aimed to review, assess and present the current evidence regarding neurogenic inflammation in tendinopathy. This may potentially provide further insights into the pathophysiology of this multifaceted and debilitating disorder and allow us to discover new therapies.

## MATERIALS AND METHODS

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.

### Search strategy

A systematic search was undertaken in November 2021 via the following databases: CINAHL PLUS EbscoHost, EMBASE, Medline OVID, Scopus, SPORTDiscus and Web of Science. The following Boolean operators were used: Tendinopathy OR Tendino* OR Tendinitis OR Tendonitis OR Tendon rupture OR Ruptured Tendon AND Neurogenic inflammat* OR Neurogenic-mediated inflammat* OR Neuro-mediated inflammat* OR Neuroinflammat* OR Neuro-inflammat* OR neur* OR nerv*.

For databases that use medical subject headings (MeSH) [AMED, CINAHL, EMBASE, MEDLINE and SPORTDiscus], free terms for the Neurogenic Inflammation (NI) were combined with the subject heading "nervous system" using the Boolean operator "OR". This method was also used for free terms and subject headings related to tendinopathy. These two groups (NI and tendinopathy) were then joined using the Boolean operator "AND". Only free terms were used for databases that did not use relevant subject headings (Biological Abstracts, Scopus and Web of Science). Review articles were used to identify eligible articles missed in the initial search. Additionally, reference list screening and citation tracking in Google Scholar were performed for each relevant article.

### Inclusion and exclusion criteria

Papers were included only if they were clinical case–control studies in humans (including those that obtained sampling of tendons for analyses) investigating the presence of neurogenic inflammation in tendinopathic tendons through the presence of cells, receptors, markers and mediators relevant to neurogenic inflammation. Eligible participants were of any age presenting with a clinical diagnosis of tendinopathy or spontaneous tendon rupture, considering the assumption that predominantly tendinopathic tendons are prone to spontaneous ruptures.[27] Diagnostic criteria of tendinopathy included a clinical presentation of chronic pain or loss of function of the affected tendon, with or without confirmatory imaging. We only included case–control studies and not other types of observational studies as we deemed it important to assess the presence of neurogenic inflammation markers in tendinopathic tissue compared with healthy tissue, as some of these may be present in both and be irrelevant.

Studies were excluded if they only assessed paratendinous tissue, were in vitro studies wherein tissue or cells were treated with cytokines or other agents or modified, animal studies, reviews, case reports or case series and studies that could not be obtained in English.

The search, selection of studies and data analysis were performed independently by two authors (SVZW and WW). Agreement on inclusion was achieved after a review of the full-text articles and a joint decision by both authors based on the inclusion/exclusion criteria. Data were then extracted using a spreadsheet that included patient demographics, symptom duration, investigations, control group type, tissue analysis method, statistical methods and methodological characteristics.

### Quality assessment

Methodological quality was assessed using a 15-point scale. This quality assessment tool was constructed using a set of questions assimilated by the authors from several sources.[20–23] It was designed so that each question would clearly and unambiguously target one important source of bias (online supplemental table 1). The first five questions (Q1–5) focus on the recruitment method employed in the studies. Questions 6–8 and 13 evaluate the relevance of each study in furthering our knowledge about neurogenic inflammation in tendinopathy. Questions 9–12, 14 and 15 assess whether the methodology employed in each study is valid and minimises any risk of bias. Studies were deemed as 'high quality' (>12), 'moderate quality' (10–12), or 'low quality' (<10) based on their overall score in the study quality assessment tool.

Each article was independently evaluated by two authors (SVZW and WW). Where disagreements existed, the opinion of a third author (DC) was sought, and a consensus was reached among the three assessors.

### Data handling

Data were extracted from each of the included papers by two of the authors (SVZW and WW) and were tabulated to facilitate analysis. The results of studies assessing the presence/upregulation/involvement of specific markers of neurogenic inflammation were pooled on a binary scale (upregulated or not upregulated), and an overall (pooled) result was obtained for each one of these markers. The three possible results for each marker were 'upregulated', 'not upregulated' or 'unclear due to conflicting evidence'. The overall result for each marker was derived from an agreement between the two first authors and the decision was based on the number of studies demonstrating a positive versus a negative outcome and the quality of these studies as assessed using our devised quality assessment tool. In the absence of a clear majority of either 'upregulated' or 'not upregulated', the overall result was deemed as 'unclear' (due to conflicting evidence). Only markers assessed by three or more studies were used for pooling. No meta-analyses were performed.

## RESULTS

### Search yield

The search of the 6 databases yielded 646 papers. On eliminating duplicates and irrelevant articles and those that did not match the inclusion and exclusion criteria, 31 case–control studies were found to be eligible for inclusion (figure 1).

Online supplemental tables 2 and 3 show the most important characteristics of the included studies. The findings of each study are summarised in online supplemental tables 4 and 5, illustrating the relevant markers of neurogenic inflammation assessed in each study.

### Study characteristics

Of the 31 studies included, 10 were related to the Achilles tendon, 7 to the patellar tendon, 2 both to Achilles and patellar tendons, 4 to the rotator cuff tendon, 4 to the extensor carpi radialis brevis (ECRB) tendon, 3 to the bicep tendon and 1 to the gluteal tendon. The included studies related to patients with painful tendinopathy,

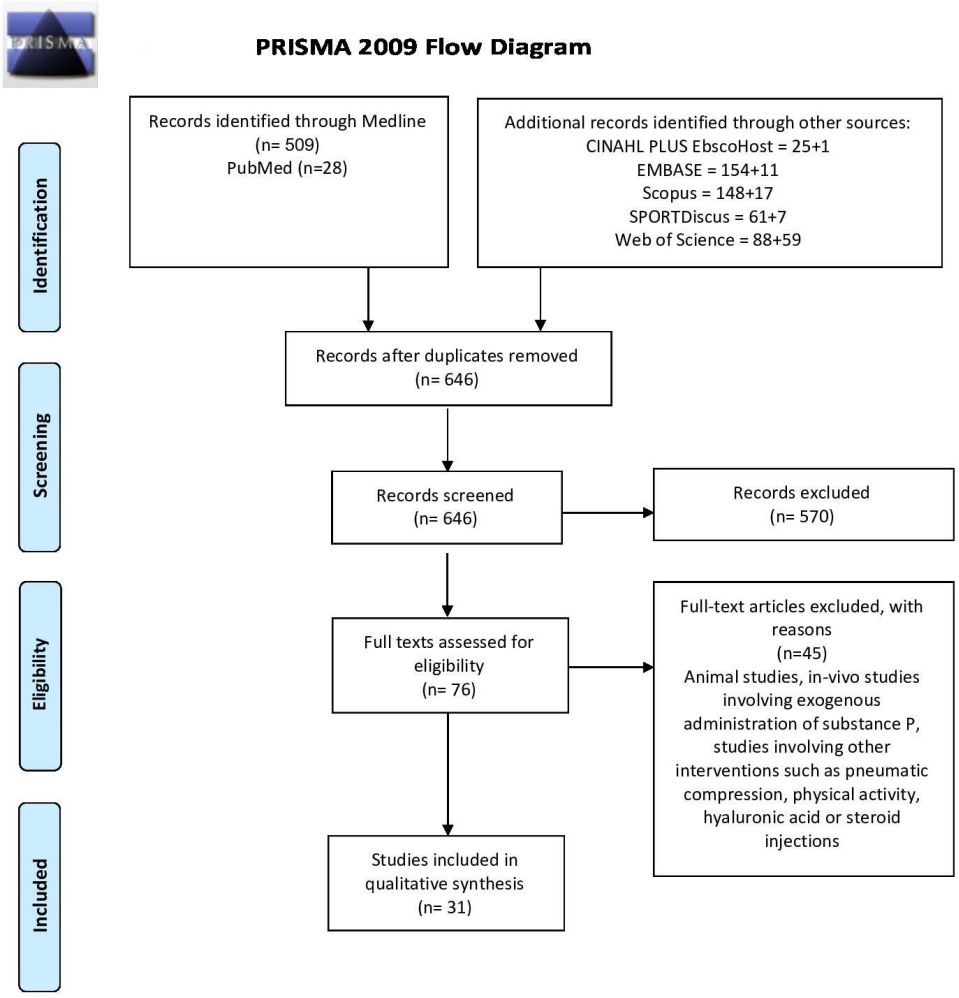


**Figure 1** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram of included studies. Adapted from: Moher D *et al*.[56]

and all studies stated specific diagnostic criteria. Samples were obtained during surgery for 23 studies, while the remaining 8 performed direct biopsy sampling. The mean ages of the overall patient groups were as follows: 44 years (Achilles), 26 years (patellar), 53 (rotator cuff), 44 years (ECRB) and 62 years (biceps). The control group consisted of healthy asymptomatic patients in 30 studies and cadaveric control in one study.

