Chronic Achilles Tendon Rupture

Surgical reconstruction and post–operative outcomes

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“Even a fool learns something once it hits him”

Homer, The Iliad, *762 B.C.*

ABSTRACT

When the treatment of Achilles tendon ruptures is delayed by more than four weeks, the rupture is classified as chronic. This thesis aims to examine the postoperative outcome of chronic Achilles tendon ruptures treated surgically in terms of long-term functional outcomes, patient-reported outcome measurements, biomolecular changes and economic cost. Additionally, a new surgical technique for treating chronic Achilles tendon ruptures will be described and evaluated.

Study I: Fifty-nine patients with a mean (SD) age of 63 (14) that were surgically treated for a chronic Achilles tendon rupture between 2014 and 2018 with a gastrocnemius aponeurosis free flap were invited to participate in this study evaluating economic costs and patient-reported outcome. The production-loss costs were extracted from the Swedish Social Insurance Agency. The presented costs were compared with the costs of acute ruptures. The mean (SD) total cost for the treatment of a chronic Achilles tendon rupture was 6,494 € (6,508). This was 1,276 € more than the treatment of an acute Achilles tendon rupture. The main reason for this difference was the higher medical costs associated with chronic Achilles tendon rupture treatment.

Study II: Twenty-two patients with a mean (SD) age of 61 (15) treated with a gastrocnemius aponeurosis free flap were evaluated one year postoperatively with a validated test battery of muscle function tests, patient-reported outcomes and clinical measurements. The median (range) ATRS was 67 (18-95) of 100. The patients performed significantly less well on the injured side compared with the healthy side in heel-rise repetitions (20 vs 24) and heel-rise height (8 vs 10 cm). Calf circumference on the injured side was significantly smaller (37 vs 38 cm) and the tendon was elongated compared with the healthy side, as shown by the ATRA (55 vs 50°) and ultrasonography (22.4 vs 20.5 cm).

Study III: Twenty-two patients with a mean (SD) age of 60 (12) treated surgically with endoscopically assisted Achilles tendon reconstruction using a semitendinosus tendon autograft were included and evaluated in terms of muscle function tests, patient-reported outcomes and clinical measurements at 12 months postoperatively. The patients reported a median (range) ATRS of 76 (45-99) of 100. The median ATRA was significantly larger on the injured side (60 vs 49.5°) and the tendon length significantly longer (24.8 vs 22 cm) as measured by ultrasonography. The calf circumference was significantly smaller (37.5 vs 39 cm) on the injured side.

Study IV: A systematic review of previous studies reporting postoperative outcomes of chronic Achilles tendon ruptures treated with a gastrocnemius aponeurosis flap or semitendinosus graft was performed. Of the 818 studies identified, 36 studies justified the inclusion criteria. A grand total of 763 individuals were selected for the descriptive analysis. The mean (SD) ATRS was 83 (14) for patients treated with a gastrocnemius aponeurosis flap and 88 (6.9) for patients treated with a semitendinosus graft. Both treatment options were shown to produce acceptable results with minimal complications and both can be regarded as valid methods for treating chronic Achilles tendon ruptures.

Study V: Thirty-five patients that were surgically treated for acute (<4 weeks), short-term chronic (1-6 months) or long-term chronic Achilles tendon ruptures (>6 months) each contributed two tissue sample biopsies at the rupture site during surgical repair; one sample from the distal tendon end and another from the proximal tendon end. RNA was extracted from the tissue samples using the TRIspin method and analysed with real-time polymerase chain reaction (RT-PCR). The mRNA levels for COL1A1 and COL3A1 were significantly higher in the short-term chronic rupture group compared with the acute group ($p<0.05$). MMP-1 and MMP-13 both had the highest levels in the acute group ($p<0.001$) compared with the long-term chronic group. Inflammatory markers such as IL-1, IL-6 and TNF-alfa all had significantly higher mRNA levels in the acute group. Significant differences between the proximal and distal tendon ends could only be found for the monocyte and macrophage marker CD163 ($p<0.05$). To summarise, the study proposes rapid matrix degradation after Achilles tendon ruptures that continues for months after the injury. This may explain part of the elongation process that occurs in acute and chronic Achilles tendon ruptures treated surgically.

To conclude, chronic Achilles tendon ruptures imply high, yet reasonable costs and acceptable functional outcomes with multiple surgical techniques. The chronic Achilles tendon ruptures also show lower mRNA levels that could imply more degradation, which indicates that the surgical treatment of Achilles tendon ruptures should not be delayed more than necessary. In spite of this, the result of this thesis shows good clinical outcomes and patient satisfaction following chronic Achilles tendon ruptures and none of the various surgical techniques presented was shown to be superior to another.

Keywords: Chronic Achilles tendon rupture. Semitendinosus graft. Tissue sample. Achilles tendon re-rupture. Surgical repair. Gastrocnemius aponeurosis free flap. Health economics. Patient-reported outcome measurements

SAMMANFATTNING PÅ SVENSKA

Hälseneruptur (hälsenebristning) är en vanlig skada. I genomsnitt drabbas 20/100 000 invånare och år. Under de senaste årtiondena har incidensen ökat. Den främsta orsaken tros vara att folk deltar i sport- och fritidsaktiviteter högre upp i åldrarna. När behandlingen av en hälseneruptur är försenad med mer än 4 veckor klassas rupturen som kronisk. Denna doktorsavhandling syftar till att undersöka resultatet av kroniska hälsenerupturer avseende långsiktiga funktionella resultat, patientrapporterade utfallsmått, biomolekylära förändringar och ekonomiska kostnader. Dessutom beskrivs och utvärderas en ny kirurgisk teknik för att behandla kroniska hälsenerupturer.

Studie I: Femtionio patienter som behandlades operativt för en kronisk hälseneruptur mellan 2014 och 2018 med en del av fascian från gastrocnemiusmuskeln erbjöds att delta i denna studie som analyserade ekonomiska kostnader och patientrapporterat utfall. Kostnaderna för produktionsbortfall hämtades från Försäkringskassan. Kostnaderna för behandling av de kroniska rupturerna jämfördes med kostnaderna för akuta rupturer. Den genomsnittliga (SD) totala kostnaden för behandling av en kronisk hälseneruptur var 68 240 SEK (68 388). Detta var 13 408 SEK mer än behandlingen av en akut hälseneruptur. Den främsta orsaken till denna skillnad var de högre medicinska kostnaderna förknippade med den operativa behandlingen av kroniska hälsenerupturer.

Studie II: Tjugotvå patienter som behandlades med en del av fascian från gastrocnemiusmuskeln utvärderades ett år postoperativt med ett validerat testbatteri av muskelfunktionstester, patientrapporterade utfall och kliniska undersökningar. Patienterna hade en median (range) ATRS på 67 (18–95) utav 100. Patienterna presterade betydligt sämre på den skadade än den friska sidan vad avser tåhävningsrepetitioner (20 vs 24) och tåhävningshöjd (8 vs 10 cm).

Vadomkretsen på den skadade sidan var signifikant mindre (37 vs 38 cm) och senan var förlängd jämfört med den friska sidan vilket visades med ATRA (55 vs 50°) och ultraljud (22,4 vs 20,5 cm).

Studie III: Tjugotvå patienter som behandlades med en ny endoskopiskt-assisterad hälsenerekonstruktion med hjälp av transplantation av semitendinosussenan inkluderades och utvärderades 12 månader postoperativt med muskelfunktionstester, patientrapporterade utfall och kliniska undersökningar. Patienterna rapporterade efter operationen en median (range) ATRS på 76 (45–99) utav 100. Medianen ATRA var signifikant större på den skadade sidan (60 vs 49,5°) och senlängden mätt med ultraljud signifikant längre (24,8 vs 22 cm). Vadomkretsen var signifikant mindre (37,5 vs 39 cm).

Studie IV: En systematisk översikt av tidigare studier som rapporterat postoperativa utfall av kroniska hälsenerupturer, som har behandlats med del av fascian från gastrocnemiusmuskeln eller ett transplantat från semitendinosussenan utfördes. Av de 818 identifierade studierna, presenterade 36 studier det postoperativa resultatet av de två metoderna. En del av fascian från gastrocnemiusmuskeln användes i 21 av studierna och ett transplantat från semitendinosussenan i 13 av studierna. Totalt 763 individer identifierades och medelvärdet (SD) ATRS var 83 (14) för patienter behandlade med en del av fascian från gastrocnemius muskeln och 88 (6,9) för patienter behandlade med ett transplantat från semitendinosussenan. Båda metoderna visades ge acceptabla resultat med minimala komplikationer och båda kan anses vara acceptabla metoder för att behandla kroniska hälsenerupturer.

Studie V: Biopsier samlades in från 35 patienter som behandlats kirurgiskt för akut (<4 veckor), kortvarigt kronisk (1–6 månader) eller långvarigt kronisk hälseneruptur (>6 månader). Varje patient bistod med två vävnadsbiopsier från bristningsstället vid kirurgisk rekonstruktion; ett prov från den distala och ett från den proximala senändan. RNA extraherades från vävnadsproverna med hjälp av TRIspin-metoden och analyserades med realtidspolymeraskedjereaktion (RT-PCR). mRNA-nivåerna för COL1A1 och COL3A1 var signifikant högre i den kortvarigt kroniska rupturgruppen jämfört med den akuta gruppen ($p<0,05$). MMP-1 och MMP-13 hade båda de högsta nivåerna i den akuta gruppen ($p<0,001$) jämfört med den långvarigt kroniska gruppen. Inflammatoriska markörer så som IL-1, IL-6 och TNF-alfa hade alla signifikant högre mRNA-nivåer i den akuta gruppen. Signifikanta skillnader mellan den proximala och distala senändan kunde endast hittas för monocyt- och mak-

rofagmarkören CD163 ($p<0,05$). Sammanfattningsvis visar studien att det sker en snabb nedbrytning av senmatrix efter en hälseneruptur som fortsätter i flera månader efter skadan. Denna nedbrytning kan i sin tur förklara delar av den förlängningsprocess som sker i kirurgiskt behandlade hälsenerupturer vilket innebär att behandlingen av hälsenerupturer inte ska försenas mer än nödvändigt.

Sammanfattningsvis har detta doktorandprojekt belyst de problem som kan uppstå i samband med att behandling av hälsenerupturer fördröjts. De problem som uppstår har både ekonomiska, funktionella och sociala konsekvenser för patienterna. Det är dock viktigt att även belysa att det finns effektiva behandlingar med goda resultat avseende funktionella tester och patienters upplevelse av symtom. Mer kunskap behövs dock för att optimera behandlingen framöver.

Nyckelord: Kronisk hälseneruptur. Semitendinosus graft. Vävnadsprov. Reruptur av hälsenan. Kirurgisk rekonstruktion. Gastrocnemius aponeurosis free flap. Hälsoekonomi. Patient-rapporterade utfallsmått.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. The economic cost and patient-reported outcome of chronic Achilles tendon ruptures

Nilsson N, *Nilsson Helander K, Hamrin Senorski E, Holm A, Karlsson J, Svensson M, Westin O. J Exp Orthop. 3;7(1):60. doi: 10.1186/s40634-020-00277-z.*

II. Patients with chronic Achilles tendon rupture have persistent limitations in patient-reported function and calf muscle function one year after surgical treatment – a case series

Nordenholm A, ***Nilsson N,*** *Hamrin Senorski E, Nilsson Helander K, Westin O, Olsson N. J Exp Orthop. 9;9(1):15. doi: 10.1186/s40634-022-00451-5.*

III. Endoscopically assisted repair of chronic Achilles tendon ruptures and re-ruptures using a semitendinosus autograft is a viable alternative to pre-existing techniques

Nilsson N, *Gunnarsson B, Carmont MR, Brorsson A, Karlsson J, Nilsson Helander K. Knee Surg Sports Traumatol Arthrosc. 30(7):2477-2484. doi: 10.1007/s00167-022-06943-2.*

IV. Both gastrocnemius aponeurosis flaps and semitendinosus tendon grafts are effective in the treatment of chronic Achilles tendon ruptures – a systematic review

Nilsson N, *Stensöta I, Nilsson Helander K, Brorsson A, Carmont MR, Concaro S. (Submitted).*

V. Delayed Treatment of Achilles Tendon Ruptures Results in a Significant Alteration in the Gene Expression of COL1A1, MMPs, TIMPs and IL-6

Nilsson N*, Alim Md A, Dietrich-Zagonel F, Concaro S, Brorsson A, Nilsson Helander K, Eliasson P. (Submitted).*

ADDITIONAL PAPERS BY THE AUTHOR ON THE SAME TOPIC

I. **Disappointment and frustration, but long-term satisfaction: patient experiences undergoing treatment for a chronic Achilles tendon rupture-a qualitative study.**

*Nordenholm A, **Nilsson N**, Krupic F, Hamrin Senorski E, Nilsson Helander K, Westin O, Karlsson J. J Orthop Surg Res. 9;17(1):217. doi: 10.1186/s13018-022-03103-7.*

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ABBREVIATIONS

AOFAS:	American Orthopaedics Foot and Ankle Score
ATR:	Achilles Tendon Rupture
ATRS:	Achilles tendon Total Rupture Score
ATRA:	Achilles Tendon Resting Angle
CATR:	Chronic Achilles Tendon Rupture
CI:	Confidence Interval
CMJ:	Counter Movement Jump
DNA:	Deoxyribonucleic Acid
EQ-5D:	EuroQol-5D
FAOS:	Foot and Ankle Outcome Score
ICC:	Intraclass Correlation Coefficient
J:	Joule
LSI:	Limb Symmetry Index
MRI:	Magnetic Resonance Imaging
N:	Newton
Pa:	Pascal
PAS:	Physical Activity Scale
PCR:	Polymerase Chain Reaction
PROM:	Patient-Reported Outcome Measurement
QALY:	Quality Adjusted Life Year
RCT:	Randomised Controlled Trial
RNA:	Ribonucleic Acid
SD:	Standard Deviation
VAS:	Visual Analogue Scale

DEFINITIONS IN SHORT

Achilles tendon re-rupture	An Achilles tendon rupture treated surgically or non-surgically that ruptures again
Chronic Achilles tendon rupture	An Achilles tendon rupture that has been left untreated for more than four weeks and results in an altered gait pattern and functional deficit for the affected patient because of tendon elongation or a remaining tendon gap
Healthcare cost	All costs related to surgery, hospital admissions, orthoses, pharmaceuticals and emergency visits
Kruskal-Wallis ANOVA	Non-parametric test to determine whether or not multiple samples originate from the same sample. It is an extension of the Mann-Whitney U test that only measures two independent samples
Limb Symmetry Index (LSI)	The ratio between the outcome on the injured side versus the uninjured side, expressed as a percentage as injured/non-injured x 100
Non-parametric bootstrapping	A data sampling methodology where data are randomly resampled to form a “new” sample of data to enable the statistical inference of a population with unknown parameters and distribution
P-value	The probability of obtaining test results as extreme as the observed result assuming the null hypothesis is true
Paired t-test	A statistical test to evaluate whether or not the mean difference between pairs of measurement is equal to zero
Production-loss cost	All costs related to sick-leave and absence from work
Reliability	The overall consistency of a measurement
Sensitivity	The probability of a test being positive if the true test result is positive

Specificity	The probability of a test being negative if the true test result is negative
Statistical power (P)	The probability that a scientific test rejects the null hypothesis when a specific alternative hypothesis is true. The power is dependent on sample size and variation
Swedish Social Insurance Agency	The national insurance agency that secures financial security in the event of injury or sickness among Swedish citizens
Tendon elongation	An acute Achilles tendon rupture that has been treated surgically or non-surgically with remaining elongation compared with the non-injured tendon on the opposite leg
Type I error	Mistaken rejection of a true null hypothesis
Type II error	Failure to reject a null hypothesis that is false
Validity	The extent to which a measurement corresponds to the true value in the real world or total population
Wilcoxon's signed rank test	Non-parametric test used to compare paired, or dependent, measurements to evaluate whether the population mean is different

01

INTRODUCTION AND BACKGROUND

The mechanism of an acute Achilles tendon rupture is complex and multifactorial. [39] The injuries most commonly occur during forced dorsiflexion of the foot during sports or recreational activities. [85] However, tendon ruptures may also occur during falls or sprains. The patient often describes a loud "snap" or "pop", acute pain and a sudden loss of ankle function. [90] In most cases, a clinical examination with tendon palpation, the "calf squeeze" test and the "resting position" test is considered sufficient to establish or preclude the diagnosis. [95] In cases where there is doubt in relation to the clinical diagnosis, it is possible to use ultrasonography or MRI to examine the continuity of the tendon. Even though most ruptures are detected through clinical evaluation, several are missed, reportedly as many as 10-25%. [27, 134] These Achilles tendon ruptures then run the risk of becoming chronic. The chronic Achilles tendon rupture is defined as a rupture left untreated for more than four weeks. [57, 105]

Chronic Achilles tendon ruptures are often characterised by a minor trauma followed by long-term ankle pain and recurrent swelling. [105] The gait pattern is often affected, as well as the patient's ability to perform heel rises. [134] In many cases, the patient has already been in contact with healthcare authorities in conjunction with the trauma. [55] The further management of the injury may then require more resources than an acute Achilles tendon rupture detected early. Acute Achilles tendon ruptures can be treated either surgically or non-surgically and with different rehabilitation protocols. The preferred method is still debatable and lately several randomised controlled trials (RCTs) and meta-analyses have been published. [35, 36, 40, 87, 93, 96, 113, 132, 144, 150, 152, 157, 160, 168, 189, 211, 219] The methods vary significantly in terms of surgical technique, immobilisation time, rehabilitation regimen and study quality. As a result, no universal consensus has been reached and the debate is still ongoing.