### Quality assessment

Five studies were deemed as 'high quality', 10 as 'moderate quality' and 16 as 'low quality'. The results of the study quality assessment are shown in online supplemental table 6.

### Main findings

The results are presented below based on the neurogenic inflammation marker assessed (table 1). Online supplemental table 5 summarises the findings of each study and shows the pooled result for each neurogenic inflammation marker separately.

#### Protein gene product 9.5

A total of nine case–control studies assessed for the involvement of protein gene product 9.5 (PGP 9.5) in tendinopathy. Four were of high quality,[15 24–26] three of moderate quality[28–30] and two of low quality.[31 32] Based on the pooled findings of these studies, PGP 9.5 is likely to be upregulated in tendinopathy.

#### Substance P

A total of 12 case–control studies assessed the involvement of substance P in tendinopathy. Four[25 26 33 34] were of high, four[27–29 34] of moderate and four[9 30 35 36] of low quality. While seven of these studies[26 28 30 32 33 37 38] suggest an upregulation of substance P, the other five studies[25 29 31 34 35] demonstrate no difference; therefore, the overall result is unclear due to conflicting evidence

#### Calcitonin gene-related peptide

Seven case–control studies assessed the involvement of CGRP in tendinopathy; one[25] was of high, three[28 29 34] of moderate and three[31 32 35 38] of low quality. Three of the studies[27 31 37] found upregulation of CGRP in tendinopathy, and four[26 29 31 35] found no differences. The overall result is, therefore, unclear due to conflicting evidence.

#### Glutamate

A total of six case–control studies assessed the involvement of glutamate in tendinopathy. Four[39–42] were of low, one[26] of moderate and one[24] of high quality. Based on the pooled findings of these studies, glutamate is likely to be upregulated in tendinopathy.

#### Glutamate receptor: mGLUT (metabotrophic glutamate receptor)

A total of four case–control studies[24–26 36] investigated the involvement of glutamate receptors (mGLUT) in tendinopathy, and all four demonstrated the upregulation of these receptors. Three[26 32 33] were of high and one[43] of low quality. mGLUT receptors are, therefore, likely to be upregulated in tendinopathy.

#### N-methyl-D-aspartate Receptors

A total of six case–control studies assessed the involvement of NMDAR in tendinopathy. Three[24–26 44] were of high, one[32] of moderate and two[38 39] of low quality. Based on the pooled findings of these studies, NMDAR is likely to be upregulated in tendinopathy.

#### Neurokinin 1 receptor

Three case–control studies assessed the involvement of NK-1R in tendinopathy. Two[37 45] were of low and one[25] of high quality. The overall result is unclear due to conflicting evidence.

#### Adrenoreceptors

A total of four case–control studies assessed the involvement of adrenoreceptors in tendinopathy. Two[25 46] were of high and two,[31 47] were of low quality. Based on the pooled findings of these studies, adrenoreceptors are likely to be upregulated in tendinopathy.

#### Tyrosine hydroxylase

A total of seven case–control studies assessed for the involvement of tyrosine hydroxylase in tendinopathy. Three[31 47 48] were of low, two[30 49] of moderate and one[25] of high quality. Based on the pooled findings of these studies, the overall result is unclear due to conflicting evidence.

#### Neuropeptide Y

A total of five case–control studies assessed for the involvement of neuropeptide Y in tendinopathy. Three[47] were of low, one of moderate and one[46] of high quality. Based on the pooled findings of these studies, neuropeptide Y is likely to be upregulated in tendinopathy.

#### Acetylcholinesterase

Three case–control studies assessed the involvement of AChE in tendinopathy. All three[39 40 50] of these were of low quality. Based on the pooled findings of the studies, the overall result is unclear due to conflicting evidence, as two showed upregulation of AChE and one no difference.

## DISCUSSION

The purpose of our study was to summarise the evidence for neurogenic inflammation in tendinopathy. We found six neuronal markers that are likely upregulated in tendinopathic samples versus control. These were PGP 9.5, NMDAR, glutamate, glutamate receptors (mGLUT), neuropeptide Y (NPY) and adrenoreceptors. Of the remaining markers, CGRP was shown not likely to be involved. However, there was conflicting evidence regarding the involvement of substance P, NK-1R, tyrosine hydroxylase and AChE in tendinopathy. These findings suggest the likely involvement of the glutaminergic (glutamate, NMDAR, mGLUT) and sympathetic

**Table 1** Overall result for each marker of neurogenic inflammation occurring from qualitative pooling of the results of all studies that assessed them

| Marker | Study | Result | Overall result |
|---|---|---|---|
| AChE | Alfredson *et al*, 2000a[41] | ↑ | Unclear |
| | Alfredson *et al*, 2001b[39] | ↑ | |
| | Danielson *et al*, 2006[57] | ↔ | |
| Adrenoreceptors | Franklin *et al*, 2014[25] | ↑ | Involved |
| | Tosounidis *et al*, 2013[45] | ↑ | |
| | Bjur *et al*, 2008[46] | ↑ | |
| | Danielson *et al*, 2007b[47] | ↑ | |
| CGRP | Sahmey *et al*, 2016[27] | ↑ | Unclear |
| | Bjur *et al*, 2005[31] | ↑ | |
| | Sahmey *et al*, 2016[27] | ↑ | |
| | Franklin *et al*, 2014[25] | ↔ | |
| | Sasaki *et al*, 2013[28] | ↔ | |
| | Singaraju *et al*, 2008[34] | ↔ | |
| | Danielson *et al*, 2007 (2)[30] | ↔ | |
| Glutamate | Schizas *et al*, 2010[26] | ↑ | Involved |
| | Alfredson *et al*, 2001[39] | ↑ | |
| | Alfredson *et al*, 2000a[41] | ↑ | |
| | Alfredson *et al*, 2000b[41] | ↑ | |
| | Alfredson *et al*, 1999[40] | ↑ | |
| | Dean *et al*, 2015[24] | ↔ | |
| mGLUT receptors | Dean *et al*, 2015[24] | ↑ | Involved |
| | Franklin *et al*, 2014[25] | ↑ | |
| | Schizas *et al*, 2012[36] | ↑ | |
| | Scott et al, 2008[42] | ↑ | |
| Neuropeptide Y | Sasaki *et al*, 2013[28] | ↑ | Involved |
| | Tosounidis *et al*, 2013[45] | ↑ | |
| | Bjur *et al*, 2009[49] | ↑ | |
| | Bjur *et al*, 2008[46] | ↑ | |
| | Danielson *et al*, 2007b[47] | ↑ | |
| NK-1R | Andersson *et al*, 2008[35] | ↑ | Unclear |
| | Forsgren *et al*, 2005[44] | ↑ | |
| | Franklin *et al*, 2014[25] | ↔ | |
| NMDAR | Franklin *et al*, 2014[25] | ↑ | Involved |
| | Schizas *et al*, 2012[36] | ↑ | |
| | Schizas *et al*, 2010[26] | ↑ | |
| | Alfredson *et al*, 2001[39] | ↑ | |
| | Alfredson *et al*, 2000 (1)[41] | ↑ | |
| | Dean *et al*, 2015[24] | ↔ | |

Continued

**Table 1** Continued

| Marker | Study | Result | Overall result |
|---|---|---|---|
| PGP 9.5 | Sahemey *et al*, 2016[27] | ↑ | Involved |
| | Dean *et al*, 2015[24] | ↑ | |
| | Franklin *et al*, 2014[25] | ↑ | |
| | Sasaki *et al*, 2013[28] | ↑ | |
| | Schizas *et al*, 2012[36] | ↑ | |
| | Xu *et al*, 2011[15] | ↑ | |
| | Danielson *et al*, 2007b[47] | ↑ | |
| | Lian *et al*, 2006[29] | ↑ | |
| | Bjur *et al*, 2005[31] | ↑ | |
| Substance P | Sahmey *et al*, 2016[27] | ↑ | Unclear |
| | Christensen *et al* 2015[32] | ↑ | |
| | Fearon *et al*, 2014[33] | ↔ | |
| | Franklin *et al*, 2014[25] | ↔ | |
| | Sasaki *et al*, 2013[28] | ↔ | |
| | Singaraju *et al*, 2008[34] | ↔ | |
| | Danielson *et al*, 2007[30] | ↔ | |
| | Schizas *et al*, 2012[36] | ↑ | |
| | Andersson *et al*, 2008[35] | ↑ | |
| | Lian *et al*, 2006[29] | ↑ | |
| | Bjur *et al*, 2005[31] | ↑ | |
| | Ljung *et al*, 1999[37] | ↑ | |
| Tyrosine hydroxylase | Zeisig*et al*, 2009[48] | ↑ | |
| | Bjur *et al*, 2008[46] | ↑ | |
| | Danielson *et al*, 2007a[30] | ↑ | Unclear |
| | Danielson *et al*, 2007b[47] | ↑ | |
| | Franklin *et al*, 2014[25] | ↔ | |
| | Lian *et al*, 2006[29] | ↔ | |

↑, upregulated; ↔, not upregulated.
AChE, Acetylcholinesterase; CGRP, calcitonin gene-related peptide; mGLUT, metabotrophic glutamate receptor; NMDAR, N-methyl-D-aspartate receptor; PGP 9.5, protein gene product 9.5.

nervous (NPY, adrenoreceptors) systems and the upregulation of nerve ingrowth markers (PGP 9.5) (figure 2). These results derived from pooling of studies of different tendinopathy locations, which may involve different pathophysiological processes and neuronal markers, therefore making definitive conclusions is difficult.

Our findings are partly consistent with two previously conducted reviews. Jewson *et al*[22] mainly investigated the involvement of the sympathetic nervous system in tendon disease; they included 13 observational studies (including cohort studies without controls) and concluded that sympathetic innervation (adrenoreceptors α1 or α2A and β1, NPY, tyrosine hydroxylase) is likely not upregulated in tendon proper but may be upregulated in paratendinous tissues in patients with tendinopathy.[22] The review by Dean *et al*[23] evaluated the correlation between pain symptoms and the trend in peripheral neural markers in painful human tendinopathy. They concluded that painful tendinopathy is accompanied by an upregulation of nerve ingrowth markers (PGP9.5, GAP43) and glutaminergic system (Glutamate, NMDAR, mGlut receptors). Specifically, substance P was particularly implicated in rotator cuff tendinopathy. This latter study was very similar to ours in that it only included case–control studies and assessed the presence of neurogenic inflammation in general; we added four studies published after the review and presented updated results, having handled data slightly differently. Similarly to Dean *et al*, we found strong evidence for the upregulation of the glutaminergic system and nerve ingrowth markers in tendinopathic tissue. However, our results were unclear regarding the upregulation of substance P. In contrast to the conclusions of Jewson *et al*, we found that the sympathetic nervous system is likely to be upregulated in tendinopathic tissue.

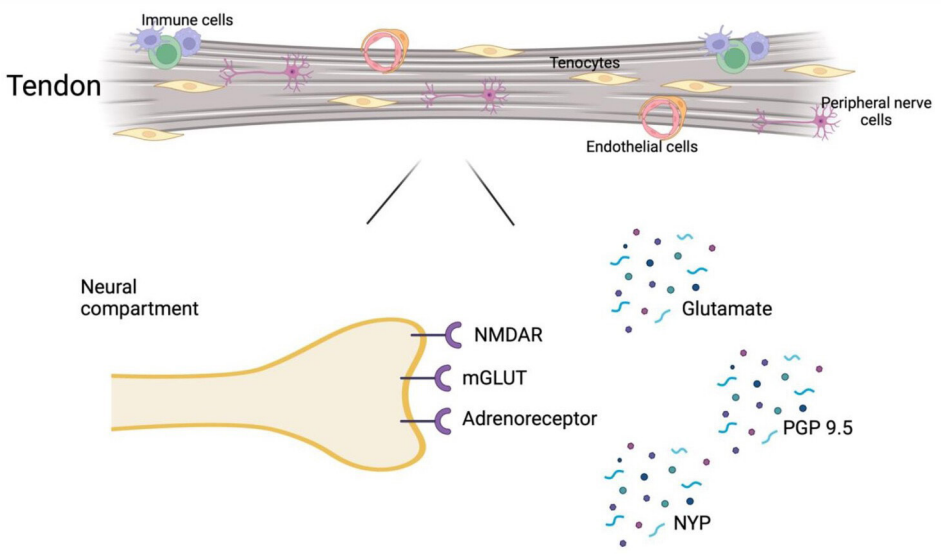


**Figure 2** Mediators of neurogenic inflammation involved in tendinopathy. The neural compartment within the tendon detailing the mediators discovered through the systematic review. In the homoeostatic state the neural compartment plays a role in proprioception. It interacts with immune cells to modulate adaptive responses in the normal tendon, but excessive stimulation leads to tissue breakdown, degeneration and neoinnervation involving the glutamatergic and autonomic systems. The systematic review found N-methyl-D-aspartate receptors (NMDAR), adrenoreceptors and glutamate receptors (mGLUT) to be upregulated in tendinopathic tissues. Furthermore the release of neuropeptides, such as neuropeptide Y (NPY), glutamate and protein gene product 9.5 (Pgp 9.5) stimulates immune cell activation, releasing various agents, which modulate a variety of cell activities in the matrix.

Neurogenic inflammation is mediated by the peripheral nervous system responding to noxious stimuli.[13] These stimuli include signals associated with tissue damage (ATP, uric acid and hydroxynonenals), environmental signals (heat, acidity and chemicals), pathogen-associated signals (bacterial or viral proteins), as well as chemokines released from immune cells.[13] These signals are detected by various receptors such as danger-associated molecular pattern receptors (TRP channels, P2X channels), pattern recognition receptors (Toll-like receptors and Nod-like receptors) and cytokine receptors, which are present on afferent neurons. Nociceptive stimulation of sensory neurons generates antidromic axon reflexes that cause the release of neuropeptides. These neuropeptides trigger an inflammatory response, including recruitment and activation of immune cells, vasodilation and exudation.[13]

Ackermann. discussed the growing evidence for the role of neural elements in tissue homoeostasis and healing in connective tissues such as tendons and ligaments.[14 51] Several studies have consistently demonstrated positive immunohistochemical staining for the protein marker PGP 9.5 in tendinopathy.[24 29] PGP 9.5 stains for all nerves and was particularly noted to be upregulated in abnormal tenocytes and perivascular areas of the tendon sample.[24 26 34 35 37] Xu *et al* hypothesised that this association between neoinnervation and angiogenesis may be involved in pain signalling in tendinopathy.[15] Sahmey *et al* suggested that in tendinopathy, tenocytes behave like neuroendocrine cells and secrete peptides such as substance P, CGRP and VEGF, which trigger an inflammatory cascade of events downstream.[27] The predominant proportion of upregulated innervation in tendinopathic samples corresponded to sympathetic innervation, evidenced by positive NPY staining.[29 31 32 46 47] Sasaki *et al* suggested that NPY may reflect central sensitisation secondary to nascent sympathetic innervation. However, only a very small proportion of tendon tissue staining was associated with sensory innervation, evidenced by decreased expression of CGRP and substance P.[29] This is consistent with the findings of this systematic review, where the overall result indicates that CGRP appears not to be upregulated in tendinopathy. Sasaki *et al* and Lian *et al* suggested that the loss of sensory innervation of the tendinosis tissue and the upregulation of sympathetic innervation are crucial in understanding chronic tendon pathology.[28 29] Lian *et al* observed the sprouting of sensory nerve endings inside the tendon properly and suggested that it reflects the intensification of nociceptive signalling secondary to recurring mechanical impetus. They further propose that the upregulation of sympathetic innervation may very well act contrary to nociceptive signalling, thus helping to modulate and reduce tendon pain.[30]

The autonomic nervous system is largely involved in regulating blood flow to the tendons during exercise, wherein acetylcholine causes vasodilation, while sympathetic neuropeptides mediate vasoconstriction.[52] Danielson *et al* reported the presence of alpha1-adrenoreceptor and tyrosine hydroxylase in tendinopathic tendons and therefore hypothesised

the local catecholamine synthesis in tendinopathy.[30] Furthermore, the same group notes that adrenergic receptors stimulation produces degenerative/apoptotic events and cell proliferation, which is known to be present in the early and late phases of tendinopathy.[31 53] They also demonstrated the presence of muscarinic receptors, choline acetyltransferase and vesicular acetylcholine transporters in tendinopathic tissue samples, which suggests an upregulation of the cholinergic system as part of the neurogenic inflammatory response in tendon disease.[50]

This systematic review suggests that the overall result concerning the upregulation of substance P in tendinopathy is conflicting. Murphy and Hart noted that substance P altered the expression of plasminogen activator and plasminogen activator inhibitor in the ligament, epiligament and synovial tissues of rabbits.[53] Han *et al*[54] observed higher substance P gene expression levels in human tendinopathic tissue compared with healthy tenocytes. Furthermore, they demonstrated that exposing healthy tenocytes to substance P resulted in increased cellular proliferation, synthesis of type 3 collagen and morphological alteration similar to what we see in tendinopathic tenocytes.[55] Burssens *et al*[55] reported exogenous substance P injection to induce fibroblast proliferation and improved collagen organisation in injured rat Achilles' tendon.