Chronic Achilles tendon ruptures are generally thought to require surgical repair with reinforcement to regain full ankle function. [1, 16, 109] Many surgical techniques have been presented, such as V-Y plasty, peroneus brevis tendon grafts, gastrocnemius aponeurosis turndown or free flaps, flexor hallucis longus grafts, synthetic grafts and semitendinosus tendon grafts. [1, 27, 57, 103, 105, 109, 119, 158, 218, 226] The cohort sizes in these studies are often small and the follow-up short term. No consensus has been reached and the management differs significantly between institutions in terms of surgical procedures and rehabilitation protocols. The surgical treatment is often individualised for each patient and depends in many cases on the results of additional ultrasonography or MRI. [120, 148]

Patients with chronic Achilles tendon ruptures lose a great deal of their ankle function in conjunction with the injury and the rehabilitation process is often long and challenging. More knowledge is needed fully to understand this group of patients and how they are affected by their injury. A missed Achilles tendon rupture has a considerable effect on both the patient and the healthcare system. Established surgical techniques and rehabilitation protocols are needed to manage these ruptures in the best possible way.

1.1. HISTORY AND MYTHOLOGY

The name Achilles originates from Greek mythology. As a child, Achilles was dipped in the River Styx by his mother Thetis. The water that ran between the world of death and the world of life was supposed to give Achilles great power and invincibility. His mother held him by the calcaneal tendon and therefore left a weak spot. This became the demigod's only flaw and resulted in his downfall in the Trojan War where an arrow shot by Paris, son of King Priam, hit him on the heel. [7] The arrow was poisonous and Achilles died shortly after. Even though Achilles became the hero of Homer's poem The Iliad, it was not until the 17th century that the tendon was given its infamous name. It was previously known as the tendon of Hippocrates, due to his early descriptions of the tendon in ancient Greece.

FIGURE 1: Sketch of Fillippo Albacini's "The Wounded Achilles".

1.2. ANATOMY OF THE ACHILLES TENDON

The Achilles tendon is the strongest tendon in the human body. [42] The tendon is formed by the conjunction of the gastrocnemius and soleus muscle in the posterior superficial compartment of the lower leg (Figure 2). The gastrocnemius and soleus muscles together form the triceps surae muscle and contribute to the plantar flexion of the foot. The gastrocnemius muscle has two heads originating from the lateral and medial posterior femoral condyle. [156] The muscle heads pass posterior to the knee joint and merge with the soleus muscle to form the Achilles tendon. The soleus muscle originates from the posterior head of the fibula, the middle third of the tibial medial border and the interosseous membrane. The gastrocnemius muscle mainly consists of type II muscle fibres that are involved in the explosive act of jumping. Meanwhile, the soleus muscle is mainly made up of type I muscle fibres that contribute to posture in walking and running. In addition to the gastrocnemius muscle and soleus muscle of the triceps surae, the plantaris muscle is also involved in the formation of the Achilles tendon. The muscle is absent in 6-8% of the population and differs in both structure and location between individuals. [208]

It is a relatively small muscle that originates from the popliteal fossa on the lateral condyle of the femur and, together with the gastrocnemius muscle, it contributes to plantar flexion of the foot and flexion of the knee. In some cases, the tendon can be used as a reinforcement during the direct repair of both chronic and acute Achilles tendon ruptures.

The mean (range) length of the tendon is 15 cm (11-26). [38, 45] From origin to insertion, the tendon passes both the ankle and the subtalar joints. The widest part averages (range) 6.8 cm (4.5-8.6), while the thinnest part, approximately 4 cm from the insertion of the tendon, averages (range) 1.8 cm (1.2-2.6). [45, 99] From origin to insertion, the tendon spirals [38] (Figure 2). The most distal part of the tendon is protected from friction along the calcaneal bone by the retrocalcaneal bursa. [15, 60] On the anterior side, Kager's fat pad is located. [60]

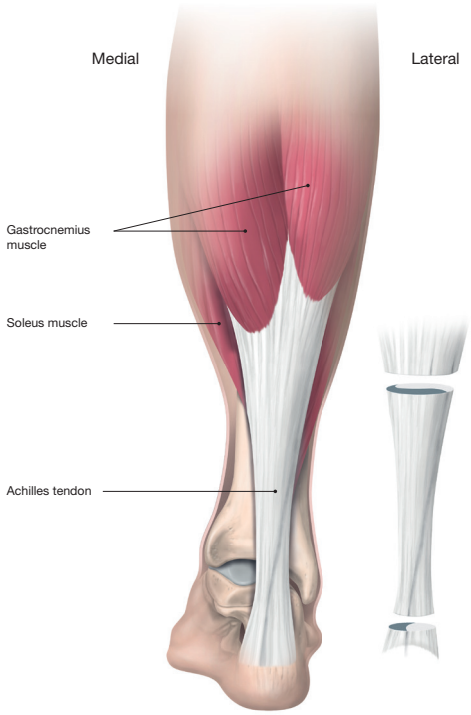

FIGURE 2: The anatomy of the Achilles tendon and the muscles of the posterior superficial compartment of the lower leg including the gastrocnemius and soleus muscles and the spiralling of the Achilles tendon.

1.2.1. Tendon structure

The Achilles tendon originates from the muscle fibres of the gastrocnemius and soleus muscle. The tendon fibres are arranged in primary, secondary and tertiary bundles. The fibres contain collagen types I (65-80%) and III (0-15%) and elastin (1-2%). [89, 165] The collagen is produced by fibroblasts and fibrocytes within the proteoglycan matrix. [88] The tendon cells produce procollagen. [238] This is then converted to tropocollagen extracellularly and cross-linked in formations of five in collagen fibrils. [97] The collagen fibre bundles are surrounded by a protective proteoglycan water matrix and numerous layers of paratenon, epitenon and endotenon (Figure 3). It is still relatively unknown how these structures are affected by an Achilles tendon rupture, pharmaceuticals and/or long-term overuse injury.

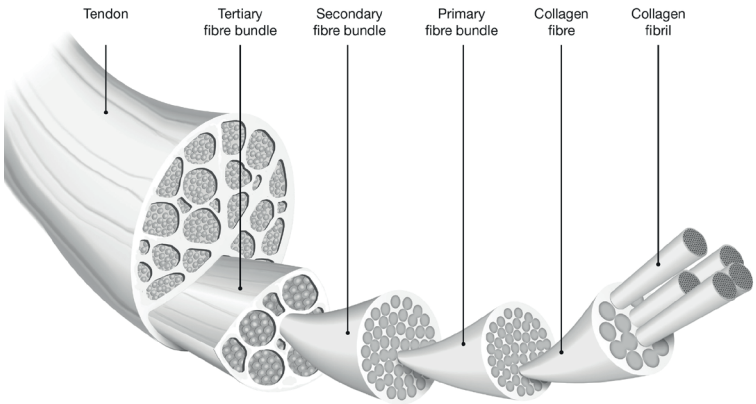

FIGURE 3: The arrangement of tendon fibres from microscopic collagen fibrils to the entire tendon with different levels of bundling.

1.2.2. Circulation

The blood supply to the Achilles tendon originates from two main arteries, the posterior tibial artery and the peroneal artery. [34, 216] The blood is then supplied to the tendon through vessels in the paratenon, in the musculotendinous junction and in the osteotendinous insertion. [5] Due to the proximity to muscle and bone in the proximal and distal third of the tendon, the blood supply is relatively effective when compared with the middle third. The middle third of the tendon receives all the blood supply from the peroneal artery through the paratenon. The proximal and distal third also receive blood supply from the

posterior tibial artery. It is hypothesised that the lower vascularisation of the middle third of the tendon is the reason for the increased rupture frequency of this area. [5, 34] In more recent studies, the microcirculation of the tendon has been evaluated and analysed with regard to rehabilitation and functional outcomes. [180] It was found that low microcirculation strongly correlates to slow healing and a longer rehabilitation period. The distribution of blood within the tendon differs a great deal between individuals, shown, for example, in the review article by Theobald et al. [216].

1.2.3. Biomechanics

Tendon stress (Pa) is commonly calculated as the quotient between the applied force (N) and the cross-sectional area of the tendon (m2). Tendon strain is measured by the percentage of tendon elongation. While walking and running, the Achilles tendon works with strains between 0-3%. When the strain surpasses 3-4%, the tendon starts to suffer microscopic damage. Strain in the region of 4-6% leads to physiological changes and contributes to a stronger tendon. When the strain exceeds 6-10%, macroscopic damage occurs and the tendon risks deforming and rupturing. [28, 136] This can be illustrated by the tendon stress-strain curve (Figure 4). [141]

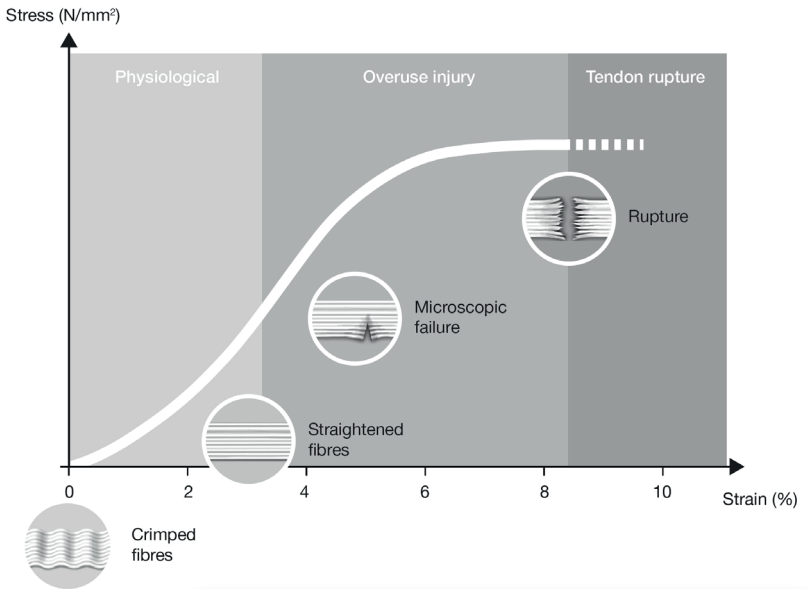

FIGURE 4: The tendon stress-strain curve describing the correlation between tendon stress (N/mm2), tendon strain (%) and tendon rupture.

When the Achilles tendon stretches, it induces the tendon stretch shortening cycle (SSC). The SSC is essential for the capability of the Achilles tendon to store and release elastic energy during movement and stress. [100] The strength and solidity of the tendon can endure forces 3.9 times the body weight while walking and 7.7 times while running. [61]

1.2.4. Tendon healing and metabolism

Tendons are considered relatively metabolically inactive, at least when compared with skeletal muscles and other parts of the musculoskeletal system. [164] The slow metabolism implies that tendons heal slowly and that tendon injuries take a long time to rehabilitate. The tendon is most metabolically active during adolescence, growth and healing and metabolic activity decreases over time. [165] Through micro-dialysis, several studies have explored the levels of glucose, lactate, glutamate, pyruvate, glycerol and other metabolites in healing tendons. In a study by Svedman et al., [214] it was shown that the longer duration of operative time (DOT) correlated with increased levels of glutamate and glycerol in tendons two weeks postoperatively, as well as less loss of physical activity and better functional outcome. All the metabolites mentioned above are important in the carbohydrate metabolism, which is involved in tissue repair and tendon healing.

The tendon healing process can be divided into three distinctive phases. The first phase is inflammatory and is characterised by the large secretion of pro-inflammatory mediators and the formation of a stabilising fibrin clot. [114] The second phase is proliferative and is composed of reparative fibroblasts and macrophages producing a large amount of stabilising collagen type III. [223] The third, and final, stage of healing is the remodelling phase, where collagen type I is produced by the tenocytes. [106] This phase starts after four to eight weeks and can last for more than a year (Figure 5). [223]

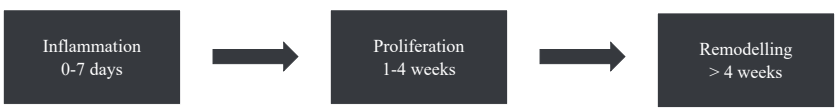

FIGURE 5: The three phases of tendon healing and corresponding time periods after injury.

Mechanical loading is thought to contribute to tendon healing during the rehabilitation of an injured Achilles tendon. [10] The absence of tendon loading during the healing period has been shown to strongly effect the healing process and the rehabilitation outcome in a negative manner. [98] In several animal studies, it has been shown that early mobilisation results in the upregulation of growth factors and increased innervation. [22, 53] In human research, similar results have been presented related to the early mobilisation of tendons, leading to positive outcomes in terms of healing. [12, 225]

1.3. ACUTE ACHILLES TENDON RUPTURE

1.3.1. Incidence and distribution

The incidence of Achilles tendon ruptures has been studied widely over the years. [59, 76, 108, 111, 135] The incidence is reportedly 48.3-55.2 per 100,000 person-years for men and 14.7-15.16 per 100,000 person-years for women, when measured in Denmark and Sweden in 2013 and 2012 respectively. [59, 76] The ratio between males and females is generally considered to be 8:2. However, the reported ratios vary significantly between studies. [163, 224] Older patients often describe trauma sustained during low-energy activity and this is more frequent among patients with chronic Achilles tendon ruptures. The incidence reportedly increases at all ages, [74, 108, 135, 147, 210] with the highest increment in patients over 60 years of age. [76] In older patients, the difference in incidence between men and women is not as significant as in younger patients. [55]

1.3.2. Trauma mechanism

An acute Achilles tendon rupture is a traumatic injury that occurs during sports or recreational activities in most cases. [85] The trauma mechanism is multifactorial. [39] There is both a degenerative component and a traumatic component with increased tendon strain. The main traumas associated with Achilles tendon ruptures are concentric contraction of the gastrocnemius and soleus with the knee extended, eccentric contraction of the gastrocnemius and soleus with the ankle dorsiflexing and forced dorsiflexion of the foot. It is argued that degenerative changes to the tendon precede the rupture. [37, 84, 125] However, this is still the subject of debate and more data are needed to fully describe the predictors of Achilles tendon ruptures. A correlation between chronic and inflammatory diseases and Achilles tendon ruptures has been reported. [72] Several pharmaceuticals, such as corticosteroids, statins and quinolone antibiotics, have also been described as potential risk factors for rupture. [71, 196, 209, 221]

1.3.3. Clinical evaluation of Achilles tendon ruptures

A clinical examination and trauma history are considered to be sufficient to establish the diagnosis of an Achilles tendon rupture. [118] The patient often describes the trauma as a sudden "snap" or "pop" from the lower leg, followed by immediate ankle pain. Many patients describe the event as if someone hit or kicked them on the heel. The instant ankle pain is then followed by a sensation of weakness and instability of the affected leg/ankle. [90] The most common activities associated with Achilles tendon ruptures are recreational sports that require abrupt jumping or quick turns of running. [85] Examples of these activities are tennis, badminton, football, running, squash, basketball and padel. In addition to a trauma report, it is also important to assess the previous medical history of the patient, including periods of ankle pain, swelling and dysfunction relating to the tendon, previous ruptures, comorbidities and previous physical activity.

Weakness and a characteristic trauma are not always present in patients. Due to surrounding swelling and other structural tissues, the tendon gap might also be absent. This is very important to consider when an ankle is examined, or ruptures will be missed. The most common site for ruptures is the middle third of the Achilles tendon. [207] Reportedly, 10-25% of all Achilles tendon ruptures are initially missed or misdiagnosed due to either "patient delay" or "doctor delay". [27, 134]

The examination consists of palpation of the tendon, the "calf squeeze" test (Thompson's test) and the "resting position" test (Matles test). Thompson's test is performed with the patient in a prone position with both feet over the edge of the examination couch. The examiner then squeezes the calves of the patient at the level of the soleus muscle. [194] If the tendon is fully intact, the squeeze will induce the plantar flexion of the foot. If the plantar flexion is absent, it indicates that the tendon is injured (Figure 6).