Several studies have confirmed glutamate, an excitatory neuropeptide, to be upregulated in tendinopathy.[26 39–41] Additionally, glutamate receptors such as NMDAR and mGLUT have also been identified and localised in tendinopathic tissue samples.[26 36 39] These changes were prominent in morphologically altered tenocytes and vasculature and were absent in control samples. A possible explanation of glutamate upregulation may be its role in cell-hyperexcitation, pain signalling and cell proliferation/differentiation.[39 51] Franklin *et al* suggested that the early inflammatory changes in tendinopathy upregulate the expression of glutaminergic receptors, which in turn results in peripheral sensitisation.[25]

This review also accounts for other neural markers, which may be implicated in tendinopathy's pathophysiology and clinical presentation. These include neuron-specific enolase, PAR receptors, KA1, Nav1.7, TRPA1 BDKRB2, S-100, BDNF, CB1, GAP 43, NGF, BDNF P75, M2 Ach Receptor, ChAT, VAChT. However, a comprehensive analysis of their upregulation was not possible due to limited studies undertaken on these specific markers.

We recognise the limitations of our review. Results were pooled without accounting for the location of tendinopathy, assuming that the potential upregulation of neurogenic inflammation markers would be consistent in all tendinopathies; subgroup analyses would result in fewer studies being pooled, which could compromise the strength of evidence. However, to the best of our knowledge, we conducted a detailed literature search and included all eligible studies and performed a thorough study quality assessment, which was accounted for in our overall results.

## CONCLUSION

We found strong evidence for the upregulation of nerve ingrowth markers, the glutaminergic and sympathetic nervous systems in tendinopathic tissue. The involvement of the parasympathetic nervous system and the upregulation of sensory nerves remains unclear. More high-quality case–control studies are needed to contribute data to future reviews that will hopefully report results with higher strength of evidence and clarify the possible involvement of markers for which evidence was conflicting.

**Twitter** Neal L Millar @tendonglasgow

**Contributors** SVZW and DC conceived and designed the study. SVZW and DC performed analysis. GAM and NLM provided expert advice. All authors analysed the data. SVZW, WW, GAM and NLM wrote the paper.

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**ORCID iD**

Neal L Millar http://orcid.org/0000-0001-9251-9907

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# Supplementary Information: Neurogenic Inflammation in Tendinopathy

| Number | Criteria | Decision Rule | Yes/No/Unclear |
|---|---|---|---|
| 1 | **Recruitment** method clearly reported? | Yes, if the study states how participants were recruited.<br><br>No, if the method of recruitment is not stated or is unclear. | |
| 2 | **Inclusion/exclusion criteria** clearly described? | Yes, if clear eligibility criteria for participant inclusion and/or exclusion are reported.<br><br>No, if eligibility criteria are not given or are unclear | |
| 3 | **Study Population:** Are the **cases and controls** recruited over the same period of time? | Yes, if it is stated that cases and controls were recruited concurrently.<br><br>No, if cases and controls were not recruited concurrently, if recruitment times were unclear or if recruitment times were not reported.<br>Score N/A if only one group. | |
| 4 | **Study Population:** Are cases and controls drawn from the same population? | Yes, if both the case and control group were drawn from the same source population.<br><br>No, if case and controls groups are from different populations or if unclear.<br>Score N/A if only one group. | |
| 5 | **Study Population:** Are the participants representative of the population from which they were recruited? | Yes, if the study states that consecutive eligible participants were used, participants were randomly selected, or all participants were used from the source population. | |

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|---|---|---|---|
| 6 | **Case**: Is the case definition explicit? | Yes, if the criteria for diagnosing injury is clearly described. OR<br>Yes, if diagnosis was made using established criteria and an appropriate reference is given (e.g. a consensus document).<br><br>No, if the criteria for diagnosis are not given or are unclear.<br>N/A if no case group. | |
| 7 | **Control:** Is the control group free from injury? | Yes, if the method of confirming that the control group is free from the target injury is reported.<br><br>No, if the method of confirming the control group is free of injury is not given or is unclear.<br>Score N/A if only case group. | |
| 8 | **Method**: Were markers of neurogenic inflammation assessed identically in the case and control group? | Yes, if the measurement of neurogenic inflammation was stated to be identical in the case and control group.<br><br>No, if there were any differences in measurement technique between the case and control group.<br>N/A if only one group. | |
| 9 | **Method:** Was the reliability of the measurement technique reported? | Yes, if reliability estimates of the measurement technique was calculated or a reliability study was cited. | |
| 10 | **Method:** Was assessor blinding reported? | Yes, if is stated that the assessor measuring sympathetic involvement was blind to injury status. | |

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|---|---|---|---|
| | | No, if the assessor is aware of injury status or if no mention is made of assessor blinding.<br>N/A if only one group. | |
| 11 | **Method:** Were the observational tests used to assess the main outcomes appropriate? | Yes, if the observational tests used were appropriate for the research question and the data with minimum 2 independent observers?<br><br>No, if no quantitative or semi-quantitative descriptive analysis was reported. | |
| 12 | **Method:** Are the distributions of principal confounders in each group of subjects to be compared clearly described? | Yes, if summaries of participant age, sex, BMI (or height and weight) are provided for the case and control group.<br><br>No if study did not provide data for at least these factors. | |
| 13 | **Data Analysis:** Are differences between neurogenic inflammation markers between the case and control group clearly reported? | Yes, if the comparison of neurogenic inflammation between case and control groups is clearly described. OR<br>Yes, if data is provided in sufficient detail to calculate a comparison between the case and control groups<br><br>No, if comparison is not clearly described.<br>No, if comparison is given as significant or non-significant without p-value or detailed data.<br>N/A if only one group | |
| 14 | **Data Analysis:** Does the study provide estimates of the random variability in the data for the main outcomes? | Yes, if an estimate of data variability is provided for sympathetic involvement. Acceptable estimates include SD, SE, and IQR. | |

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|---|---|---|---|
| | | No, if an estimate of data variability is not provided.<br>Range of scores not acceptable.<br>N/A if no statistical analysis performed. | |
| 15. | **Study Design:** Study limitations addressed? | Yes, if study limitations appropriately addressed.<br><br>No, if study limitations are not listed. | |

**Supplementary Table 1: Methodological Quality Assessment Tool**. *N/A, Not applicable; SD, standard deviation; SE, standard error of the mean; IQR, Interquartile range*

| S.No. | Study | Type of study | Specimen | Tendon | Tissue | Detection |
|---|---|---|---|---|---|---|
| **1.** | Schmalzl J et al., 2019 | Case-Control | Biopsies from living tissue | Long head of biceps tendon | Tendon stump | Immunohistochemistry |
| **2.** | Sahmey et al 2016 | Case Control | Biopsies from living tissue | Supraspinatus tendon | Tendon and tendon sheath | Immunohistochemistry |
| **3.** | Christensen J et al 2015 | Case-Control | Biopsies from living tissue | Achilles tendon | Mid-portion of Achilles Tendon (from ventral side) | Immunofluorescence |
| **4.** | Dean et al 2015 | Case control | Biopsies from living tissue | Supraspinatus tendon | Within 1cm of bony insertion into greater tuberosity | Immunohistochemistry |
| **5.** | Fearon et al 2014 | Case Control | Biopsies from living tissue | Greater trochanteric bursa and gluteal tendon | Mid tendon | Immunohistochemistry |
| **6.** | Franklin et al 2014 | Case Control | Biopsies from living tissue | Rotator Cuff tendon | Supraspinatus tendon | Immunohistochemistry |
| **7.** | Sasaki et. al., 2013 | Case Control | Biopsies from living tissue | Extensor Carpi Radialis Brevis | Capsular aspect of ECRB tendon | Immunohistochemistry |
| **8.** | Tosounidis et al 2013 | Case Control | Biopsies from living tissue and cadavers | Long head of Bicpes Brachii (LHB) tendon | Tendon proper and surrounding tissue | Immunohistochemistry |
| **9.** | Schizas et al 2012 | Case Control | Biopsies from living tissue | Patellar tendon | Tendon proper and peritendinous loose CT | Immunohistochemistry |
| **10.** | Bagge et al 2012 | Case Control | Biopsies from living tissue | Achilles tendon | Mid-portion | In Situ Hybridization and Immunohistochemistry |

| | | | | | | |
|---|---|---|---|---|---|---|
| **11.** | Bjorklund et al 2011 | Case Control | Biopsies from living tissue | Achilles tendon | Tendon mid-portion (tendon proper and paratendinous connective tissue) | Immunofluorescence |
| **12.** | Xu et al 2011 | Case Control | Biopsies from living tissue | Rotator Cuff tendon - torn Supraspinatus tendon and matched intact Subscapularis tendon | Torn edges of Supraspinatus and intact subscapularis tendons | Immunohistochemistry |
| **13.** | Schizas et al 2010 | Case Control | Biopsies from living tissue | Patellar tendon | | Immunofluorescence |
| **14.** | Bagge et al 2009 | Case Control | Biopsies from living tissue | Achilles tendon | Ventral part of mid tendon (tendon mid-portion) | Immunofluorescence |
| **15.** | Bjur et al 2009 | Case Control | Biopsies from living tissue | Achilles tendon | Mid portion + paratendinous tissue | Immunohistochemistry |
| **16.** | Zeisiget al 2009 | Case Control | Biopsies from living tissue | Extensor carpi radialis brevis (ECRB) tendon | Muscle origin at the lateral epicondyle (TE) & origin of the flexor muscles at the medial | Immunofluorescence |

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| | | | | | epicondyle (GE) | |
| **17.** | Singaraju et al 2008 | Case Control | Biopsies from living tissue and cadavers | Long head of the biceps brachii (LHBB) tendon | Portion of the LHBB tendon above the bicipital groove | Immunohistochemistry |
| **18.** | Andersson et al 2008 | Case Control | Biopsies from living tissue | Achilles tendon | Mid-portion tendon | Immunofluorescence & In Situ Hybridization |
| **19.** | Bjur et al 2008 | Case Control | Biopsies from living tissue | Achilles tendon | Mid-portion tendon | Immunofluorescence & In Situ Hybridization |
| **20.** | Scott et al 2007 | Case Control | Biopsies from living tissue | Patellar and Achilles tendon | | Immunofluorescence & In-Situ Hybridisation |
| **21.** | Danielson et al 2007 (1) | Case Control | Biopsies from living tissue | Patellar tendon | | In-Situ Hybridisation |
| **22.** | Danielson et al 2007 (2) | Case Control | Biopsies from living tissue | Patellar tendon | Tendon proper | Immunofluorescence |
| **23.** | Danielson et al 2006 | Case control | Biopsies from living tissue | Patellar tendon | Proximal patellar tendon | Immunofluorescence & Immunostaining using EnVision detection |
| **24.** | Lian et al 2006 | Case Control | Biopsies from living tissue | Patellar tendon | | Immunofluorescence |
| **25.** | Bjur et al 2005 | Case Control | Biopsies from living tissue | Achilles tendon | Mid-portion tendon | Immunohistochemistry |

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|---|---|---|---|---|---|---|
| **26.** | Forsgren et al 2005 | Case control | Biopsies from living tissue | Patellar or Achilles tendon | Proximal part of Patellar tendon Mid-portion of Achilles tendon | Immunofluorescence |
| **27.** | Alfredson et al 2001 (1) | Case Control | Biopsies from living tissue | Achilles tendon | | Microdialysis – high performance liquid chromatography and Immunohistochemistry |
| **28.** | Alfredson et al 2001 (2) | Case Control | Biopsies from living tissue | Patellar tendon | | Microdialysis- high performance liquid chromatography and Immunohistochemistry |
| **29.** | Alfredson et al 2000 | Case Control | Biopsies from living tissue | Extensor Carpi Radialis Brevis tendon | | In situ microdialysis |
| **30.** | Alfredson et al 1999 | Case control | Biopsies from living tissue | Achilles tendon | | In situ microdialysis |
| **31.** | Ljung et al 1999 | Case Control | Biopsies from living tissue | Extensor Carpi radialis brevis tendon | Dorsal aspect of tendon insertion | Immunohistochemistry |

**Supplementary Table 2: Study Characteristics – part A**

| Study | Group | Tendon Status | Sample Size | Sex | Age, years (mean and/or range) |
|---|---|---|---|---|---|
| 1. Schmalzl J et al., 2019 | Case (arthroplasty, rotator cuff surgery, Isolated biceps surgery) | Tendinopathy | 11 | 9 M 2 F | 60-82 |
| | Control (arthroplasty, rotator cuff surgery, Isolated biceps surgery) | No Tendinopathy | 11 | 4 M 7 F | 46-67 |
| 2. Sahmey et al 2016 | Case (rotator cuff surgery) | Tendinopathy | 4 | 2 M 2 F | 39-53 |
| | Control (arthroscopic re-stabilisation) | No Tendinopathy | 1 | 1 M | 20 |
| 3. Christensen et al., 2015 | Case (Achilles' tendinosis surgery) | Tendinopathy | 17 | 6 M 11 F | 27-68 |
| | Control (healthy individuals) | No Tendinopathy | 4 | 4 M | 21- 48 |
| 4. Dean et al 2015 | Case (subacromial decompression surgery) | Tendinopathy | 9 | 7M 2F | 51 +/- 8.2 |
| | Control (5-years after subacromial decompression ('pain-free') | No Tendinopathy | 9 | 6M 3F | 52 /- 7.8 |
| 5. Fearon et al 2014 | Case (gluteal tendon reconstructive surgery and bursectomy) | Severe Tendinopathy (SD 12.65) | 34 | - | - |
| | Control (total hip arthroplasty) | Mild Tendinopathy (SD 10.43) | 29 | - | - |

| Study | Group | Tendon Status | Sample Size | Sex | Age, years (mean and/or range) |
|---|---|---|---|---|---|
| 6. Franklin et al 2014 | Case (arthroscopic or open tendon repair) | Tendinopathy | 64 | 39 M<br>25 F | 50- 78 |
| | Control (post-traumatic shoulder instability) | No Tendinopathy | 16 | 14 M<br>2 F | 17-29 |
| 7. Sasaki et. al., 2013 | Case (recalcitrant tennis elbow) | Tendinopathy | 8 | 2 M<br>6 F | 38-66 |
| | Control (ECRB capsule of Osteochondritis Dissecance of Capitellum) | No Tendinopathy | 2 | 1 M<br>1 F | 15 |
| 8. Tosounidis et al 2013 | Case: RC tear and biceps tendinitis | Tendinopathy | 14 | 6 F<br>8 M | 51-76 |
| | Control A (shoulder hemiarthroplasty for management of complex proximal humerus fractures) | No Tendinopathy | 17 | 1 M<br>16 F | 56-81 |
| | Control B (specimens from cadavers with no history of shoulder pain, trauma or systemic disease) | No Tendinopathy | 10 | 2 M<br>8 F | 60- 82 |
| 9. Schizas et al 2012 | Case (jumper's knee) | Tendinopathy | 10 | 9 M<br>1 F | 19-32 |
| | Control (tibial shaft fractures undergoing intramedullary nailing) | No Tendinopathy | 8 | 5 M<br>3 F | 19-60 |

| Study | Group | Tendon Status | Sample Size | Sex | Age, years (mean and/or range) |
|---|---|---|---|---|---|
| 10. Bagge et al 2012 | Case (Achilles' tendinosis surgery) | Tendinopathy | 2 | 1 M<br>1 F | 29<br>52 |
| | Control | No Tendinopathy | 2 | 2 F | 47 |
| 11. Bjorklund et al 2011 | Case (Achilles' tendinosis surgery) | Tendinopathy | 17 | 8 M<br>9 F | 28-70<br>47-68 |
| | Control (healthy tendon) | No Tendinopathy | 7 | 3 M<br>4 F | 39-46<br>21-47 |
| 12. Xu et al 2011 | Case (rotator cuff tear repair) | Tendinopathy | 26 | 14 M<br>12 F | 30-73 |
| | Control (shoulder instability) | No Tendinopathy | 10 | 3 F<br>7 M | 17-59 |
| 13. Schizas et al 2010 | Case ((jumper's knee) | Tendinopathy | 10 | 9 M<br>1 F | 19-32 |
| | Control (tibia fractures- intramedullary nailing without current or previous knee pain) | No Tendinopathy | 8 | 5 M<br>3 F | 16-53 |
| 14. Bagge et al 2009 | Case (Achilles' tendinosis) | Tendinopathy | 15 | 9 M<br>6 F | 23-59 |
| | Control (healthy tendon) | No Tendinopathy | 5 | 2 M<br>3 F | 39-47 |

| Study | Group | Tendon Status | Sample Size | Sex | Age, years (mean and/or range) |
|---|---|---|---|---|---|
| 15. Bjur et al 2009 | Case (Achilles' tendinosis surgery) | Tendinopathy | 37 | 17 M<br>20 F | 26-61 |
| | Control (pain-free Achilles' tendons) | No Tendinopathy | 8 | 3 M<br>5 F | 21-47 |
| 16. Zeisiget al 2009 | Case (tennis elbow surgery & golfer's elbow surgery) | Tendinopathy | 7 & 4 | 4 M<br>3 F &<br>2 M<br>2 F | 32-52<br><br>24-40 |
| | Control (pain free healthy individuals) | No Tendinopathy | 6 | 5 M<br>1 F | 24-40 |
| 17. Singaraju et al 2008 | Case (arthroscopically assisted biceps tenodesis) | Tendinopathy and tenosynovitis | 6 | 3 M<br>3 F | 44-60 |
| | Control (healthy cadavers) | No Tendinopathy and tenosynovitis | 6 | 5 M<br>1 F | 42-81 |
| 18. Andersson et al 2008 | Case (IF; chronic painful mid-portion Achilles tendinosis) | Tendinopathy | 20 | 9 M<br>11F | 26-67 |
| | Control (IF; healthy pain free Achilles' tendons) | No Tendinopathy | 7 | 4 M<br>3 F | 33-46 |
| | Case (ISH; chronic painful mid-portion Achilles tendinosis) | Tendinopathy | 9 | 3 M<br>6 F | 37-56 |