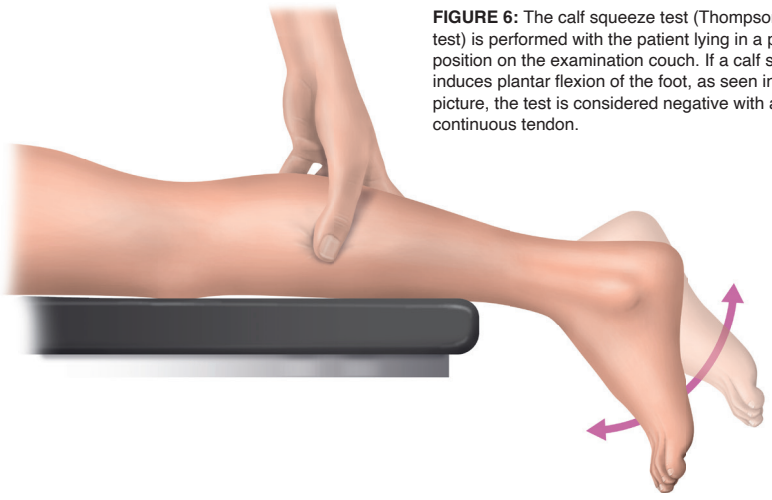

FIGURE 6: The calf squeeze test (Thompson's test) is performed with the patient lying in a prone position on the examination couch. If a calf squeeze induces plantar flexion of the foot, as seen in the picture, the test is considered negative with a likely continuous tendon.

The Matles test is another dynamic test of the Achilles tendon performed in a prone position. The patient starts with both knees extended and with the feet over the edge of the examination couch. The patient is then asked to flex both knees at 90 degrees while the examiner observes the position of each foot. [142] If one of the feet is more dorsiflexed than the other, this indicates that the tendon is discontinuous or ruptured (Figure 7).

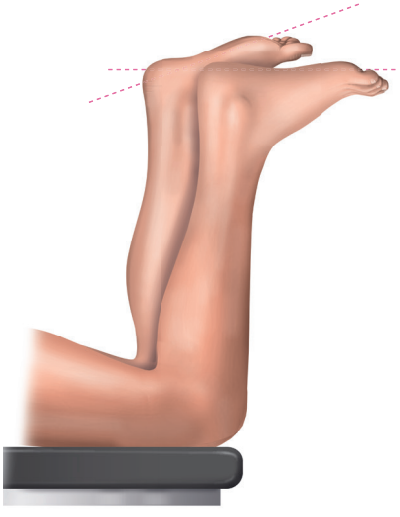

FIGURE 7: The Matles test is also performed in a prone position on the examination couch. The test is considered positive when larger dorsiflexion is seen on the injured side.

For a more quantitative measurement of the tendon lengthening that occurs after an Achilles tendon rupture, the Achilles Tendon Resting Angle (ATRA) could be used. [31] The measurement is made with the patient in a prone position with the knee flexed at 90 degrees. The angle is measured with a goniometer that is placed between the central fibula and the centre of the fifth metatarsal head (Figure 8). The angle is presented in degrees.

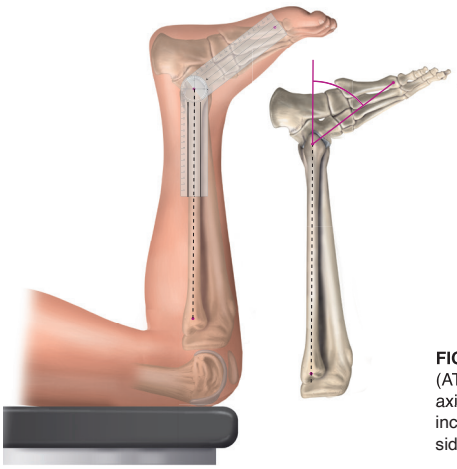

FIGURE 8: The Achilles Tendon Resting Angle (ATRA) is measured as the angle between the long axis of the fibula and the fifth metatarsal head. An increased angle compared with the contralateral side indicates a longer or discontinuous tendon.

The sensitivity of the calf squeeze test is reported to be 0.96 and the specificity 0.93, while the resting positioning test (Matles test) has an estimated sensitivity of 0.88. [118] Even though both tests present high sensitivity and specificity, imaging of the tendon is sometimes deemed necessary to establish or exclude the diagnosis. Plain radiography is sufficient to diagnose fractures or bone abnormalities. If the Achilles tendon rupture diagnosis is uncertain, ultrasonography or MRI may be used to specify the diagnosis. These instruments are also useful in the pre-operative mapping of the tendon. However, they should not be used to exclude the diagnosis. In a study by Westin et al., [228] it was shown that patients with a diastasis of >10 mm ran a significantly higher risk of re-rupture when treated non-surgically. It was also shown that patients treated non-surgically presented a significantly lower ATRS score and performed significantly less well in heel-rise tests when the diastasis exceeded 5 cm. To conclude, the authors emphasised that ultrasonography may be a useful tool in the clinical treatment algorithm and decision-making for acute Achilles tendon ruptures.

1.3.4. Treatment and outcome

The preferred treatment strategy for Achilles tendon ruptures is still the subject of debate. The management can be divided into surgical or non-surgical treatment, with different rehabilitation protocols. Many randomised controlled trials (RCTs) and meta-analyses have been conducted to determine the best treatment. [20, 35, 36, 40, 47, 73, 87, 93, 96, 113, 132, 144, 150, 152, 157, 168, 189, 211, 219] It has been shown that patients treated non-surgically have comparable results to those treated surgically, but the re-rupture rate is significantly higher in the group treated non-surgically. [157, 168]

In a recent study by Myhrvold et al., [155] a total of 554 patients were randomised to non-surgical treatment, open repair, or minimally invasive surgery. That study revealed no superior improvement in the ATRS in patients treated surgically compared with patients treated non-surgically.

1.4. CHRONIC ACHILLES TENDON RUPTURE

1.4.1. Incidence and distribution

Despite the fact that medical history and clinical examinations are sufficient to establish the diagnosis of an Achilles tendon rupture, several ruptures are being missed. [118, 134] In multiple studies and reviews, the incidence of initially missed Achilles tendon ruptures is regarded as 10-25%. [27, 134] There are, however, insufficient original studies that feature patient data on the incidence of an initially missed diagnosis. Many reviews of chronic Achilles tendon ruptures refer to a retrospective comparative study from 1976 by Inglis et al. [78] where it was shown that 17 of 79 patients (22%) had an initial misdiagnosis. The main aim of that study was, however, to compare surgical and non-surgical treatment in acute Achilles tendon ruptures and the large number of missed Achilles tendon ruptures was an accidental finding.

It is also important to consider that not all chronic Achilles tendon ruptures are missed Achilles tendon ruptures. Some patients appear to develop a chronic Achilles tendon rupture without a distinct trauma. It is also possible that patients who develop a chronic Achilles tendon rupture have a more degraded tendon than those that suffer an acute Achilles tendon rupture.

Healthcare needs to increase the awareness of these ruptures in order to detect them earlier. In a study by Fridén et al., [55] it was shown that most

Achilles tendon ruptures with delayed treatment in Sweden were mainly missed in general practice due to an inadequate physical examination. A total of 46 patients, 24 men and 22 women, were identified in this retrospective register study with data from the Health and Social Care Inspectorate in Sweden. Most patients' (n=37) injury was missed in general practice. It was also shown that most patients were older (>50% over 60 years old) and that the risk of a missed Achilles tendon rupture may be higher among women (24 males and 22 females) compared with acute Achilles tendon ruptures (8:2).

In studies, the median age of patients affected by chronic Achilles tendon ruptures is generally higher than that presented for patients with acute Achilles tendon ruptures. [215] The higher age potentially results in poorer functional outcomes, as shown for acute ruptures in a study by Westin et al. [230]

1.4.2. Trauma mechanism

Patients with chronic Achilles tendon ruptures display different symptoms compared with patients with acute ruptures. Long-term pain and recurrent swelling are more frequent in patients with chronic ruptures. [105] The gait pattern is often affected, together with weakness at push-off and poor balance, as well as the capability to perform heel rises. [134] A clinical examination and trauma reports are therefore not always sufficient to establish the diagnosis. [134] MRI or ultrasonography is therefore often used as a complement to the clinical evaluation. [105] The imaging methods are also important implements in the preparation for surgery. The drawback with ultrasonography is that it is user dependent. In a recent systematic review by Dams et al., [43] a total of 37 studies of ultrasonography as a diagnostic tool for acute Achilles tendon ruptures were analysed. The conclusion was that it plays an adjunct role in Achilles tendon rupture diagnosis and in the choice between surgical or non-surgical treatment. It is, however, important to remember the adjunct role of imaging, as the risk of false negatives is otherwise high. The main difficulties with MRI are the high economic costs and limited availability.

The delayed treatment implies larger tendon-end diastasis with additional scar tissue within the tendon. [37] In acute Achilles tendon ruptures, the option is either surgical or non-surgical treatment. However, the ruptures that are delayed by more than four weeks, and can therefore be defined as chronic, almost always require a surgical intervention to recover lower leg function.

[1, 16, 57, 105, 109] In many cases, the surgical intervention is more extensive and entails a higher risk of complications, such as infections and inadequate wound healing, than the treatment of acute ruptures. [133]

1.4.3. Treatment and outcome

As different from an acute Achilles tendon rupture, chronic ruptures most likely require a surgical intervention to regain ankle function and push-off strength. [120] Normal end-to-end sutures are generally not considered an acceptable treatment and reinforcements are commonly recommended for patients with chronic Achilles tendon ruptures. [1, 16, 109] Many different surgical techniques are available in the literature; they include V-Y plasty, gastrocnemius aponeurosis turndown or free flaps, different tendon transfers, such as peroneus brevis, flexor hallucis longus and plantaris, as well as semitendinosus grafts, synthetic grafts and allografts. [1, 27, 57, 103, 105, 109, 119, 158, 218, 226] Depending on the size of the rupture, other individual factors, and the experience of the surgeon, one of the surgical procedures is therefore chosen. In most cases, the intervention for a chronic Achilles tendon rupture is more demanding and associated with more risks than the intervention for an acute Achilles tendon rupture. [133] The reason behind the more problematic interventions after a chronic Achilles tendon ruptures is the retracted tendon ends and degraded changes in the tendon due to more spontaneous ruptures. [37]

To date, there is insufficient information on the outcome of chronic Achilles tendon ruptures. A study by Nilsson-Helander et al. [158] evaluated the functional and patient-reported outcome of chronic Achilles tendon ruptures as well as re-ruptures. That study showed that patients with chronic Achilles tendon ruptures, or re-ruptures, recovered well but did not recover full function. In a systematic review by Hadi et al., [66] several different surgical techniques used for chronic Achilles tendon ruptures and their outcomes were presented. The review was published in 2013 and included 34 publications with a total of 484 patients. The review outlined many different surgical techniques. It was, however, shown that the literature lacks a common and validated outcome measurement and that larger cohorts with matched control groups are missing. Consequently, more knowledge is needed in order to fully understand the nature of these ruptures and how to manage them in the future.

1.5. RE-RUPTURE AND EXCESSIVE TENDON ELONGATION

1.5.1. Incidence and distribution

A re-rupture is a feared and serious complication of an acute Achilles tendon rupture. These re-ruptures emerge after the initial treatment of a primary rupture and have traditionally been regarded as an indication of treatment failure. Re-ruptures are expected to require surgical repair, longer rehabilitation processes and possibly an extended time off work. [158] Re-ruptures and chronic Achilles tendon ruptures can be regarded as injuries resembling one another; the reasons are different, but the outcomes can be regarded as similar.

Another complication that can affect patients negatively after an Achilles tendon rupture is excessive tendon elongation. The length of the tendon can be measured using multiple direct and indirect techniques. In the literature, different measurements of dorsiflexion range and ankle angle have been presented. [18, 31] Ultrasonographic methods and MRI have also been discussed, but without any consensus. [101, 201] Excessive tendon elongation has been shown to effect gait pattern and the ability to perform heel rises. [202] These are symptoms similar to those presented for chronic Achilles tendon ruptures. The main difference is that excessive tendon elongation is regarded as a complication of acute Achilles tendon ruptures, while chronic ruptures are regarded as a chronic injury with a different traumatology.

1.5.2. Trauma mechanism

Re-ruptures can occur after both the non-surgical and surgical treatment of Achilles tendon ruptures. The risk of re-rupture is, however, considerably higher when the primary Achilles tendon rupture is treated non-surgically. The incidence is reportedly 9.8% for non-surgical treatment and 3.7% for surgical treatment. [47] In more recent randomised controlled studies (RCTs) with earlier mobilisation, lower re-rupture rates have been reported for both groups, as shown by 6.2% and 0.6% respectively in the study by Myhrvold et al. [155] and 10% and 0% respectively in the study by Olsson et al. [168]

Tendon elongation is mainly associated with early mobilisation after surgical repair or non-surgical immobilisation. However, no major differences between surgical and non-surgical treatments have been shown. [192, 193] The most tendon elongation occurs during the first six weeks of immobilisation and rehabilitation. [86, 153]

1.5.3. Treatment and outcomes

Multiple studies have reported the long-time outcome after the surgical repair of Achilles tendon re-ruptures. [143, 172, 195] Most studies have included a small number of patients and the outcomes have been non-validated tests. Like chronic Achilles tendon ruptures, more knowledge and data are required to fully understand these ruptures and their implications for the affected patients. In a study conducted by Westin et al., [229] it was shown that patients with re-ruptures had a poorer patient-reported outcome but a similar, or superior, functional outcome in comparison with primary ruptures. Analogous to chronic Achilles tendon ruptures, many different surgical techniques are presented in the literature. [128, 158] The information on the long-term functional outcomes is, however, still insufficient.

The treatment of tendon elongation aims to shorten and strengthen the Achilles tendon in similar ways to chronic Achilles tendon ruptures. In a systematic review by Diniz et al., [49] it was shown that tendon elongation is a relevant problem in relation to the treatment of acute Achilles tendon ruptures, but that more information is needed to fully evaluate the implications and their relationship to functional and patient-reported outcomes.

1.6. ANATOMY OF POTENTIAL TENDON GRAFTS

Multiple potential grafts and tendon transfers to treat chronic Achilles tendon ruptures and re-ruptures are presented in the literature. The anatomy of the most commonly used grafts is presented below. Other potential grafts include Achilles tendon allografts, flexor digitorum longus grafts and gracilis tendon grafts.

1.6.1. Semitendinosus tendon

Together with biceps femoris and semimembranosus, the semitendinosus muscle is one of the three muscles that constitute the hamstring muscle group in the posterior compartment of the thigh. This muscle is the most medially located of the three. It has received its name due to the long insertional tendon that stretches down towards its insertion at the upper part of the medial surface of the tibia. The muscle originates from the lower medial facet of the lateral section of the ischial tuberosity. The semitendinosus acts to (1) extend the thigh and hip, (2) flex the leg at the knee and (3) internally rotate the knee when the leg is flexed. For all functions, there are multiple agonists including gluteus maximus, semimembranosus, biceps femoris (long and short head),

adductor magnus and popliteus. Because of the long tendon of insertion and multiple agonists, the tendon is especially useful as a graft in both knee and ankle surgery. A semitendinosus tendon graft is routinely used in knee surgery for ligament reconstructions and it has also been shown to have good outcomes when used to treat chronic Achilles tendon ruptures with few long-lasting impairments. [122, 177, 204]

1.6.2. Flexor hallucis longus tendon

The flexor hallucis longus is a muscle located on the posterior side of the fibula within the deep posterior compartment of the lower leg. The muscle originates from the lower two-thirds of the posterior fibula and inserts at the distal phalanx of the great toe. The main function of the muscle is to flex all the joints of the first digit when raising the foot. The muscle is also involved in stabilising the foot during heel rises and when walking on tiptoes. The tendon is frequently used in the reconstruction of chronic Achilles tendon ruptures and other foot and ankle pathologies. [170]

1.6.3. Peroneus brevis tendon

The peroneus brevis muscle is a short muscle that is located in the lateral part of the lower leg, together with the peroneus longus and peroneus tertius. The peroneus brevis originates from the distal two-thirds of the fibula and is inserted on the styloid process of the fifth metatarsal. The muscle is mainly involved in the eversion of the foot, as well as assisting during plantar flexion. In chronic Achilles tendon ruptures, the tendon may be used to reinforce the rupture site. [130]

1.7. SURGICAL TECHNIQUES FOR CHRONIC ACHILLES TENDON RUPTURES

Reinforcement surgery is generally recommended when treating chronic Achilles tendon ruptures and Achilles tendon re-ruptures. Several different surgical techniques have been presented in the literature. Patients who are not surgically treated have also been evaluated. The main methods used are V-Y tendon plasty, peroneus brevis tendon grafts, gastrocnemius aponeurosis turndown or free flaps, flexor hallucis longus grafts, synthetic grafts and semitendinosus grafts. No consensus has been reached in terms of the optimal treatment. Table 1 presents different surgical techniques and studies describing and evaluating them in the literature. Comments related to the pros and cons of the techniques, as presented by the authors, have also been added. A short presentation of the different techniques now follows in text.

TABLE 1: Overview of the different surgical techniques that have been presented for chronic Achilles tendon ruptures before this thesis.