| Study | Group | Tendon Status | Sample Size | Sex | Age, years (mean and/or range) |
|---|---|---|---|---|---|
| | Control (ISH; healthy pain free Achilles' tendons) | No Tendinopathy | 3 | 3 | 47 |
| 19. Bjur et al 2008 | Case (IF; chronic painful mid-portion Achilles tendinosis) | Tendinopathy | 21 | 8 M<br>13 F | 43 (mean age)<br>47 (mean age) |
| | Control (IF; healthy pain free Achilles' tendons) | No Tendinopathy | 8 | 4 M<br>4 F | 37 (mean age)<br>40 (mean age) |
| | Case (ISH; chronic painful mid-portion Achilles tendinosis) | Tendinopathy | 2 | | |
| | Control (ISH; healthy pain free Achilles' tendons) | No Tendinopathy | 1 | | |
| 20. Scott et al 2007 | Case (Patellar + Achilles tendinopathy) | Tendinopathy | 1 + 13 | 19 M | 18-54 |
| | Control (healthy pain free individuals) | No Tendinopathy | 8 + 7 | 10 F | |
| 21. Danielson et al 2007 (1) | Case (chronic painful tendinosis) | Tendinopathy | 2 | 1 M<br>1 F | 22<br>23 |
| | Control (pain-free patellar tendon) | No Tendinopathy | 1 | 1 M | 22 |
| 22. Danielson et al 2007 (2) | Case (unspecified surgical treatment) | Tendinopathy | 7 | 6 M | 22-32 |

| Study | Group | Tendon Status | Sample Size | Sex | Age, years (mean and/or range) |
|---|---|---|---|---|---|
| | | | | 1 F | |
| | Control (normal control tissue- skin incision) | No tendinopathy | 15 | 14 M<br>1 F | 20-47 |
| 23. Danielson et al 2006 | Case (proximal patellar tendinopathy) | Tendinopathy | 7 | 6M<br>1F | 27 (22-32) |
| | Control (pain-free and normal patellar tendons) | No Tendinopathy | 16 | 15M<br>1F | 32.1, 20-47 |
| 24. Lian et al 2006 | Case (jumper's knee) | Tendinopathy | 10 | | 24-34 |
| | Control (tibia fracture undergoing marrow nailing) | No Tendinopathy | 10 | | 19-43 |
| 25. Bjur et al 2005 | Case (chronic painful mid-portion Achilles' tendinosis) | Tendinopathy | 21 | 8 M<br>13 F | 35-54<br>34-56 |
| | Control (normal Achilles' tendons) | No Tendinopathy | 9 | 4 M<br>5 F | 35-60<br>22-46 |
| 26. Forsgren et al 2005 | Case (Achilles' & Patellar tendinosis) | Tendinopathy | 6<br>12 | | |
| | Control (normal tendons) | No Tendinopathy | 13<br>5 | | |
| 27. Alfredson et al 2001 | Case (chronic painful Achilles tendinosis) | Tendinopathy | 9 | 3 M<br>6 F | 45 (mean) |

| Study | Group | Tendon Status | Sample Size | Sex | Age, years (mean and/or range) |
|---|---|---|---|---|---|
| | Control (normal (pain-free) Achilles tendons) | No Tendinosis | 2 | 1 M<br>1 F | 39 |
| 28. Alfredson et al 2000 (1) | Case ((Jumper's knee) | Tendinopathy | 5 | 4 M<br>1 F | 23-31 |
| | Control (healthy tendon) | No Tendinopathy | 5 | 4 M<br>1 F | 27-43 |
| 29. Alfredson et al 2000 (2) | Case (surgical treatment of tennis elbow) | Tennis Elbow | 3 | 3 M<br>1 F | 29-54 |
| | Control (painful elbow) | No tendinopathy | 2 | 2 F | 28-43 |
| 30. Alfredson et al 1999 | Case (Achilles tendinosis) | Tendinopathy | 4 | 4M | 40.7, 34-53 |
| | Control (healthy tendon) | No Tendinopathy | 5 | 5M | 37.2, 27-42 |
| 31. Ljung et al 1999 | Case (tennis elbow) | Tendinopathy | 6 | 3 M<br>3 F | 38-52 |
| | Control (healthy tendon) | No tendinopathy | 6 | 5 M<br>1 F | 24-39 |

**Supplementary Table 3: Study Characteristics – part B**

| No. | Study | Result |
|---|---|---|
| **1.** | Schmalzl J et al., 2019 | NSE immunohistochemical staining observed high density of free nerve endings at the transition zone to the paratenon in inflamed tendons compared to the no tendinitis group. |
| **2.** | Sahmey et al 2016 | a) CGRP, PGP9.5 (a neuroendocrine marker) and SP immunoreactions also occurred in abnormal chondrocyte-like cells.<br>b) SP-labelled fibres, more intimately associated with vessels, were only detected in some tendinopathic tendon<br>c) a greater expression of SP in tendons that exhibited immature vessels.<br>d) Synaptophysin-immunoreactive nerves were closely related to vessels in tendinopathy |
| **3.** | Christensen J et al 2015 | a) Double staining of the PAR receptors and SP showed that nerve fibres and fascicles expressing the PAR-receptors often co-localised with SP, however not all the nerve fibres expressing PARs were positive for SP.<br>b) Protease activated receptors are expressed in the Achilles tendon and surrounding tissues<br>- PAR 1 and 4 predominantly in nerves, whilst PAR-2 by tenocytes<br>- all 4 PAR receptors colocalised with SP positive nerve fibres |
| **4.** | Dean et al 2015 | - Results<br>o No difference in glutamate between groups (p=.86)<br>o No difference in NMAR1 between groups (p=.61)<br>o Significantly higher PGP-9.5 in painful group (3.75 vs 0.87) (p=.0079)<br>o Significantly higher mGluR2 in painful group (0.064 vs 0.0019) (p=.05)<br>o No difference in mGluR1<br>o Significantly higher mGluR7 in pain-free group (0.18 vs 0.005) (p=.0019)<br>o Significantly higher Kainate receptor 1 (KA1) in painful group (4.55 vs 0.85) (p=.0028)<br>o *mGluR = metabotrophic glutamate receptor<br>o Correlations<br>▪ No strong correlation between PGP-9.5 & glutamate receptor expression<br>▪ No strong correlation between PGP-9.5 & TNF-alpha expression<br>o Explored other inflammatory cells (macrophages etc), but doesn't seem as relevant |
| **5.** | Fearon et al 2014 | - significantly greater presence of SP in the bursa but not in the tendon (p-.223) of subjects with GTPS vs controls<br>- SP was expressed in fibroblast like cells embedded within the bursa stroma or within the tendon and in close association with vessels both in bursa and in tendon |
| **6.** | Franklin et al 2014 | a) Glutamate → P < 0.001 tear vs control<br>b) NMDAR1 → P < 0.001 tear vs control<br>c) mGluR2 → P=.008 overexpressed in tears vs control<br>d) mGlur7 → dramatically reduced (P<0.001)<br>e) mGlur8 → significant in small tears vs large/medium tears (1-3 cm tears)<br>f) NK-1 → lower vs control (p=0.007)<br>g) BDKRB2 → reduced in tears (p=0.354)<br>h) PGP9.5 → significant difference between small tear and large tear (p=.021) |

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|---|---|---|
| | | i) Nav1.7 → no significant difference<br>j) TRPA1 → significantly reduced in small tears vs large ones (p=.001)<br>k) No changes in SP and CGRP expression<br>l) Increased expression of alpha-2a adrenergic receptors<br>m) TH → reduced (p=.0235) |
| **7.** | Sasaki et. al., 2013 | a) intensity of the PGP 9.5 and NPY was stronger in the tendinosis tissue compared to control tissue.<br>b) decreased immunoreactivity of CGRP and SP in tendinosis tissue.<br>c) increased sympathetic innervations + loss of sensory innervations of the tendinosis tissue at the ECRB capsule<br>d) perivascular sensory innervation was limited in the tendinosis tissue whilst there were marked immunoreactions for sympathetic nerve markers |
| **8.** | Tosounidis et al 2013 | a) showed S-100 and NPY, adrenergic in 11/14 cases of RC tear and biceps tendinitis<br>b) Alpha 1 adrenergic immunoreactions were positive in a subpopulation of cells that expressed NPY |
| **9.** | Schizas et al 2012 | a) increased tissue immunodensity of NMDAR1, phosphor-NMDAR1 and mGluR5, SP vs control<br>b) NMDAR1 predominant in peritendinous tissue whilst phospho-NMDAR1 in tendon proper<br>c) presence of sprouting nerve fibres in tendon proper (Positive PGP 9.5 staining)<br>d) mGlur5 distinctive of late-stage tendinopathy, predominant on altered tenocytes and free nerve fibres in tendinopathy biopsies<br>e) SP present on both peritendinous and tendon proper tissue<br>f) SP on sprouting nerve fibres in 5 out 10 biopsies exhibiting signs of late stage tendinosis vs absent in control<br>g) the occurrence and immunodensity of NMDAR1 correlated with that of SP in tendinopathic samples vs not so in control<br>h) co-localisation between NMDAR1 and SP and phosphor-NMDAR1 and SP both in the tendinopathic and control biopsies, however only tendinopathic biopsies exhibited co-localisation of SP and phosphor-NMDAR1 within the tendon proper. |
| **10** | Bagge et al 2012 | - ISH results-<br>- tendinosis tenocytes showed specific BDNF mRNA reaction<br>- specific mRNA reactions were noted for tenocytes in non-tendinosis patients<br>- IHC results-<br>- large number of tenocytes showed BDNF immunoreactivity in both tendinosis and non-tendinosis groups<br>- BDNF is produced in the tenocytes of the human Achilles tendon, however BDNF immunolabelling and BDNF mRNA is not confined to all tenocytes in the Achilles tendon |
| **11** | Bjorklund et al 2011 | - IHC-F results<br>a) Difference in CB1 expression between groups was statistically significant (P<.05) with it being higher in the tendinosis group vs control |
| **12** | Xu et al 2011 | - IHC results<br>a) Immunoreactivity for PGP9.5 and GAP43 was rarely seen in the tendon tissue proper, but rather in the paratendinous tissue and endotenon between collagen bundles and near blood vessels |