Surgical technique	Authors (year) and journal	Comments
Direct repair	Yasuda et al. (2016) J Bone Joint Surg Am [234]	Could be applicable in patients with small tendon gaps and a short time from injury to repair
V-Y tendon plasty	Yang-Jing Lin et al. (2019) Orthop Surg [115]	Easy and fast. Especially useful in ruptures with smaller tendon gaps
Peroneus brevis tendon transfer	Maffulli et al. (2012) J Bone Joint Surg Am [130]	The method uses a peroneus graft, which could negatively affect the eversion of the patient's foot
Gastrocnemius aponeurosis flap	Nilsson-Helander et al. (2008) Knee Surgery, Sports Traumatology, Arthroscopy [158]	The technique does not include distant graft harvest and does not appear to lead to any harvest site complications
Semitendinosus graft	Yue-Jie Song et al. (2020) Knee Surgery, Sports Traumatology, Arthroscopy [205]	The method could be of importance in treating distal ruptures and ruptures with gaps of > 6 cm.
Flexor hallucis longus graft	Ozer et al. (2018) J Foot Ankle Surg [170]	Applicable with larger tendon gaps. Easy access to graft site
Synthetic graft	Shoaib et al. (2017) Foot Ankle Surg [198]	No donor site complications and shorter and simpler operations
Achilles tendon allograft	Ofili et al. (2016) J Foot Ankle Surg [166]	Large bridging without donor site morbidity
Flexor digitorum longus graft	Mann et al. (1991) J Bone Joint Surg Am [139]	Easy access to graft site
Gracilis tendon transfer	Maffulli et al. (2012) J Bone Joint Surg Am [131]	Applicable for large tendon defects
Non-surgical	Winson et al. (2020) Foot (Edinb) [232]	No wound complications. Long and difficult rehabilitation process

Direct repair is sometimes regarded as efficient in treating chronic Achilles tendon ruptures. In a study by Yasuda et al. [234] using Krackow sutures, a total of 30 consecutive patients were included and there was an improvement in the mean AOFAS from 82.8 points preoperatively to 98.1, 33 months postoperatively. The postoperative ATRS was 92.0 points at the final follow-up.

The V-Y tendon plasty is a surgical technique that uses tendon-length reduction plastic with or without reinforcement. In a study performed by Yang-Jing Lin et al., [115] patients treated with the technique presented a mean postoperative ATRS of 94 points of 100 and a post-operative AOFAS score of 96.5 points of 100. In another study by Guclu et al. [65] using a similar surgical technique,

the reported AOFAS score was 95 points. The first study included a total of 20 consecutive patients and the second 17 patients. The mean follow-up time was 33 months and 16 years respectively.

The peroneus brevis tendon transfer uses a peroneus brevis graft from the ipsilateral ankle to reconstruct the gap between the distal and the proximal tendon end. The technique has, for instance, been presented and described by Maffulli et al. [130] with a 15-year follow-up. The study showed that 16 patients treated surgically with the technique presented a mean postoperative ATRS score of 89.5 points at the last follow-up. The technique has also been described and evaluated by Kosaka et al., [102] where 16 consecutive patients presented a mean postoperative AOFAS score of 86.9 points of 100.

The gastrocnemius aponeurosis flap has been described in multiple studies. In a study by Nilsson-Helander et al., [158] it was shown that 28 consecutive patients treated with the technique presented a median ATRS score of 86 points of 100 at a follow-up 29 months after surgical repair. A similar technique has also been described by Seker et al. [197] who reported a postoperative AOFAS score of 98.5 points of 100.

The semitendinosus graft is a graft that does not originate from the ankle. Multiple studies have presented different techniques, including endoscopically assisted and open repair, using a semitendinosus graft when treating chronic Achilles tendon ruptures and Achilles tendon re-ruptures. [14, 82, 112, 121, 205] The outcomes are mainly presented as postoperative ATRS or AOFAS scores. In all the studies, the self-evaluated outcome is generally high, with AOFAS and ATRS scores over 90 points of 100. It is common to all these studies to include small cohort sizes with varying outcome measurements.

The flexor hallucis longus (FHL) graft is another tendon graft commonly used and evaluated in the literature. The method has been described by Ozer et al. [170] The outcome showed a mean AOFAS score of 93.8 points at a six-month follow-up. In another study performed by Pendse et al. [174] using a similar technique, the outcome was a mean AOFAS score of 96.7 points at a mean follow-up time of 27 months. The most prominent difficulties for patients treated with an FHL graft is the potential weakening of the foot, with reduced stability and affected balance and gait. [67]

Synthetic grafts are not used to the same extent as biological autografts. In a study by Shoaib et al., [198] seven consecutive patients were treated with V-Y tendon augmentation reinforced with a synthetic Artelon® graft. The patients treated with this technique presented postoperative AOFAS scores of 91 points of 100 and ATRS scores of 92 points of 100 at a mean follow-up time of 29 months.

Achilles tendon allograft: Ofili et al. [166] reviewed 14 cases of allografts used to treat chronic Achilles tendon ruptures. The study showed that all the patients were able to perform a single heel rise at a mean follow-up time of 16.1 months. The mean tendon (range) gap was 7 cm (4-15 cm). No validated outcome measurement or patient-reported outcome score was presented.

Flexor digitorum longus graft has only been used in a few studies. Mann et al. [139] reported a high level of satisfaction in a case series of seven patients. However, two patients required additional reconstruction and one had a persisting limp at a medium-term follow-up at 39 months postoperatively.

Gracilis tendon transfer is a less frequently used technique. It uses the gracilis tendon of the hamstring to reconstruct the injured Achilles tendon. It has been shown to be efficient by Maffulli et al. [131] In their study, all 21 patients were able to perform a single-leg heel rise on the injured side and reported a mean (SD) ATRS score of 90.1 points (5.8) at a mean follow-up time of 10.9 years.

Non-surgical treatment: a non-surgical approach to chronic Achilles tendon ruptures may be applicable in some cases. A recent study by Winson et al. [232] evaluated the functional and patient-reported outcomes of 19 patients with chronic Achilles tendon ruptures that had been rehabilitated using a physiotherapy treatment practice called the Swansea Morriston Achilles Rupture Treatment protocol (SMART). [75] The mean ATRS score increased from 64.7 to 83 points in the patient group during a period of 6.6 years. No control group was included. Compared with studies of surgically treated injuries, the initial ATRS score of 64.7 points is relatively high, which may make these patients especially suitable for non-surgical treatment with a structured rehabilitation protocol. At the time of writing this thesis, the data on non-surgically treated chronic Achilles tendon ruptures are very limited. In addition to rehabilitation and physiotherapy, different walking aids and ankle-foot orthoses can be used to prevent disproportionate dorsal flexion of the foot when walking. [138]

Previous systematic reviews and meta-analyses:

In 2013, Hadi et al. [66] performed a systematic review of the previously presented surgical techniques in the literature. They identified eleven different surgical techniques: (1) peroneus brevis tendon transfer, (2) flexor hallucis longus graft, (3) V-Y tendinous flap, (4) gastrocnemius turndown flaps, (5) flexor digitorum longus tendon, (6) gracilis tendon transfer, (7) semitendinosus graft, (8) synthetic graft, (9) direct repair, (10) dual techniques and (11) interposed tissue. The most common outcome measurements were the AOFAS, which is non-validated for Achilles tendon ruptures, and the validated ATRS scores. All the reported techniques presented satisfactory results. However, it was difficult to make any direct comparisons due to the limited number of patients, no control groups and heterogeneous groups of patients in all the studies. The systematic review still contributes to the research field by summarising the existing techniques and presenting them in a structured, easily accessible manner.

In 2020, Apinun et al. [9] performed a meta-analysis of original articles presenting outcomes after the surgical repair of chronic Achilles tendon ruptures using an FHL graft alone or an FHL graft with augmentation. They found six studies using each technique. The meta-analysis revealed that the mean (SD) AOFAS score improved from 57.1 (3.6) preoperatively to 93.0 (22.7) points postoperatively in the FHL group and from 66.9 (3.2) to 95.3 (4.7) points in the group treated with additional augmentation. However, no significant differences were found between the groups and the need for further prospective controlled studies was emphasised.

1.8. HEALTH ECONOMICS IN ACHILLES TENDON RUPTURES

Increasing healthcare costs are a problem for healthcare professionals and more cost-effectiveness analyses (CEAs) are needed to understand expenditure and to prioritise treatment [162]. The main reasons for increasing costs are the population growth and the rapid development of new diagnostic techniques and treatment options. The healthcare of today therefore faces new challenges when it comes to economic prioritisation [162]. The treatment of Achilles tendon ruptures is no exception. When analysing health economics, costs can be divided into healthcare costs and production-loss costs. The healthcare costs are the

costs related to healthcare, medicine and surgical procedures. Production-loss costs, on the other hand, are the costs associated with sick leave and the loss of working hours.

There have been discussions about whether surgical or non-surgical techniques are most cost effective when managing an acute Achilles tendon rupture. A study by Westin et al. [231] reported that surgical treatment is more expensive per se but that it can be equally cost effective when quality of life is considered. The study was performed on 100 patients (86 men and 14 women) who were randomised to either surgical or non-surgical treatment. The costs were divided into direct costs (healthcare costs) and indirect costs (production-loss due to sick leave). The costs were then evaluated in terms of quality-adjusted life-years (QALY). The mean cost of surgical treatment was 7,322 €, while it was 6,008 € for non-surgical treatment. When these data were analysed together with QALY using the incremental cost-effectiveness ratio, it was decided that surgical treatment could be cost effective in 57% of cases at a level of 50,000 € per QALY.

Moreover, Carmont et al. [29] found that the percutaneous repair of the Achilles tendon is cost effective compared with open repair. In their study, they retrospectively included 49 patients treated with percutaneous repair and 35 patients who were treated using an open surgical technique. They found that patients treated with the percutaneous technique had fewer complications (10.4%) than patients treated openly (14.3%). This resulted in lower healthcare costs, as patients required a shorter time in hospital and fewer clinical visits. Another reason for the lower costs was that patients treated percutaneously had a significantly shorter occupancy time in the surgical theatre compared with patients treated using the open technique. The direct cost of each surgical technique was £574.04 for percutaneous repair and £935.6 for open repair. This corresponds to 648.72 € and 1,057.32 € using the average exchange rate in 2018 (GBP = 1.1301 €). However, in this study, production-loss costs due to sick leave or cost/QALY were not included. The direct costs in the study performed by Westin et al. [231] were 742 € for non-surgical treatment and 3,146 € for surgical treatment.

Comparisons between studies are not appropriate, as they use different exchange rates and include different expenses as costs. This was also emphasised by Truntzer et al. [217] who reported that the non-surgical treatment of acute Achilles tendon ruptures resulted in significantly lower costs than

surgical treatment. They retrospectively included 1,979 patients treated surgically and 3,065 treated non-surgically. The mean cost of the surgical treatment was $4,292, while it was $2,432 for non-surgical treatment. This corresponds to 3,637 € and 2,061 € using the average exchange rate in 2018 (USD = 0.8475 €). Other health-economic analyses include Ebinesan et al. [50] and Goel et al. [62] comparing percutaneous repair and open surgery, as well as Hutchison et al. [75] analysing the cost effectiveness of using the Swansea Morriston Achilles Rupture Treatment (SMART) protocol. No previous study has analysed the cost of treating chronic Achilles tendon ruptures or re-ruptures.

1.9. BIOMOLECULAR TENDON CHANGES AFTER ACHILLES TENDON RUPTURES

The extracellular matrix in the Achilles tendon and other tendons is mainly composed of collagens and proteoglycans. [88] The collagens are cross-linked in fibres and give the tendon strength and solidity. Meanwhile, the main purpose of the proteoglycans is to retain water within the tendon and give the tendon grease and spacing for less friction and more endurance during activity. [97] In response to loading, the tendon increases its anabolic activity with multiple growth factors. The growth factors that have been mainly associated with anabolic processes within the Achilles tendon are fibroblast growth factors (FGF), insulin-like growth factors (IGF), platelet-derived growth factors (PDGF), bone morphogenetic proteins (BMP) and transforming growth factors (TGF). [80] It has, however, also been shown that catabolic processes are initiated in loading or overloading. [70] The main factors associated with these changes are the matrix metalloproteinases (MMPs). [104]

The known gene expressions that have been hypothesised to affect the healing ability of general tendons are the matrix metalloproteinases (MMPs) and the tissue inhibitor of metalloproteinases (TIMPs). [56, 68] The MMPs are further sub-grouped depending on which part of the extracellular matrix (ECM) they degenerate. The main subgroups are collagenases (MMP-1, MMP-8 and MMP-13), gelatinases (MMP-2 and MMP-9), stromelysines (MMP-3 and MMP-10 etc) and metalloelastases (MMP-12). [21] TIMPs are less specific and are able to inhibit different MMPs in both their active and passive phases. [63] They do, however, also play a role in cell proliferation and other anabolic processes.

In a previous study by Minkwitz et al., [146] it was shown that the expression of MMP-9, -13 and COL1A1 increased significantly with a longer time from injury to surgical repair, whereas MMP-3 and -10 expression decreased. They studied how this expression differed in tendons ruptured two to four days, five to six days and > 7 days prior to surgery. In another study by Eliasson et al., [53] it was shown that rats with unloaded and transected tendons, using botulinum toxin injection in the calf muscle, reduced the expression of procollagen III and tenascin-C compared with rats that were allowed loading after the tendon was transected. The mechanical loading of healing tendons is therefore considered important in the process of redeveloping the extracellular matrix (ECM) after an Achilles tendon rupture. A chronic Achilles tendon rupture implies a long period of periodic unloading of the tendon. These tendons are thereby hypothesised to have even lower gene expression than acute Achilles tendon ruptures. This could be one of the factors that lead to inferior functional outcomes in this group of patients.

02

GAPS IN KNOWLEDGE

The main gaps in knowledge in terms of chronic Achilles tendon ruptures are insufficient information on incidence, economic impact (Study I), functional outcome (Studies II and III) and patient-reported outcome (Studies I, II and III). The definition of chronic Achilles tendon ruptures differs in the literature and should be more precisely defined. It is also important to present and evaluate new and innovative surgical techniques (Study III). Consensus on the preferred surgical procedure should also be more strongly stated (Study IV). It is also important to map the biomolecular changes that occur in chronic Achilles tendon ruptures to optimise the surgical technique and rehabilitation protocol in the future (Study V).

03

AIMS AND OBJECTIVES

Study I: To evaluate the economic impact and patient-reported outcome of chronic Achilles tendon ruptures treated with reconstructive surgery

Study II: To explore the patient-reported and functional outcomes of patients treated surgically for chronic Achilles tendon ruptures using a free gastrocnemius aponeurosis flap

Study III: To describe a surgical technique to treat chronic Achilles tendon ruptures and re-ruptures with delayed presentation using a semitendinosus autograft. It also aims to describe the functional and patient-reported outcomes in patients when this technique is used

Study IV: To investigate the advantages and disadvantages of two different surgical techniques performed (gastrocnemius aponeurosis flaps and semitendinosus grafts) in patients with chronic Achilles tendon ruptures through a systematic review

Study V: To explore how the delayed treatment of Achilles tendon ruptures affects the biomolecular tendon structure in chronic Achilles tendon ruptures

04

MATERIALS AND METHODS

The materials and methods section of this thesis begins with a summary of the inclusion process of patients throughout the PhD project. Each of the methods used will then be presented separately, followed by the Roman numerals of the studies in which they have been applied. The structure for this presentation can also be found in Table 2.

Table of methods used

TABLE 2: Summary of methods used throughout the PhD project with corresponding head numbers and studies.

Head number	Method	Studies	Comments
4.2	Patient-reported outcomes (PROMs)	I, II, III, IV	ATRS. PAS. FAOS. AOFAS. Leppilahti.
4.3	Functional outcomes	II, III, IV	Hopping. Heel-rise work test. Counter-movement jump (CMJ). Concentric power.
4.4	Clinical measurements	II, III, IV	ATRA. Calf circumference. Dorsiflexion range. Tendon length evaluated using ultrasonography.
4.5	Health economics	I	Healthcare costs vs. production-loss costs.
4.6	Surgical techniques	I, II, III, IV	Gastrocnemius aponeurosis free flap. Endoscopically assisted semitendinosus graft.
4.7	Systematic review	IV	Search strategy. Inclusion and exclusion criteria. Study selection. Result extraction. Quality assessment.
4.8	Tissue samples, RNA extraction and RT-PCR	V	Tissue sample collection. Tissue homogenisation. RNA extraction. Reverse transcription. Quality and quantity control of RNA. RT-PCR.
4.9	Statistical methods	I, II, III, V	Wilcoxon's signed-rank test. Kruskal-Wallis ANOVA. Dunn's multiple comparisons test.

4.1. STUDY POPULATION AND DATA COLLECTION

Studies I-II include patients who were treated surgically for a chronic Achilles tendon rupture at Sahlgrenska University Hospital and Kungsbacka Hospital between 2014 and 2018. A total of 69 patients were identified using medical records. All the included patients were treated with the same surgical technique previously described by Nilsson-Helander et al. [158] using a free gastrocnemius aponeurosis flap. Two experienced orthopaedic surgeons performed all the procedures. After surgical repair of the tendon, the injured foot was immobilised in a cast followed by a lower leg brace for a total of eight to nine weeks. All the patients were invited to participate in the economic comparison study by mail. A total of 40 patients responded and were thus included in Study I. One year after the operation, a total of 22 patients agreed to participate in functional testing, clinical measurements and a patient-reported outcome evaluation in Study II.

In Study III, patients treated with the endoscopically assisted ipsilateral free semitendinosus tendon transfer at Sahlgrenska University Hospital and Princess Royal Hospital, Shrewsbury and Telford Hospital NHS Trust were evaluated one year postoperatively with regard to function and patient-reported outcomes. A total of 22 patients were identified and included between 2018 and 2022. The treatment method was chosen by an experienced orthopaedic surgeon with consent from the patient. The study also includes a comprehensive description of the surgical technique.

Patients who were treated surgically for an acute or chronic Achilles tendon rupture at Sahlgrenska University Hospital between 2018 and 2022 were invited to participate in the biomolecular study (Study V). Tissue samples were collected during the surgical repair of Achilles tendon ruptures and later examined through RNA extraction and RT-PCR. Patients with acute (0-28 days), short-term chronic (28 days to 6 months) and long-term chronic (>6 months) Achilles tendon ruptures were all included and analysed. A total of 35 patients were included. The tissue samples were analysed based on an mRNA level analysis of matrix metalloproteinase (MMP), tissue inhibitor of metalloproteinase (TIMP) and collagen (COL1A1 and COL3A1) at the University of Linköping. After exclusion due to low RIN values in the analysis, 27 patients remained.

To summarise, this thesis includes patients from two distinctive cohorts. The first cohort of patients was presented at the designated hospitals between 2014

and 2018 and analysed retrospectively. The second cohort was presented between 2018 and 2022. The way patients have been included throughout the project, together with demographic data, is illustrated in Table 3, describing the inclusion process.

Table of patients included

TABLE 3: Summary of the sex and age of the included patients. Categorical variables are presented as n (%) and continuous variables as the mean ± SD. Comments are made on the inclusion process for each study.

Study	Patients included	Sex		Age	Comments
		Male	Female		
Study I	40	29 (72.5%)	11 (27.5%)	62.7 ± 13.8	29 of 69 patients did not respond to the invitation to participate in the study.
Study II	22	14 (63.6%)	8 (36.4%)	62.3 ± 13.0	All the patients were prospectively included in a previous study analysing the gait pattern pre- and postoperatively.
Study III	22	13 (59%)	9 (41%)	60 ± 12.0	Patients treated with a semitendinosus graft
Study V	27	17 (63%)	10 (37%)	50.4 ± 14.6	Patients with acute and chronic Achilles tendon ruptures treated surgically

Additionally, the project also includes a systematic review of different surgical techniques used to treat chronic Achilles tendon ruptures (Study IV). The latest thorough systematic review was presented in 2013. [66] Since then, several interesting surgical techniques have been presented using more validated outcome measurements. The present systematic review aimed to explore these more recent techniques and their outcomes. The main trend in new surgical techniques is endoscopically assisted techniques with less risk of wound complications and postoperative infections.

4.2. PATIENT-REPORTED OUTCOMES (STUDIES I, II, III, IV, V)

In this thesis, multiple patient-reported outcome questionnaires (PROMs) were used to evaluate the symptomatology of patients treated surgically for chronic Achilles tendon ruptures. In Studies I, II, III and IV, the validated Achilles tendon Total Rupture Score (ATRS) was most prominently used. Study II, which

uses a more comprehensive test battery, also included the Physical Activity Scale (PAS) and the Foot and Ankle Outcome Score (FAOS). In the systematic review, as well as in the discussions, the Leppilahti and AOFAS scores were added to provide a more extensive view of previously published studies related to chronic Achilles tendon ruptures. The main limitation of self-evaluation is recall bias and different interpretations of questions.

The Achilles tendon Total Rupture Score (ATRS) is an injury-specific patient-reported outcome score developed by Nilsson-Helander et al. [159] The instrument is well tested for validity and reliability. [159] It is commonly used in studies that evaluate the outcome of Achilles tendon treatment and rehabilitation. The score involves 10 questions with Likert scales ranging from 0 to 10, where 0 represents the worst possible symptoms and 10 no symptoms at all. As a result, a score of 100 implies a fully recovered patient. The ATRS has been translated and culturally adapted to English [32], Turkish [91], Danish [58], Dutch [169], French [25] and Persian [8] et cetera. The ATRS has been tested for validity and reliability, with high internal consistency (ICC = 0.96) and intraclass correlation (ICC = 0.98). [159] The ATRS is the only injury-specific questionnaire evaluating acute Achilles tendon ruptures.

The Physical Activity Scale (PAS) evaluates the regularity and level of activity among patients. The scale was first reported by Saltin et al. [190] in 1968 and has been frequently used since then. The score used in these studies is a modified score from 1986 developed by Grimby et al. [64] The measurement scale consists of six levels of activity. The first level means predominantly sedentary, while the sixth level means intense workouts on numerous days a week. The scale has not been tested for reliability and validity for Achilles tendon ruptures.

The Foot and Ankle Outcome Score (FAOS) is used to evaluate ankle instability related to ankle ligament injuries. It is, however, also useful when evaluating ankle function after an Achilles tendon rupture. The score was initially developed by Roos et al. [187] as a knee injury score. It was later additionally adapted and validated for foot and ankle injuries in a new score called the FAOS. [186] The score consists of five subscales that measure activities of daily living (ADL), quality of life (QoL), pain, other symptoms and sport and recreational activity. The score is rated from 0 to 100 for each subscale.
A score of 0 implies major suffering and 100 no problems at all. The FAOS has been tested for reliability and validity for ankle injuries but not specifically for Achilles tendon ruptures.

The American Orthopaedic Foot and Ankle Score (AOFAS) is another non-specific patient-reported outcome score related to foot and ankle insufficiency. It was originally developed and validated for general foot and ankle complaints, but it has also been widely used to evaluate patient function after acute and chronic Achilles tendon ruptures. [206] The score consists of four scales that evaluate the function of the ankle-hindfoot, midfoot, hallux metatarsophalangeal-interphalangeal (MTP-IP) or lesser metatarsophalangeal-interphalangeal regions. Each of these scales includes questions on pain and function. The scale also includes physical examination items of function and alignment, making the AOFAS a mixed scale. The total score is 40 points for pain, 50 points for function and 10 points for alignment. A total of 100 points mean full recovery and 0 points mean serious limitations.

The Leppilahti score is a scoring scale that was first developed by Leppilahti et al. [107] in 1998 to evaluate clinical outcomes after surgically repaired Achilles tendon ruptures. The scoring scale uses both subjective and objective outcome measurements. The scale includes seven items relating to pain, stiffness, calf muscle weakness, footwear restrictions, active range of motion between ankles, subjective satisfaction and isokinetic muscle strength. Each item gives the patient 0-15 points, apart from footwear restrictions (0-10), where 0 points mean severe limitations and 15 points mean full recovery. When all the items are summarised, the patient is given a score of 0-100 points. The Leppilahti scoring scale is widely used, but it has not been checked for validity or reliability, as similar scoring systems do not exist.

4.3. FUNCTIONAL OUTCOMES (STUDIES II, III AND IV)

In Studies II and III, multiple functional follow-up tests were used objectively to quantify the lower leg function of the Achilles tendon after the treatment and rehabilitation of chronic Achilles tendon ruptures. Both studies used the validated test battery developed by Silbernagel et al. [199] The test battery uses MuscleLab® (Ergotest Technology, Oslo, Norway) and includes hopping, the heel-rise work test, concentric heel-rise power and the Counter-Movement Jump (CMJ). The healthy and the injured side were compared in terms of the difference and the Limb Symmetry Index (LSI) in both studies. The Limb Symmetry Index (LSI) is defined as the value on the injured side divided by the value on the healthy side x 100 expressed in per cent. In the discussions and the review, other functional outcome measurements will also be presented.

The functional tests are well used and established in evaluating acute Achilles tendon ruptures. They have been used in many previous studies and are tested for validity. [199] The main limitation of these tests is that they can be challenging for patients affected by an Achilles tendon rupture. This applies in particular to the elderly patients described in these studies. Many patients were not able to perform all the tests. This will obviously affect the outcome of the studies. The tests are not considered applicable in the early follow-up, eight weeks to six months, due to the risk of re-rupture.

The hopping test is registered as the mean hopping height, mean hopping frequency and mean flight time/contact ratio of 10 jumps performed by the patient. [199] The height is listed in centimetres. The hopping is performed on one leg in a skipping motion with a frequency of two jumps/second. The action is like the one performed when skipping with a rope. Patients are requested to perform 15 jumps on each leg, starting with the healthy side. The first three and the last two jumps are excluded which gives 10 jumps for analysis. The 15 jumps are performed twice for each leg with a 15-second rest between sets. The result is presented as the mean of each parameter with the LSI between the healthy and the injured side.

The heel-rise work test was first described by Silbernagel et al. [200] The test is performed with the patient standing on one leg on top of a platform with a 10-degree incline with an encoder connected to the heel (Figure 9). The patient is then asked to raise the heel as high as possible with a frequency of 30 rises per minute. The heel-rise repetitions, the heel-rise height and the heel-rise work are registered. The test ends when the patient is no longer able to maintain the requested frequency or perform heel-rises higher than two centimetres. The test starts with the healthy foot followed by the injured one. The data presented are the number of heel-rise repetitions, the highest heel-rise height and the total heel-rise work in Joules (body weight x total distance x gravity).

The heel-rise height is a simplified functional outcome measurement where only the maximum heel-rise height is measured with a tape measure from the floor to the patient's heel on a flat surface. [33]

The Counter-Movement Jump is performed with the patient standing on the ground on one leg with their hands on the waist or behind the back. [199] The patient is then asked to perform a vertical jump as high as possible. The whole

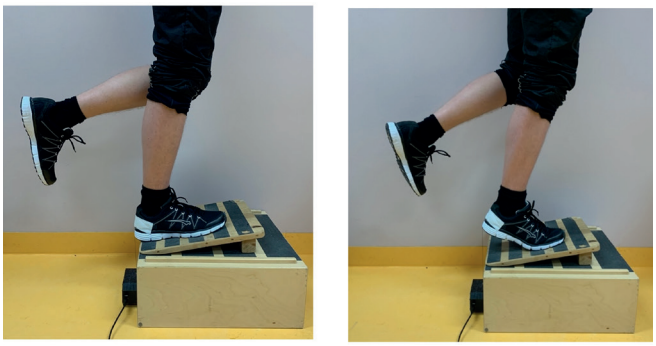

FIGURE 9: The heel-rise test was performed with a linear encoder attached to the heel while the patient performed single-leg heel rises with a 10° incline.

movement is performed on one leg. The patient is requested to perform at least three jumps on each leg after familiarisation. The height of the highest jumps is then presented with the absolute value and the LSI.

The concentric power is evaluated with the patient performing single-leg heel rises in a weight machine with successively added weights. The instruction to the patient is to perform the heel rise "as quickly and powerfully as possible without bending the knee". At first, 13 kg is added and the weight is then increased by 10 kg for each interval until a decrease in power output is noted. Each interval is three repetitions with 15 seconds of rest between each repetition. The highest force in Watts is determined and compared with the other limb for the same external load. [200]

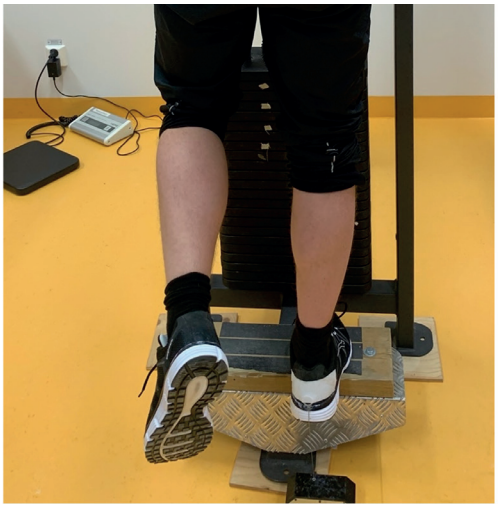

FIGURE 10: The concentric power was evaluated with the patient in a weight machine with the load on the shoulders.

4.4. CLINICAL MEASUREMENTS (STUDIES II, III AND IV)

In addition to patient-reported outcomes and functional testing, the patients included in Studies II and III were also evaluated using multiple clinical measurements that estimate the tendon length and range of motion (ROM) of the ankle. The measurements applied in these studies were the Achilles Tendon Resting Angle (ATRA), dorsiflexion range, calf circumference and tendon length measured with ultrasonography. As previously described for patient-reported outcomes and functional tests, additional clinical measurements will be assessed in conjunction with the result of the systematic review and the overall discussion of this thesis.

The Achilles Tendon Resting Angle (ATRA) is an indirect measurement that evaluates the length of the Achilles tendon. Patients with a longer tendon have a larger resting angle and, in several cases, poorer ankle function. [237] The ATRA was developed and validated by Carmont et al. [31] The measurement is performed with the patient in a prone position with the knee bent 90 degrees and the foot relaxed. The angle is measured using a goniometer (Medi GmbH, Bayreuth, Germany). One arm of the goniometer is placed along the fibular shaft, towards the centre of the fibular head, with the axis on the tip of the fibula and the other arm centred on the head of the fifth metatarsal bone. The angle measured is then the angle between the lateral malleolus and the distal fifth metatarsal bone. The result is presented in degrees. The test is thought to provide an accurate restoration of tendon length, with a standard error of the mean of 2.5°, and is well tested for reliability through test-retest reliability (ICC 0.91). [31] A potential source of error is ankle stiffness affecting the angle.

The dorsiflexion range is another way to measure the movability of the ankle. The patient is asked to stand with the leg which is going to be studied as far behind the other leg as possible without lifting the heel above the ground. The patient is then invited to lean forward in a stance position. The angle is measured with a digital inclinometer (Baseline® Digital Inclinometer 12-1057). The main difference between the dorsiflexion range and the ATRA is that patients in the dorsiflexion range tension the tendon upon axial load. This means that the test cannot be performed before three months after initiated treatment. The angle of the foot is registered both with the knee extended and with the knee flexed. The tests have been tested for test-retest reliability and inter-rater reliability by Munteanu et al. [154] and Bennell et al. [18]

The calf circumference is measured as the largest possible calf circumference with the patient lying in a prone position with the feet and calves outside the examination couch. The test is performed with a tape measure with 0.5 cm increments. It is important that the tape measure does not squeeze the skin around the calf. The calf circumference is an indirect measurement of muscle volume and strength. The test has been described in a randomised controlled trial by Möller et al. [149] A similar test has been tested for reliability by Carmont et al. [31] and the test was regarded as accurate as no significant differences were found in a reliability test.

The tendon length is measured with ultrasonography. In the following studies, the LOGIC E US (GE) or LOGIC P9 US (GE) wide-band linear array probe with 5-13 MHz and extended field of view (EFOV) was used. The length between the calcaneal osteotendinous junction (OTJ) and the gastrocnemius musculotendinous junction (MTJ) was used. The technique is reliable and valid according to a study performed by Silbernagel et al. [201] The measurement has been tested for validity through analysis of ultrasonography-measured tendon length in relation to tape-measured tendon lengths of dissected tendons from cadavers as the golden standard. The study showed an ICC of 0.987-0.997 in inter-rater reliability, an ICC of 0.898-0.944 in test-retest and an ICC of 0.895 when compared with anthropometric measurements. There were no significant differences in tendon length between measurements on ultrasonography EFOV and anthropometric measurements on dissected tendons from cadavers. There were no significant differences in terms of inter-rater reliability between limb reliability and test-retest reliability. In the studies in this thesis, the mean of two to three measurements of each leg was used.

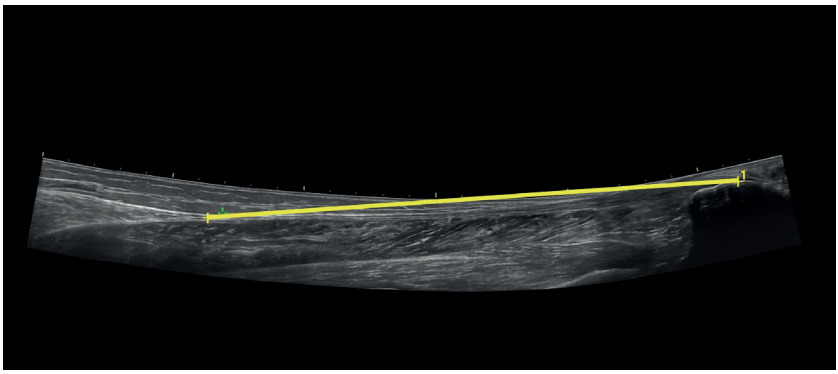

FIGURE 11: The extended field of view (EFOV) measuring the length between the gastrocnemius musculotendinous junction (MTJ; to the left) and the calcaneal osteotendinous junction (OTJ; to the right).