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| | | b) Large groups of nerve fascicles observed in torn and matched tendon groups vs control<br>- GAP43 and PGP9.5 observed within tendon proper and or intimately associated with blood vessels<br>c) Quantitative analysis showed that number of PGP9.5 and GAP43 immunoreactive nerves were significantly higher in matched subscapularis tendons vs control subscapularis tendons (P<0.05) and torn supraspinatus tendons (P<.0002 and P<.0001). |
| **13** | Schizas et al 2010 | Quantitative Assessment<br>1) **NMDAR1**<br>a) No vessels within the tendon proper,<br>b) vascular **NMDAR1** in the tendon proper (5.9%) of tendinopathic group exhibited similar levels as in the paratendinous tissue<br>c) 9 fold increase in vascular NMDAR 1 in tendinopathic tenocytes vs control<br>**2) Glutamate**<br>a) Glutamate occurrence in vessels and cells was elevated 10 times in tendinopathic group<br>b) Increased number of glutamate positive tenocytes in tendinopathic tendons vs control (p= 0.009)<br>c) Vascular glutamate localised in the paratendinous tissue higher vs control<br>3) **Correlation of NMDAR1 and glutamate occurrence**: no correlation found in either 2 groups<br>Combined IF and DAB staining<br>1) NMDAR1<br>a) Increased NMDAR1 immunoreaction in painful tendons, localisation of increased NMDAR1 immunostaining in tendinopathic samples<br>b) Occurrence of NMDAR1 with PGP9.5- elevated in tendinopathy vs control<br>2) Glutamate<br>a) Glutamate was elevated in tendinopathic tissue vs control<br>b) Occurrence of glutamate with PGP9.5- in tendinopathy vs control<br>c) Increased glutamate positive tenocytes in tendinopathic tissue vs control<br>3) Co-localisation of NMDAR1 and glutamate:<br>a) Demonstrated in nerve fibres in all tendinopathic tissues vs none in control |
| **14** | Bagge et al 2009 | There are marked immunoreactions for the neurotrophins NGF and BDNF and for the p75 receptor, but not for TrkA or TrkB, in the tenocytes of the human Achilles tendon |
| **15** | Bjur et al 2009 | a) NPY- immunoreactions were seen in the nerve fascicles, and mildly in the perivascular nerve fibres, but none in the tenocytes.<br>b) Y1 receptor- immunoreactions present in both non-tendinosis and tendinosis groups, seen in tenocytes and blood vessel walls<br>- stronger immunoreactions present in tendinosis group vs control (p<0.01)<br>c) Y2 Receptor- no immunoreactions in blood vessels wakks, tenocytes or nerve fascicles.<br>- |
| **16** | Zeisiget al 2009 | - showed presence of catecholamine-synthesising enzyme TH in the fibroblasts of the tissue samples from 4/7 patients with TE and 2/4 patients with GE, and no detectable levels of this enzyme were found in fibroblasts of control tissue from the lateral epicondyle (0/6).<br>- no evidence of such production in patients with TE or GE was found in the present study using staining for the ACh-synthesising enzyme ChAT.<br>- no evidence of nerves positive for ChAT, whereas several nerve structures displaying TH-immunohistochemical reactions were detected. |
| **17** | Singaraju et al 2008 | The IHC staining detecting CGRP and substance P was found globally throughout the tendon body in the proximal and distal sections of both groups with no significant differences between the control and experimental tendons. |

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| **18** | Andersson et al 2008 | - The nerve fascicles were seen to contain nerve fibers showing SP-immunoreactions.<br>- The results of the present study show that tenocytes of Achilles tendons display expression of SP and NK-1 R.<br>- Expression at both protein and mRNA levels was shown for the NK-1 R, whilst SP was demonstrated at the mRNA level.<br>- The labelling was detected for a subpopulation of the tenocytes, the semi-quantitative estimations suggesting higher expression levels of both NK-1 R and SP in tendinosis tendons compared with normal tendons. |
| **19** | Bjur et al 2008 | - TH and NPY innervation perivascularly in both control and tendinosis tendons for both paratendinous CT and tendon tissue proper<br>- Distinct occurrence of alpha 1 adrenoreceptors including in tenoyctes in tendinosis specimens<br>- Tendinopathic tenocytes had the occurrence of TH-LI<br>- TH immunoreactions were more common in tenocytes than nerves<br>- TH mRNA- ISH reactions were observed for tenocytes<br>- Limited sympathetic innervation but abundant adrenoceptors |
| **20** | Scott et al 2007 | IHC-F results<br>- VGlut1- no immunoreactions in tendon<br>- VGlut2 immunofluorescence was observed in tendon- in tenocytes<br>- Semiquantitative grading revealed a significantly greater expression of VGLuT2 in tenocytes from tendinosis patients than in those of controls (p=.005).<br>In situ Hybridisation<br>- VGluT2 mRNA expression in tenocytes |
| **21** | Danielson et al 2007 (1) | ISH results<br>- Tenocytes of the tendinosis specimens displayed a stronger and more frequent AP reactions vs control<br>- Occurrence of mRNA for TH in tenocytes is positive |
| 22 | Danielson et al 2007 (2) | - IHC results: General and Sensory Innervation Patterns<br>1) PGP 9.5- specific reactions for PGP 9.5 abundant in areas of loose CT<br>2) SP/ CGRP or SP-CGRP-LI were overall rarely detected in specimens of the tendinosis tendons; this corresponded to the normal tendon tissue proper<br>3) Sympathetic innervation patterns<br>- Normal tendons: lower NPY and TH immunoreactive nerve fibres than in the loose paratendinous CT<br>- Tendinosis Tendons: NPY & TH immunoreactive nerve fibres abundant in loose CT and around blood vessels<br>- not a lot of difference btw normal and tendinosis samples<br>4) Adrenergic Receptors<br>- nerve fascicles in the tendinous tissue displayed an immunoreaction pattern for adrenergic receptors similar to that of the loose paratendinous CT of controls, with greatest immunoreaction being for alpha 1 adrenoreceptor<br>- alpha-1 adrenoreceptors in tendinosis tendon vasculature was more marked vs control by semiquantitative analysis<br>- limited immunoreactions for alpha 2A adrenoreceptors in both healthy and tendinosis tissue |