4.5. HEALTH ECONOMICS (STUDY I)

The health-economic costs for each patient in Study I were calculated as the sum of the surgical budget, other healthcare expenses and the economic loss due to sick leave. Healthcare costs were extracted from the accounting database at Sahlgrenska University Hospital and production-loss costs were collected from the Swedish Social Insurance Agency for each individual patient. The data were later compared with the results presented in the economic study performed by Westin et al. [231] in 2018. In that study, the economic cost of the surgical and non-surgical treatment of acute Achilles tendon ruptures were analysed. The data used were based on the randomised controlled study performed by Olsson et al. [168] The healthcare and production-loss cost of chronic Achilles tendon ruptures were examined in comparison with the cost of both the surgical and non-surgical treatment of acute Achilles tendon ruptures. The cost of physiotherapist visits was excluded from this comparison due to limited information on the number of visits for some patients.

The economic costs were classified as either healthcare costs or production-loss costs. Table 4 shows the healthcare costs that were considered in the study. The production-loss costs depend on the number of sick-leave days and the gross wage of the patients registered by the Swedish Social Insurance Agency. All costs were converted from SEK to € using the 2018 exchange rate (1 € = 8.86 SEK).

TABLE 4: The health-economic costs considered in Study I.

Item	Cost per unit (€)
Accident and Emergency visit	221.0 €
Inpatient night	567.4 €
Surgeon cost/min	6.0 €
Operation cost/min	17.2 €
Clinical/reception visit	221.0 €
Day surgeon bed	282.2 €
Ankle brace*	203.2 €

* The cost of a lower leg plaster cast pre- and postoperatively was regarded as insignificant to the study.

4.6. SURGICAL TECHNIQUES (STUDIES I, II AND III)

In Studies I and II, all the included patients were treated using the same open gastrocnemius aponeurosis free flap technique, as previously presented by Nilsson-Helander et al. [158] in 2008. In Study III, a surgical technique using an endoscopically assisted reconstruction with an ipsilateral semitendinosus was performed on all the included patients. In the review and discussions, multiple other techniques will be mentioned and discussed.

Open gastrocnemius aponeurosis free flap technique (I and II)

The method was first described by Nilsson-Helander et al. [158] The method is based on an augmentation with a free gastrocnemius aponeurosis flap. To summarise, the patient is placed in a prone position. The surgical procedure is performed under general or spinal anaesthesia with a haemostatic tourniquet. A straight 20 cm incision is made along the centre of the calf, from the middle third of the gastrocnemius muscle and down towards the heel. Distally, the incision curves medially to avoid damage to the sural nerve. The tendon ends are then located in the surgical wound and the scar tissue between them is removed. Whenever possible, the tendon ends are sutured together using a modified Kessler suturing technique. A free aponeurosis flap from the gastrocnemius muscle is then prepared. The length and width of the graft depends on the size of the tendon gap. In general, it is approximately 5-10 x 3-5 cm. The flap is then sutured over the initial gap to strengthen the tendon. All patients receive prophylactic antibiotics and thrombosis prevention. The technique is not considered to be applicable on tendon gaps larger than 6 cm. [158]

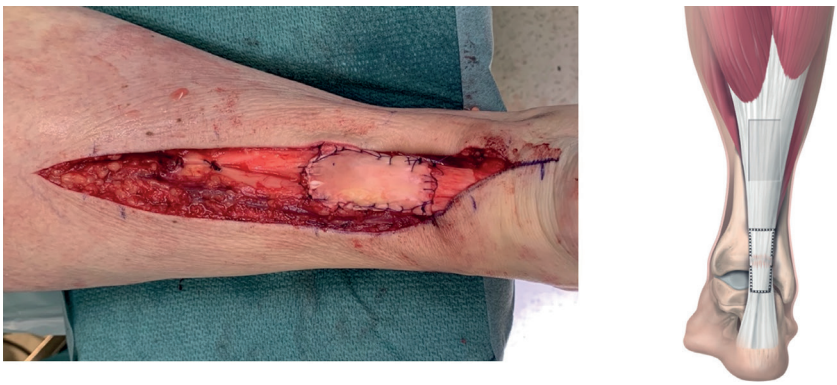

FIGURE 12: Intra-operative picture showing the free gastrocnemius aponeurosis flap attached to the ruptured Achilles tendon (left) and an illustration of the technique (right).

Following the surgery, the patient's foot is placed in an equinus position with a below-the-knee plaster cast. The cast is worn in an equinus position for three weeks, followed by three weeks in a mid-plantar flexed position. After six weeks, the cast is replaced by a pneumatic walking boot (Aircast AirSelect Standard) with 3-4 heel wedges (Figure 13). The total number of weeks of ankle immobilisation is therefore eight to nine weeks. Partial weight-bearing is allowed after three weeks and full weight-bearing after six weeks.

FIGURE 13: Aircast AirSelect Standard and wedges used for the postoperative management after surgical repair using both surgical techniques.

Endoscopically assisted semitendinosus graft technique (Study III)

This is a surgical technique developed for the less invasive repair of chronic Achilles tendon ruptures and re-ruptures. The method uses an endoscopically assisted technique and the semitendinosus tendon is used as an autograft. The patient is placed in a semi-prone position to enable access to the Achilles tendon and the semitendinosus graft. With the knee flexed at a 90-degree angle, a 2-3 cm longitudinal incision is made over the pes anserinus. The semitendinosus tendon is then harvested. The graft is cleaned and prepared with whip-stitch sutures at both ends. For the preparation of the endoscopically assisted calcaneoplasty, a three-incision approach with a posteromedial, a posterolateral and an accessory posterolateral incision is used. A guidewire is then placed through a midline calcaneal incision under endoscopic visualisation. The guidewire is then drilled through the calcaneus with an exit on the plantar surface. A 7 mm channel is subsequently drilled over the guidewire.

The proximal end of the tendon is identified and a 4-5 cm long longitudinal central incision is made over its position. The proximal end is identified and debrided. The graft is then sutured and fixed to the proximal end through a coronal tenotomy. The graft and the proximal end of the Achilles tendon are then tunnelled down to the calcaneus. The graft is threaded through the insertion hole and drawn out through the plantar exit wound. Appropriate pull is applied to achieve the right tension in the reconstructed Achilles tendon. The graft is fixated with a 6-8 mm screw at the end of the calcaneus. The technique can be used on tendon gaps larger than six centimetres and it is especially useful in distal ruptures.

Following surgery, the injured leg is placed in a circumferential plaster cast with the foot in full plantar flexion. The patient is initially mobilised with no weight-bearing using two crutches for three weeks. An outpatient clinical visit is made three weeks after the operation to change the circumferential plaster cast to a pneumatic walking boot (Aircast AirSelect Standard) with three to four heel wedges (Figure 13). Partial weight-bearing is allowed after three weeks and full weight-bearing after six weeks. The pneumatic walking boot is removed eight to nine weeks postoperatively.

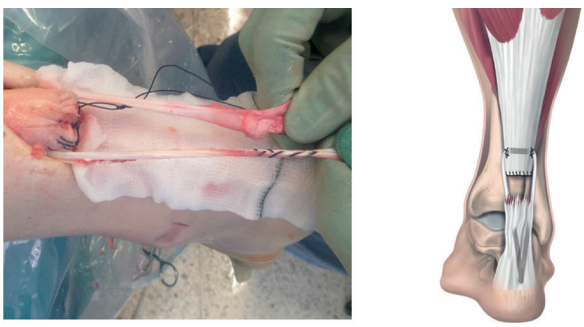

FIGURE 14: Intra-operative image showing the coronal tenotomy and the fixation of the semitendinosus graft to the proximal tendon end (left) and an illustration of the technique (right).

4.7. SYSTEMATIC REVIEW (STUDY IV)

Study IV is a systematic review that maps the current knowledge and results of a gastrocnemius aponeurosis flap and a semitendinosus graft to treat chronic Achilles tendon ruptures. The search strategy, inclusion and exclusion cri-

teria, study selection, result extraction and quality assessment are presented below. The systematic review was registered and published in PROSPERO (CRD42022294130).

Search strategy

The initial search strategy was extensive and included all the articles that presented outcomes after chronic Achilles tendon rupture reconstruction. All the searches were performed in June 2021, with a complementary search in September 2022, in PubMed, Scopus and the Cochrane Library using the search queries outlined in Table 5. All the searches were made with the assistance of an experienced librarian at the Biomedical Library, University of Gothenburg.

TABLE 5: Search query and search results in the PubMed, Scopus and Cochrane databases

PubMed	Search string	Limits	Number of records
#1	(Achilles Tendon[mh] OR "Achilles tendon" [tiab] OR "Achilles tendons"[tiab] OR "Calcaneal Tendon"[tiab] OR "Calcaneal Tendons"[tiab] OR "Tendo calcaneus"[tiab])		12 515
#2	(chronic*[tiab] OR neglect*[tiab] OR miss*[tiab] OR delay*[tiab])		2 204 598
#3	#1 AND #2		1 750
#4	(Rupture[mh] OR rupture[tiab] OR ruptures[tiab] OR tear*[tiab])		192 888
#5	(Treatment Outcome[mh] OR treatment[tiab] OR therapy[tiab] OR outcome*[tiab] OR "clinical efficacy" [tiab] OR "clinical effectiveness" [tiab] OR complication*[tiab] OR re-rupture*[tiab] OR rerupture*[tiab])		8 235 689
#6	#3 AND #4 AND #5		526
Scopus	**Search string**	**Limits**	**Number of records**
#1	TITLE-ABS-KEY ("Achilles tendon" OR "Achilles tendons" OR "Calcaneal Tendon" OR "Calcaneal Tendons" OR "Tendo calcaneus")		16 771
#2	TITLE-ABS-KEY (chronic* OR neglect* OR miss* OR delay*)		4 433 517
#3	#1 AND #2		2 439
#4	TITLE-ABS-KEY (rupture OR ruptures OR tear*)		387 825
#5	TITLE-ABS-KEY (treatment OR therapy OR outcome* OR "clinical efficacy" OR "clinical effectiveness" OR complication* OR re-rupture* OR rerupture*)		14 409 980
#6	#3 AND #4 AND #5		803
#7	#3 AND #4 AND #5	Limit to: Article, Review	762

TABLE 5: Continued

Cochrane	Search string	Limits	Number of records
#1	("Achilles tendon" OR "Achilles tendons" OR "Calcaneal Tendon" OR "Calcaneal Tendons" OR "Tendo calcaneus"):ti,ab,kw		1004
#2	(chronic* OR neglect* OR miss* OR delay*):ti,ab,kw		232 193
#3	#1 AND #2		206
#4	(Rupture OR ruptures OR tear*):ti,ab,kw		13 308
#5	(Treatment OR therapy OR outcome* OR "clinical efficacy" OR "clinical effectiveness" OR complication* OR re-rupture* OR rerupture*):ti,ab,kw		1 344 590
#6	#3 AND #4 AND #5		31
#7	#3 AND #4 AND #5	Limit to: Trials	30

Inclusion and exclusion criteria

Included studies:

1. All studies, descriptive and comparative, presenting the results of either gastrocnemius aponeurosis flap augmentation or reconstruction using a semitendinosus graft to treat chronic Achillies tendon ruptures in adults (> 18 years)
2. Both validated and non-validated outcome measurements in terms of function and complication rates were included.

Studies were excluded if they were:

1. Case reports with fewer than 10 patients
2. Reviews
3. Non-English articles
4. Expert opinions

Study selection

The initial search was performed by the authors and resulted in a total of 1,340 studies. After duplicate removal, 818 individual studies remained. The identified studies were uploaded into Rayyan® for abstract review. Two of the authors then autonomously and systematically reviewed the titles and abstracts of the original studies for inclusion and exclusion criteria and filtered them accordingly. Any disagreement between the two authors was settled by consensus after discussions. After the initial filtering, 182 studies remained. These studies were then imported as full-text versions. After the full-text filtering, 70 studies remained. Out of these, 36 presented the outcome after either gastrocnemius aponeurosis flap or semitendinosus graft and were therefore included in the systematic review.

Result extraction

Three reviewers extracted data using structured extraction protocols focusing on the number of patients included, mean age, follow-up time, outcome measurements and presented result. The results that were analysed were complication rates, PROMs (AOFAS, Leppilahti and ATRS etc.) and functional outcome measurements. Any disagreement between the three reviewers was settled through discussions and reaching consensus.

Quality assessment

The quality assessment was performed using the Methodological Index Of Non-randomised Studies (MINORS) developed by Slim et al. [203] The assessment tool uses eight methodological items including (1) a clearly stated aim, (2) the inclusion of consecutive patients, (3) the prospective collection of data, (4) endpoints appropriate to the aim of the study, (5) an unbiased assessment of the study endpoint, (6) a follow-up period appropriate to the aim of the study, (7) loss to follow-up less than 5% and (8) the prospective outcome of the study size. For each methodological item, the study receives a score of 0-2, where 0 means not reported, 1 reported but inadequate and 2 reported and adequate. The total score is given on a scale from 0-16. The MINORS assessment tool has been proven to have high reliability and validity and has been widely used in previous reviews and meta-analyses in orthopaedic patients. [44, 173, 203, 233]

	Methodological items for non-randomised studies (MINORS)
1.	**A clearly stated aim:** the question addressed should be precise and relevant in the light of the available literature.
2.	**Inclusion of consecutive patients:** all patients potentially fit for inclusion (satisfying the criteria for inclusion) have been included in the study during the study period (no exclusion or details about the reasons for exclusion).
3.	**Prospective collection of data:** data were collected according to a protocol established before the beginning of the study.
4.	**Endpoints appropriate to the aim of the study:** unambiguous explanation of the criteria used to evaluate the main outcome, which should be in accordance with the question addressed by the study. Moreover, the endpoints should be assessed on an intention-to-treat basis.
5.	**Unbiased assessment of the study endpoint:** a blind evaluation of objective endpoints and double-blind evaluation of subjective endpoints. Otherwise, the reasons for not blinding should be stated.
6.	**Follow-up period appropriate to the aim of the study:** the follow-up should be sufficiently long to allow the assessment of the main endpoint and possible adverse events.
7.	**Loss to follow-up less than 5%:** all patients should be included in the follow-up. Otherwise, the proportion lost to follow-up should not exceed the proportion experiencing the major endpoint.
8.	**Prospective calculation of the study size:** information on the size of the detectable difference of interest with a calculation of the 95% confidence interval, according to the expected incidence of the outcome event, and information about the level for statistical significance and estimates of power when comparing the outcomes.

FIGURE 15: The methodological items used to assess risk of bias in non-randomised studies (MINORS). [203] The first eight items as listed above were used in this systematic review.

4.8. TISSUE SAMPLES AND BIOMOLECULAR ANALYSIS (STUDY V)

Study V takes a different approach towards chronic Achilles tendon ruptures. Instead of conclusively evaluating postoperative outcomes, the study aims to map the molecular changes in the Achilles tendon related to delayed treatment. The study uses tissue samples from Achilles tendons taken during surgical repair. The sample is then homogenised and the RNA is extracted and analysed in terms of gene expression using RT-PCR with primers for MMPs, TIMPs, collagens and inflammatory markers.

Tissue sample collection: All the tissue samples in Study V were collected during the surgical reconstruction of the Achilles tendon. No further invasive surgery other than clinically necessary was performed. The patients were categorised into three groups depending on the time from injury to surgical repair. The created groups were patients with an acute rupture (0-28 days), a short-term chronic rupture (1-6 months) and a long-term chronic rupture (> 6 months). Two (2 x 2 x 2 mm) tissue samples were collected, one from the proximal tendon end and one from the distal tendon end. The tissue samples were immediately placed in coded test tubes, snap frozen in liquid nitrogen and stored at -80˚C. The tissue samples were later sent to the Department of Biomedical and Clinical Sciences at the University of Linköping for RNA extraction and analysis according to a transfer agreement. The unidentifiable coded test tubes were always separated from the code key.

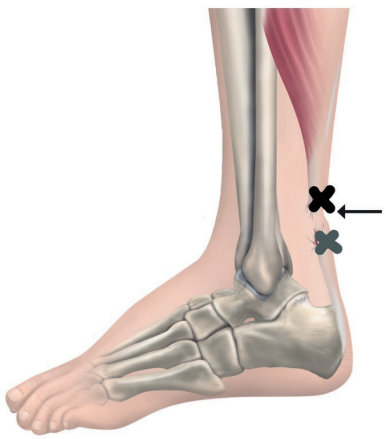

FIGURE 16: Two Achilles tendon tissue samples (2 x 2 x 2 mm) were collected at the rupture site, one from the distal (light) and one from the proximal (dark) tendon end during the surgical repair of both acute and chronic Achilles tendon ruptures.

Homogenisation of samples and RNA extraction: RNA was extracted using the TRIspin method [183], a combination of the TRIzol method and the RNeasy Total RNA-Kit (Qiagen, MERCK Eurolab, Stockholm, Sweden). All the samples were weighed before homogenisation. Samples heavier than 100 mg were divided into smaller samples lighter than 100 mg. The samples were then pulverised one by one using a Mixer Mill (Retsch, Haan, Germany) and a tungsten ball. The samples and Mixer Mills were first cooled in a container filled with liquid nitrogen for at least five minutes. They were then homogenised in the dismembranator at 2,600 rpm for 45 seconds. If samples were not fully homogenised, they were put through the process repeatedly until full homogenisation was achieved. Secondly, 1,000 µl of the TRIzol Reagent (Life Technologies, Gibco BRL) was added to each of the pulverised samples. The samples were thawed at room temperature for 45-90 minutes and transferred to new test tubes before a 30-min incubation. These test tubes were later put in a -80°C freezer awaiting further extraction.

After the samples were rethawed, 100 µl of 1-Bromo-3-chloropropane (BCP; Molecular Research Centre) was added to the samples, followed by vortex and incubation at room temperature for two to 15 minutes. The samples were then centrifuged at 12,000 g and a temperature of 4°C for 15 minutes. The top, aqueous, layer was then obtained and transferred to new tubes with 4 µl glycogen. Isopropanol of the same volume was then added to the samples. The samples were then mixed by inversion and incubated at room temperature for five to 10 minutes. After incubation, the samples were centrifuged at 12,000 g at a temperature of 4°C for eight minutes. The supernatant was removed with a pipette and replaced by 1,000 µl 70% ethanol added to the Eppendorf tubes. The samples were then once again centrifuged at a speed of 7,500 g at a temperature of 4°C for five minutes. The supernatant was removed and the samples were centrifuged at 7,500 g at a temperature of 4°C for one minute, followed by a new supernatant removal. To the remaining pallet, 100 µl of RNAse free was added, followed by five to 10 minutes of incubation at room temperature and vortex. Later, 10 µl of 3M NaAc pH 5.5 and 200 µl of 96% ethanol were added to the test tubes. The samples were then further cleaned using ethanol and centrifuged repeatedly according to the attached protocol, eluded in 10 µl of RNAse-free water and stored at -80°C.

RNA concentration and purity were assessed with a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), while quality was assessed with an RNA 6000 Nano kit (Agilent Technologies, Böblingen, Ger-

many). RNA integrity numbers (RIN) above 5 were regarded as acceptable for reverse transcription and RT-PCR. Samples for which the quality check failed were further cleaned using the RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

Reverse transcription and real-time PCR: The purified RNA samples were then analysed with reverse transcription and RT-PCR (real-time polymerase chain reaction). The process started with 400 ng of RNA from each sample that was transcripted into cDNA using a high-capacity cDNA reverse transcription set (Applied Biosystems, Warrington, UK). The samples were diluted in tris-EDTA buffers. Matching primers for IL-6, COL3A1, COL1A1, MMP-1, MMP-13, IL-1, MMP-2, MMP-9, MRC1, MCEMP-1, TIMP-1, TIMP-2, TIMP-3, TIMP-4, TNF-alfa and CD163 were purchased from Applied Biosystems. The amplification was performed using 15 µl reactions with TaqMan Fast PCR Master Mix. The quantification was made using a standard curve from Universal Human Reference RNA (Invitrogen). Wells with no template were added as negative controls.

4.9. STATISTICAL METHODS (STUDIES I, II, III, IV, V)

All the data were analysed using IBM SPSS Statistics for Mac (Version 25, IBM Corp., Armonk, New York, USA). Patient-reported outcomes were analysed as ordinal variables and were therefore statistically tested for significance using non-parametric tests. The economic costs and demographics were calculated as continuous variables and were presented as the means with standard deviation and 95% confidence intervals. The tests for statistical significance between economic costs were performed assuming that all the collected data were normally distributed.

Wilcoxon's signed-rank test was used to compare the injured side with the healthy side in all the studies, as these are dependent, non-parametric data that lack normality and equal variance. The relative gene expression in the distal and proximal tendon ends was also compared using Wilcoxon's signed rank test, as they had many outliers and diverse variance. The differences between acute, short-term and long-term chronic Achilles tendon ruptures were tested for statistical significance using the Kruskal-Wallis ANOVA test and Dunn's multiple comparisons test, as these also consisted of non-parametric data. P-values lower than 0.05 were considered statistically significant in all the included studies.

No sample size calculation was used in Study V, as the distribution among patients was unknown. In the clinical studies (Study I, II and III), all the available patients were included and, as a result, no sample size calculation was performed for those either. This could have caused multiple type-II errors. The differences in most studies were, however, large, with high power, even though the number of patients was small.

4.10. ETHICAL CONSIDERATIONS (STUDIES I, II, III, IV, V)

Study I: Ethical approval was obtained from the Regional Ethical Review Board in Gothenburg, Sweden (DNR 554-15). All the patients gave their written, informed consent before inclusion. The main ethical consideration is that the study collects sensitive patient data in terms of income and work absence due to sick leave.

Study II: Ethical approval was obtained from the Regional Ethical Review Board in Gothenburg, Sweden (DNR 554-15). All the patients gave their written, informed consent before inclusion. The tests take time and can induce minor muscular pain. The study also collects sensitive patient data in terms of health.

Study III: Ethical approval was obtained from the Swedish Ethical Review Authority (DNR 2020-02971). All the patients gave their written, informed consent before inclusion. The study did not affect the treatment choice. As a result, the only thing that distinguished the included patients from the non-included patients was the follow-up with functional tests. These tests take time for the patient and may induce minor muscular pain. The patient data contain information on health which can be considered intrusive to the involved patients.

Study IV: Systematic review with no patient inclusion or data collection. The advantage of systematic reviews is that they improve the quality of previously collected data and promote new ideas for better studies in the future. In the end, this will benefit the patients without any risk of harm.

Study V: Ethical approval was obtained from the Swedish Ethical Review Authority (DNR 2020-02971). All the patients gave their written, informed consent before inclusion. Biosamples were collected from the patients during

surgery. The samples were later coded and stored in a biobank with an isolated code key locked away in a different location. Even though the samples were coded, it is always possible to trace the samples back to the patient through the code key. This is in fact an intrusion of integrity that needs to be considered. However, the risk is minimal.

05

RESULTS AND STUDY SUMMARIES

5.1. STUDY I

The economic cost and patient-reported outcome of chronic Achilles tendon ruptures

Aim: To evaluate the economic impact and patient-reported outcome of chronic Achilles tendon ruptures treated with reconstructive surgery.

Methods: This was a cross-sectional cohort study that compared the economic cost between patients treated for chronic Achilles tendon ruptures and patients treated for acute Achilles tendon ruptures at Sahlgrenska University Hospital and Kungsbacka Hospital. A total of 40 patients treated surgically for a chronic Achilles tendon rupture between 2014 and 2018 were included in the study. Economic costs were obtained through medical records and the Swedish Social Insurance Agency. All the patients also evaluated their symptoms pre- and postoperatively using the validated patient-reported outcome score, the ATRS. The data were later compared with the results presented in a previous study performed by Westin et al. [231] on acute Achilles tendon ruptures.

TABLE 6: Summary of statistics on demographic and clinical variables of interest for patients with chronic Achilles tendon ruptures and acute Achilles tendon ruptures. Categorical variables are presented as n (%) and continuous variables as the mean (SD).

Item	Chronic rupture (n = 40)	Surgical acute (n = 43)	Non-surgical acute (n = 50)
Patient sex			
Male	29 (72.5%)	34 (79.1%)	46 (92.0%)
Female	11 (27.5%)	9 (20.9%)	4 (8.0%)
Age	62.7 (13.8)	38.9 (8.7)	39.7 (9.7)
Hospital admission			
Yes	33 (82.5%)	3 (7.0%)	2 (3.8%)
No	7 (17.5%)	40 (93.0%)	51 (96.2%)
Physician visits	4.7 (1.6)	4.79 (1.15)	3.90 (1.84)
Surgery time in minutes	85.6 (20.8)	87.0 (22.8)	0

Results: The results showed that the mean total cost of chronic Achilles tendon ruptures was 6,500 € (Table 7; Figure 17). This was 1,300 € more than the cost of acute ruptures. Chronic Achilles ruptures are consequently more expensive injuries than acute Achilles tendon ruptures. The patients with chronic Achilles ruptures recovered good lower leg function postoperatively, as shown by the increase in the mean ATRS from 16 to 77 points of 100 (Table 8).

TABLE 7: Comparison of the mean (CI 95%; lower-upper) economic cost (EUR) per patient between chronic Achilles tendon ruptures and the surgical and non-surgical treatment of acute Achilles tendon ruptures. The significant difference compared with chronic Achilles tendon ruptures is presented in a bold font.

Variable	Healthcare costs	Production-loss costs	Total costs
Chronic rupture	3,821 (3,580 – 4,061)	2,673 (555 – 4,792)	6,494 (4,413 – 8,576)
Non-surgical treatment of acute rupture	**742 (696 – 787)**	3,730 (2,230 – 5,230)	**4,472 (2,972 – 5,971)**
Surgical treatment of acute rupture	**3,146 (2,986 – 3,306)**	2,853 (1,728 – 3,978)	5,999 (4,862 – 7,135)

TABLE 8: Mean retrospective preoperative and postoperative ATRS (points) in patients with chronic Achilles tendon ruptures. The mean difference between the two variables is also presented.

ATRS	Mean	SD	95% CI (lower–upper)	Range (min–max)
Preoperative	16.2	13.0	12.5 20.9	0.0 – 62.0
Postoperative	73.3	22.8	64.0 79.5	14.0 – 100.0
Difference	57.1	22.5	49.9 64.2	10.0 – 91.0

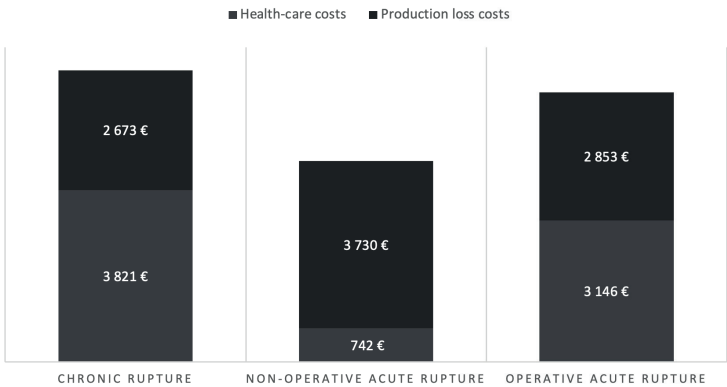

FIGURE 17: Diagram depicting the differences in the healthcare, production-loss and total costs (EUR) of chronic Achilles tendon ruptures and the surgical and non-surgical treatment of acute Achilles tendon ruptures including both working and non-working patients with chronic Achilles tendon ruptures.

The production-loss cost presented here includes both working patients and patients that have retired from work. When working patients (n=22) were analysed exclusively, the mean (SD) production-loss cost was 6,831 € (4,861) instead of 2,673 € (6,625). The total cost of chronic Achilles tendon ruptures is therefore considerably higher when it affects working patients, as shown by Figure 18.

Conclusion: This study showed that chronic Achilles tendon ruptures required considerable extra healthcare costs in comparison with acute Achilles tendon ruptures. These expenses could have been avoided if the ruptures had been detected at an earlier time. On the other hand, there were no statistically significant differences between acute and chronic Achilles tendon ruptures in terms of production-loss costs. The operational costs could therefore be considered economically efficient for patients affected negatively by chronic Achilles tendon ruptures.

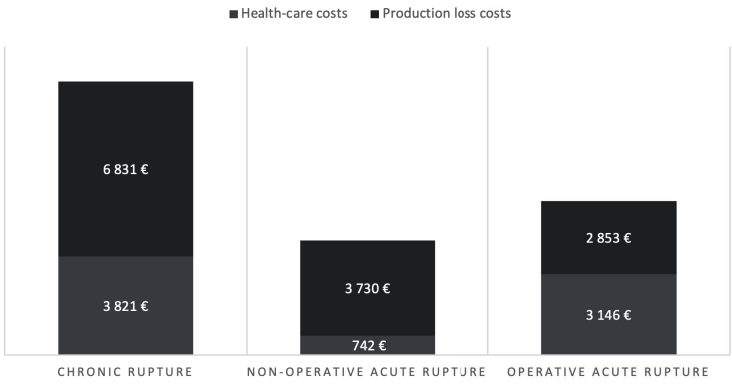

FIGURE 18: Diagram depicting the differences in healthcare, production-loss and total costs (EUR) of chronic Achilles tendon ruptures and the surgical and non-surgical treatment of acute Achilles tendon ruptures including only working patients with chronic Achilles tendon ruptures.

5.2. STUDY II

Patients with chronic Achilles tendon rupture have persistent limitations in patient-reported function and calf muscle function one year after surgical treatment – a case series

Aim: To explore the functional outcomes of patients treated surgically for chronic Achilles tendon ruptures using a gastrocnemius aponeurosis free flap.

Method: This study was a case series of patients treated surgically for chronic Achilles tendon ruptures using a gastrocnemius aponeurosis free flap. A total of 22 patients were included. The patients were evaluated in terms of functional outcomes (hopping, heel-rise work test and the counter-movement jump), clinical measurements (ATRA, dorsiflexion range, calf circumference and tendon length measured by ultrasonography) and patient-reported outcomes (ATRS, PAS and FAOS) at one year postoperatively.

Results: The 22 patients (14 men and 8 women) undergoing surgery due to chronic Achilles tendon ruptures recovered well after surgical reconstruction. They did not, however, recover full lower leg function as shown by patient-reported outcomes (Table 9).

TABLE 9: The mean (SD) and median (range) for patient demographics and the postoperative ATRS, PAS and FAOS among patients with chronic Achilles tendon ruptures. The FAOS is presented in five subscales.

Item	Mean (SD) Median (range)
Age	61 (15) 67 (28; 82) n=22
Patient sex	n=22
Male	14 (63.6%)
Female	8 (36.4%)
ATRS	62 (26) 67 (18; 95) n=22
PAS	3.5 (1.1) 3.0 (1.0; 6.0) n=21
Level 1	4.5%
Level 2	4.5%
Level 3	45.5%
Level 4	22.7%
Level 5	13.6%
Level 6	4.5%
FAOS ***Symptoms and stiffness***	79 (22) 89 (25; 96) n=22
FAOS ***Pain***	86 (22) 96 (14; 100) n=22
FAOS ***Function in daily living***	87 (19) 94 (31; 100) n=22
FAOS ***Sport and Recreation***	62 (31) 65 (5; 100) n=22
FAOS **Quality of Life**	66 (29) 72 (0; 100) n=22

ATRS, Achilles tendon total rupture score; PAS, physical activity scale; FAOS, foot and ankle outcome score.

The performances in the functional tests for the injured versus the uninjured side are shown in Table 10. The injured side showed a poorer functional outcome in the heel-rise test in comparison with the healthy side. The median (IQR) LSI was 84% (23) for repetitions, 68% (29) Joules for work and 79% (21) centimetres for height ($p<0.0001$). No significant differences were found for the counter-movement jump or the hopping height. However, a total of five patients were unable to perform jump tests due to other conditions such as osteoarthritis in the knees or hips. Moreover, two patients were not able to perform a single heel rise on the injured side.

TABLE 10: Performance in functional tests for the injured versus the healthy side among patients with chronic Achilles tendon ruptures one year after the surgical repair. Performances are presented by the median (IQR) and range. The differences between the sides are presented as the median (IQR) LSI, effect size and p-value.

	Injured side	Healthy side	LSI%	Difference between injured and healthy side	
	Median (IQR) Min;max	Median (IQR) Min;max	Median (IQR) Min;max	Effect size	p-value
Heel-rise repetitions	20 (10) 2; 49 n=20	24 (12) 17; 55 n=20	84 (23) 12; 133	0.46*	**0.004**
Heel-rise height (cm)	8 (7) 3; 15 n=20	10 (8) 7; 17 n=20	79 (21) 36; 99	0.62**	**<0.001**
Heel-rise total work (Joule)	872 (1740) 49; 3438 n=20	1590 (2145) 737; 4143 n=20	68 (29) 5; 138	0.59**	**<0.001**
CMJ (cm)	5 (7) 2; 20 n=16	5 (10) 3; 17 n=16	98 (36) 65; 204	0.16	0.365
Hopping (ratio)	0.37 (0) 0.30; 0.53 n=10	0.48 (0) 0.31; 0.62 n=10	90 (19) 66; 97	0.63**	**0.005**

* small effect; ** medium effect; *** large effect

The injured side had a significant tendon elongation compared with the healthy side. The injured tendon was (SD) 1.77 cm (1.98) longer on average than the tendon on healthy side ($p=0.002$). No significant differences were found for the dorsiflexion tests (leg extended/leg flexed). However, the calf circumference was significantly smaller and the ATRA significantly higher on the injured side. The mean (SD) differences were 1.9 cm (1.5) and 7.3 degrees (7.0) respectively ($p<0.001$). The results of the clinical measurements are shown in Table 11.

TABLE 11: Clinical measurements for the injured versus the healthy side among patients with chronic Achilles tendon ruptures one year after surgical repair. All the items are presented as the median (IQR). The differences between the sides are presented by the effect size and the p-value.