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| | | - no general difference in the occurrence of beta1-adrenoreceptor in tendinosis vs control<br>- tenocytes exhibited adrenergic receptor LI, alpha 2A adrenoreceptor-LI with more distinct immunoreaction compared to control<br>5) TH-like immunoreactions (-LI) in tenocytes<br>- immunoreactions more distinct in tendinosis tendon samples vs normal<br>6) The amount of sympathetic innervation did not match the quantity of adrenergic receptors in the tendon tissue proper of the patellar tendon, particularly in tendinosis.<br>- These findings suggest that locally produced catecholamines can be mediators that bind to the frequently occurring adrenergic receptors |
| 23 | Danielson et al 2006 | - Results<br>o M2 receptor<br>▪ Immunoreaction in blood vessel walls observed in both groups, more pronounced in tendinosis group particularly in those with hyper cellularity<br>▪ No immunoreaction in tenocytes & nerve fascicles observed in controls<br>▪ Immunoreaction in tenocytes & nerve fascicles observed in specimens, particularly in those with hypercellularity<br>o Choline acetyltransferase (ChAT)<br>▪ No immunoreaction in blood vessels or tenocytes in control<br>▪ Immunoreaction in blood vessels observed in tendinosis, particularly in those with profound hyper cellularity or abnormally appearing tenocytes<br>▪ No immunoreaction in tenocytes in specimens<br>o Vesicular Acetylcholine transporter (VAChT)<br>▪ No immunoreaction in blood vessels or tenocytes in control<br>▪ Some immunoreaction in tenocytes seen in specimens, more so in those with hypercellularity or with abnormal appearance<br>▪ No immunoreaction in blood vessels in specimens<br>o Acetylcholinesterase (AChE)<br>Immunoreaction observed in blood vessels & tenocytes of both groups, with no convincing differences between both groups |
| 24 | Lian et al 2006 | Semiquantitative Analysis tendon vs control<br>- higher occurrence of SP= 0.567<br>- higher occurrence of PGP= 0.098<br>- lower occurrence of TH = 0.018 |
| 25 | Bjur et al 2005 | IHC results<br>a) Innervation patterns- PGP9.5 was seen in tendinosis tissue, the staining was seen intimately associated with fine blood vessels unlike control<br>b) Immunoreactions against CPRP and SP were also detected in thin nerve fascicles and as freely coursing nerve fibres, sometimes being closely located to fine blood vessels<br>c) In normal tendon specimens, the immunoreaction for CGRP was more marked than that for SP<br>d) CGRP/SP immunoreaction was only observed in the association with a subpopulation of the blood vessels |

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| 26 | Forsgren et al 2005 | - A variety of NK-1R antibodies were used<br>- Results (pretty rubbish in my opinion).<br>o NK-1R immunoreaction found in blood vessel walls (greater extent) in both groups<br>o NK-1R immunoreaction found in nerve fibers/ fascicles (lesser extent) in both groups<br>NK-IR immunoreaction occurred to various extents in both tendinosis groups, with greater presence in tendinosis specimens with pronounced vascularization. |
| 27 | Alfredson et al 2001 | Results (Achilles tendon)<br>- Micro dialysis- presence of free glutamate in all tendons<br>a) Tendinosis- 78-250umol/l<br>b) No tendinosis- 16-34umol/l<br>- NMDAR1 receptor detected in all tissues<br>AChE and NMDAR1 reactions often localised to similar structures |
| 28 | Alfredson et al 2000 (1) | Results (Patellar tendon) Microdialysis- HPLC and IHC:<br>- The mean concentration of glutamate was significantly higher than the mean concentration for glutamate in control<br>- No significant differences between the mean conc of PGE2 in tendonosis vs control<br>Glutamate NMDAR1 receptors present in all tendons (localised to AChE structures) |
| 29 | Alfredson et al 2000 (2) | Results: Microdialysis<br>ECRB tendons had higher conc of glutamate vs control ($p<.001$) |
| 30 | Alfredson et al 1999 | - Microdialysis results<br>o Glutamate concentration was significantly higher in tendinosis (196 ± 59 µmol/l) vs controls (48 ± 27 µmol/l) across all timepoints over 4hr period ($p<.05$)<br>o No significant difference in mean concentrations of glutamate over 4hrs between 2 groups<br>- No significant difference in PGE2 or mean PGE2 between 2 groups |
| 31 | Ljung et al 1999 | - A quantitative analysis of the vessels and nerves in patients with tennis elbow compared to those in control was not possible.<br>- The extensor carpi radialis brevis muscle is supplied with SP and CGPR |

Supplementary Table 4: A summary of the most important findings of each study.

| Article No. | SP | CGRP | NMDAR Receptors | Glutamate | Glutamate Receptors mGlut | PGP 9.5 | NK-1R | Tyrosine hydroxylase | Neuropeptide-Y (NPY) | NPY Receptors | AChE | Adrenoreceptors | Others |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. Schmalzl J et al., 2019 | | | | | | | | | | | | | Neuron-Specific Enolase (NSE) |
| 2. Sahmey et al 2016 | ✓ | ✓ | | | | ✓ | | | | | | | |
| 3. Christensen et al., 2015 | ✓ | | | | | | | | | | | | PAR receptors |
| 4. Dean et al 2016 | | | ✓ | ✓ | ✓ | ✓ | | | | | | | KA1 |
| 5. Fearon et al 2014 | ✓ | | | | | | | | | | | | |
| 6. Franklin et al 2014 | ✓ | ✓ | ✓ | | ✓ | ✓ | ✓ | ✓ | | | | ✓ | Nav1.7 TRPA1 BDKRB2 |
| 7. Sasaki et al 2013 | ✓ | ✓ | | | | ✓ | | | ✓ | | | | |
| 8. Tosounidis et al 2013 | | | | | | | | | ✓ | | | ✓ | S-100 |
| 9. Schizas et al 2012 | ✓ | | ✓ | | ✓ | ✓ | | | | | | | |
| 10. Bagge et al 2012 | | | | | | | | | | | | | BDNF |
| 11. Bjorklund et al 2011 | | | | | | | | | | | | | CB1 |
| 12. Xu et al 2011 | | | | | | ✓ | | | | | | | GAP 43 |
| 13. Schizas et al 2010 | | | ✓ | ✓ | | | | | | | | | |
| 14. Bagge et al 2009 | | | | | | | | | | | | | NGF BDNF P75 |
| 15. Bjur et al 2009 | | | | | | | | | ✓ | ✓ | | | |
| 16. Zeisiget et al 2009 | | | | | | | | ✓ | | | | | |
| 17. Singaraj et al 2008 | ✓ | ✓ | | | | | | | | | | | |
| 18. Andersson et al 2008 | ✓ | | | | | | ✓ | | | | | | |
| 19. Bjur et al 2008 | | | | | | | | ✓ | ✓ | | | ✓ | |
| 20. Scott et al 2007 | | | | | ✓ | | | | | | | | |
| 21. Danielson et al 2007 (1) | | | | | | | | ✓ | | | | | |

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| 22. | **Danielson et al 2007 (2)** | ✓ | ✓ | | | | ✓ | | ✓ | ✓ | | | ✓ | |
| 23. | **Danielson et al 2006** | | | | | | | | | | | ✓ | | M2 Ach Receptor ChAT, VAChT |
| 24. | **Lian et al 2006** | ✓ | | | | | ✓ | | ✓ | | | | | |
| 25. | **Bjur et al 2005** | ✓ | ✓ | | | | ✓ | | | | | | | |
| 26. | **Forsgren et al 2005** | | | | | | | ✓ | | | | | | |
| 27. | **Alfredson et al 2001** | | | ✓ | ✓ | | | | | | | ✓ | | |
| 28. | **Alfredson et al 2000 (1)** | | | ✓ | ✓ | | | | | | | ✓ | | |
| 29. | **Alfredson et al 2000 (2)** | | | | ✓ | | | | | | | | | |
| 30. | **Alfredson et al 1999** | | | | ✓ | | | | | | | | | |
| 31. | **Ljung et al 1999** | ✓ | ✓ | | | | | | | | | | | |

Supplementary Table 5: Markers of neurogenic inflammation assessed in each study

| High Quality (Score: >12) | Quality Assessment Score | Moderate Quality (Score: 10-12) | Quality Assessment Score | Low Quality (Score: <10) | Quality Assessment Score |
|---|---|---|---|---|---|
| | 11 | Sahemey R et. al, 2016) | 10 | Schmalzl et. al, 2019 | 9 |
| Dean B. J. et al, 2015 | 15 | Zeisig E et. al, 2009 | 11 | Bagge J et. al, 2009 | 6 |
| Franklin S. L. et al, 2014 | 14 | Lian O et. al, 2006 | 11 | Bjur D, et. al, 2009 | 9 |
| Fearon A. M. et al, 2014 | 15 | Sasaki K. et. al, 2013 | 12 | Andersson G; et. al, 2008 | 4 |
| Tosounidis T. et al, 2013 | 15 | Bagge et. al, 2012 | 10 | Bjur D et. al, 2008 | 6 |
| Xu Y et. al 2011 | 13 | Schizas N. et. al, 2010 | 10 | Scott A et. al, 2007 | 9 |
| | | Singaraju V. M. et. al, 2008 | 10 | Danielson P et. al, 2007 (1) | 7 |
| | | Christensen J et al, 2015 | 11 | Danielson P et. al, 2007 (2) | 7 |
| | | Schizas et al, 2012 | 11 | Danielson, P, et. al, 2006 | 7 |
| | | Bjorklund et. al, 2011 | 11 | Bjur D et. al, 2005 | 7 |
| | | | | Forsgren, S et. al, 2005 | 5 |
| | | | | Alfredson H et. al, 2001 | 5 |
| | | | | Alfredson H et. al, 2000 (1) | 9 |
| | | | | Alfredson, et. al, 2000 (2) | 9 |
| | | | | Ljung B. O et. al, 1999 | 6 |
| | | | | Alfredson, H. et. al, 1999 | 8 |

Supplementary table 6. Results of study quality assessment