	Injured side	Healthy side	Difference between injured and healthy side	
	Median (IQR) Min;max	Median (IQR) Min;max	Effect size	p-value
ATRA (degrees)	55 (3) 50; 76 n=21	50 (9) 40; 69 n=21	0.54**	**<0.001**
Tendon length (cm)	22.4 (2.9) 17.9; 26.1 n=16	20.5 (2.0) 17.0; 26.1 n=16	0.48*	**0.006**
Dorsiflexion extended knee (degrees)	32 (12) 17; 40 n=20	30 (12) 21; 41 n=20	0.17	0.279
Dorsiflexion flexed knee (degrees)	32 (14) 18; 48 n=20	35 (17) 17; 44 n=20	0.12	0.448
Calf circumference (cm)	37 (4) 30; 47 n=21	38 (4) 33; 46 n=21	0.52**	**0.001**

* Small effect and ** medium effect

Conclusion: The results of this study showed that patients with chronic Achilles tendon ruptures performed less well on the injured side versus the uninjured side despite surgical repair and one year of rehabilitation. At the same time, the patient-reported outcomes were low regarding the FAOS, the ATRS and the PAS. These facts imply that patients with chronic Achilles tendon ruptures are still significantly affected one year after surgery.

5.3. STUDY III

Endoscopically assisted repair of chronic Achilles tendon ruptures and re-ruptures using a semitendinosus autograft is a viable alternative to pre-existing techniques

Aim: To describe a surgical technique for the treatment of chronic Achilles tendon ruptures and re-ruptures using a semitendinosus autograft. The study also aimed to present the functional and patient-reported outcomes of the technique.

Method: This was a case series of 22 patients treated surgically for chronic Achilles tendon ruptures with an endoscopically assisted semitendinosus graft. The main outcomes of the study were the ATRS, ATRA, tendon length, heel-rise height and heel-rise endurance, all evaluated one year postoperatively.

Results: Twenty-two patients (9 women and 13 men) underwent reconstruction using the endoscopically assisted semitendinosus autograft technique at Sahlgrenska University Hospital, Gothenburg or Princess Royal Hospital, Shrewsbury and Telford Hospital NHS Trust. The median age was 60 years (range 34-73) and the median time to surgery was 4.5 months (range 1.5-9.0). The demographics for the total group are presented in Table 12.

TABLE 12: Demographics for Swedish and British patients, including type of rupture and treatment delay in months.

	Mean (SD) Median (range)
Age	60 (12) 64 (34; 73) n=22
Patient sex	n=22
Male	13 (59%)
Female	9 (41%)
Patient nationality	n=22
Swedish	8 (36.4%)
British	14 (63.6%)
Delay in treatment (months)	11.1 (28.7) 5.0 (1.5; 139) n=22

The patients reported a median ATRS score of 76 (range 45-99) at the 12-month follow-up (Table 13). A total of 18 patients were able to perform a single-leg heel rise on the healthy side and 16 (89%) were also able to perform heel rises on the injured side. The median height and number of repetitions were significantly lower on the injured side, as shown in Table 14.

TABLE 13: Postoperative ATRS at 12 months for all the patients included in the study.

	Median (IQR)	Range	Number
ATRS	76 (35.5)	45; 99	n=22

TABLE 14: Postoperative clinical measurements relating to the ATRA, heel rises, calf circumference and tendon length at 12 months. Tendon length measurements with ultrasound were only made in patients evaluated in Sweden.

	Injured side	Healthy side	LSI%	Difference injured vs healthy side
	Median (IQR) Min;max	Median (IQR) Min;max	Median Min;max	p-value
ATRA (degrees)	60 (15) 49; 75 n=20	49.5 (6) 40; 61 n=20	83 69; 96	***<0.001***
Heel-rise height (cm)	5.5 (5.75) 1.0; 11.0 n=17	9.0 (2.75) 5.0; 11.5 n=17	61 20; 100	***<0.001***
Heel-rise reps	11 (18) 2.0; 22.0 n=11	26 (14) 2.5; 27 n=11	42.3 7.6; 81.5	***<0.001***
Calf circumference (cm)	37.5 (6) 30; 46 n=20	39 (6.4) 30.0; 50.0 n=20	96 87; 100	***<0.001***
Tendon length (cm)	24.8 (6) 20; 28.2 n=6	22 (5.2) 18.4; 24.2 n=6	91 86; 92 n=6	***<0.001***

In Sweden, additional tests including the concentric power test and heel-rise work were performed. Six patients were able to complete the concentric power test with a median LSI of 70 (range 21.8-103.3). The heel-rise work test was performed by seven patients with a median LSI total work of 25.6 (range 3.9-60.8) and a median LSI of repetitions of 53.1 (range 7.7-81.5). These results are presented in Table 15.

TABLE 15: Functional tests using MuscleLab® and ultrasonography in Sweden

	Injured side	Healthy side	LSI%	Difference between injured and healthy side
	Median (IQR) Min;max	Median (IQR) Min;max	Median Min;max	p-value
Concentric power (W)	7 (223) 0; 317 n=7	224 (281) 10; 945 n=7	70 0; 99	***0.004***
Heel-rise total work (J)	65 (412) 0; 962 n=7	1325 (1220) 227; 1650 n=7	3.9 0; 60.8	***<0.001***

Conclusion: The main finding in this study was that the endoscopically assisted reconstruction of chronic ATRs and re-ruptures with a semitendinosus graft is a viable alternative to other surgical techniques, with positive early postoperative functional results and patient-reported outcomes at the 12-month follow-up. This minimally invasive incision technique may be more suitable for patients with a pre-existing risk of wound complications.

5.4. STUDY IV

Both gastrocnemius aponeurosis flaps and semitendinosus tendon grafts are effective in the treatment of chronic Achilles tendon ruptures – a systematic review.

Aim: To explore the advantages and limitations of surgical techniques using gastrocnemius aponeurosis flaps and semitendinosus grafts to treat patients with chronic Achilles tendon ruptures through a systematic review.

Methods: A systematic search for the reconstructive treatment of chronic Achilles tendon ruptures was performed in June 2021, with a second updated search in September 2022. All the relevant publications were independently reviewed by two to three authors. The identified articles were reviewed using a multistage assessment. All the validated and non-validated outcome measurements were regarded as inclusion criteria. Data extraction and quality analysis were conducted in groups. The parameters extracted from the articles were the number of patients, mean age, follow-up time, outcome measurements, outcome result and complications. Additionally, the first eight questions in the MINORS quality assessment were used to evaluate the quality and risk of bias for each study.

Results: After the full-text filtering, 86 studies remained and were included in the systematic review. Of these, 36 studies used gastrocnemius aponeurosis flaps or semitendinosus tendon grafts to treat chronic Achilles tendon ruptures. The excluded studies included 22 presenting postoperative outcomes after reconstruction using a flexor hallucis longus (FHL) graft [2, 4, 6, 17, 41, 52, 94, 110, 137, 140, 145, 170, 174, 181, 184, 188, 212, 222, 227, 235, 239], two using a gracilis tendon graft [123, 131], one using an Achilles tendon allograft [166], eight using a peroneus brevis tendon graft [30, 77, 102, 127, 129, 130, 175], one using a plantaris tendon graft [236], eight using direct repair [19, 26, 79, 103, 117, 151, 179, 234], two using the yurt bone technique [13, 83], one using polyester tape [81] and one using Hyalonect [54].

All the included studies were case series that used either a gastrocnemius aponeurosis flap or a semitendinosus graft to treat the chronic Achilles tendon rupture. Thirteen studies used semitendinosus grafts and 21 studies used gastrocnemius flaps as the management option. There were two articles that used both management options and a combined technique.

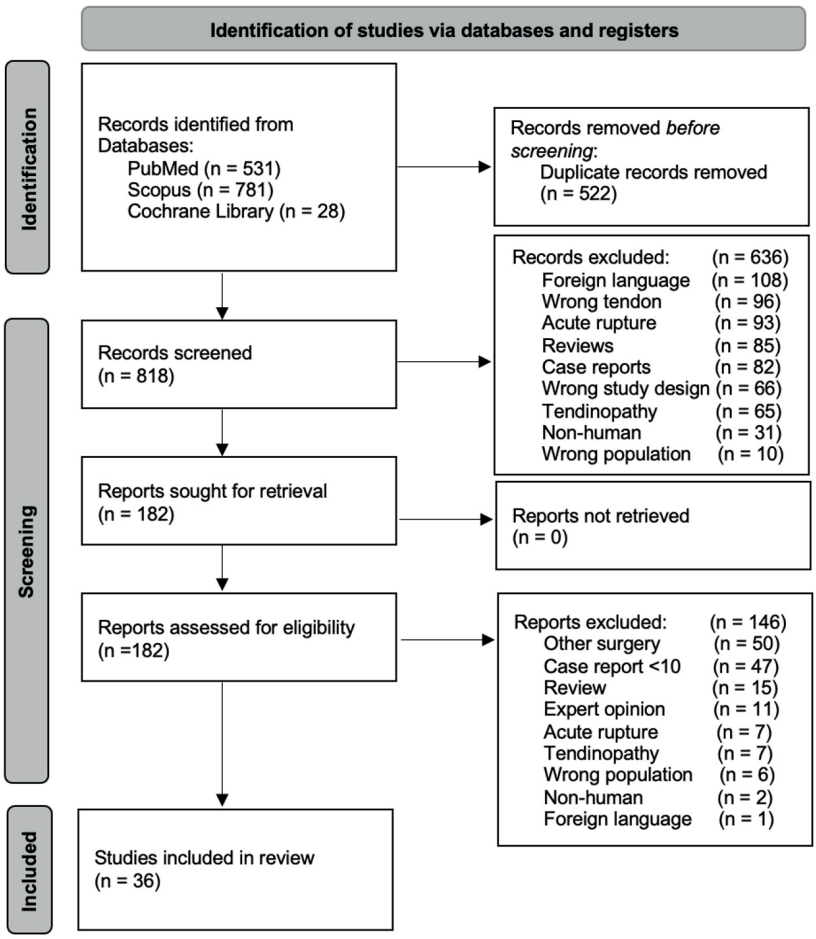

FIGURE 19: The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart for the inclusion of studies, the PRISMA 2020 statement [171]

The mean age of patients treated with a gastrocnemius aponeurosis flap was 44.5 years, while it was 44.0 years for patients treated with a semitendinosus graft. The patients were followed up for a mean time of 40 months and 28 months respectively. The mean (SD; n) postoperative ATRS for patients treated with a gastrocnemius aponeurosis flap was 83 points (14; 6 studies) and the mean AOFAS was 96 points (1.7; 12 studies). The patients treated with a semitendinosus graft achieved similar scores on the ATRS 88 (6.9; 7 studies) and AOFAS 92 (5.6; 9 studies). However, it is important to remember that patients treated with a semitendinosus graft had lower preoperative values compared with patients treated with a gastrocnemius aponeurosis flap.

A comparison of the complications between gastrocnemius aponeurosis flaps and semitendinosus grafts can be found in Table 16.

TABLE 16: Overview of the complications for semitendinosus tendon grafts (ST grafts) and gastrocnemius flaps (G flaps) presented as the number of complications and the percentage of the total number of patients.

Overview of the complications			
Complications	**ST grafts n (%)**	**G flaps n (%)**	**Total n (%)**
Wound infection	5 (1.7)	22 (4.6)	27 (3.5)
Delayed wound healing	2 (0.7)	16 (3.3)	18 (2.4)
Deep vein thrombosis	1 (0.3)	6 (1.3)	7 (0.9)
Wound dehiscence	1 (0.3)	6 (1.3)	7 (0.9)
Deep infection	-	5 (1.0)	5 (0.7)
Persistent pain from the operation wound	5 (1.7)	-	5 (0.7)
Hypertrophic scar	-	3 (0.6)	3 (0.4)
Weakness from the donor site	2 (0.7)	-	2 (0.3)
Sural nerve injury	4 (1.3)	-	4 (0.5)
Re-rupture	-	1 (0.2)	1 (0.1)
Persistent pain from the donor site	1 (0.3)	-	1 (0.1)
Suture abscess	-	1 (0.2)	1 (0.1)
Sural nerve hypoesthesia	-	1 (0.2)	1 (0.1)
Aseptic superficial skin necrosis	-	1 (0.2)	1 (0.1)
Septic partial tendon necrosis	-	1 (0.2)	1 (0.1)

The MINOR scores in the included studies are shown in Table 17. The maximum points are 16 for non-randomised articles. The included studies had a high risk of bias according to MINORS, with a median of 8 of 16 (range 2-13).

TABLE 17: MINORS scale score for each of the included studies ranked by their total score.

Authors and publication year	MINORS scale score
Nilsson Helander et al. 2008	13
Maffulli et al. 2014	12
Sadek et al. 2015	11
Sarzaeem et al. 2012	11
Nordenholm, Senorski et al. 2022	11
El Shazly et al. 2014	10
Maffulli et al. 2018	10
Bansal et al. 2021	10
Jain et al. 2020	9
Pavan Kumar et al. 2013	9
Lins et al. 2013	9
Song et al. 2018	9
Dumbre et al. 2014	9
Lin et al. 2019	9
Nilsson et al. 2022	9
Nordenholm, Nilsson et al. 2022	9
Elgohary et al. 2016	8
Kaul et al. 2020	8
Khiami et al. 2013	8
Bąkowski et al. 2020	8
El Shewy et al. 2009	8
Raju et al. 2022	8
Massoud 2017	7
Seker et al. 2016	7
Ozan et al. 2017	7
Li et al. 2021	7
Takao et al. 2003	7
Tsukada et al. 2022	7
Koh et al. 2019	6
Bai et al. 2019	6
Muliera et al. 2003	6
Gunaratne et al. 2021	6
Guclu et al. 2016	5
Gedam et al. 2016	4
Werken et al. 1983	2

Conclusion: Surgical reconstruction using either semitendinosus tendon grafts or gastrocnemius aponeurosis flaps is considered to be effective in treating chronic Achilles tendon ruptures, with good patient-reported outcomes and few re-ruptures. The complication profiles are different, with patients treated with a gastrocnemius aponeurosis flap being more prone to postoperative infections and wound complications, whereas patients treated with a semitendinosus graft have more sural nerve injuries.

5.5. STUDY V

Delayed Treatment of Achilles Tendon Ruptures Results in a Significant Alteration in the Gene Expression of COL1A1, MMPs, TIMPs and IL-6

Aim: To explore both how the delayed treatment of Achilles tendon ruptures affects the biomolecular tendon structure and the differences in gene expression between the proximal and distal tendon ends.

Method: Thirty-five patients that were treated surgically for an acute or chronic Achilles tendon rupture at Sahlgrenska University Hospital (Gothenburg, Sweden) between December 2020 and January 2022 were included in the study. Each donated two 2 x 2 x 2 mm tissue samples at the rupture site during surgical repair; one sample from the distal tendon end and another from the proximal tendon end. RNA samples were analysed using real-time polymerase chain reaction (RT-PCR).

Results: After the exclusion of low RIN values, the patients with chronic Achilles tendon ruptures were divided into a short-term chronic group (1-6 months) and a long-term chronic group (>6 months). Thirteen of the 27 patients had an acute Achilles tendon rupture, six had a short-term chronic Achilles tendon rupture and eight had a long-term chronic Achilles tendon rupture (Table 18).

TABLE 18: Demographics of patients that had been treated surgically for an acute (0-28 days) or chronic Achilles tendon rupture (>28 days) at Sahlgrenska University Hospital (Gothenburg, Sweden) between December 2020 and January 2022, from which Achilles tendon tissue samples were collected for this study.

	Acute rupture (n=13)	Short-term chronic rupture (n=6)	Long-term chronic rupture (n=8)
Age, yrs Median (range)	46 (26–72)	49 (24–74)	59 (51–75)
Sex, n (%)			
Male	9 (69%)	4 (67%)	4 (50%)
Female	4 (31%)	2 (33%)	4 (50%)
Delay in treatment in days Median (range)	6 (4–11)	91.5 (30–183)	275 (214–1128)

*Categorical variables are presented as n (%) and continuous variables as the mean (SD) and median (range).

The mRNA levels of COL1A1 and COL3A1 were significantly higher in the short-term chronic rupture group compared with the acute rupture group ($p=0.02$; $p=0.04$). MMP-1 and MMP-13 had the highest mRNA levels in the acute phase compared with MMP-2 and MMP-9 that had the highest levels in the short-term chronic group. Generally, the level of activity was significantly lower in the long-term chronic group compared with the acute group for all MMPs ($p<0.05$ for all). TIMP-1 had the highest levels in the acute rupture group and were significantly lower in the short-term chronic group ($p=0.02$). The pattern for TIMP-1 diverged compared with the other TIMPs, as other TIMPs peaked in the late chronic group.

IL-1, IL-6 and TNF-a all had the highest mRNA levels in the acute group and were significantly lower in overall terms in the short-term chronic and long-term chronic groups. Both CD163 and MCEMP1 were highest in the acute rupture group, with a significant difference compared with both the short-term chronic and long-term chronic group. CD163 is a marker of monocytes/macrophages, while MCEMP1 is a marker of mast cells. MRC1, macrophage marker, was the only gene without any difference between the groups ($p=0.9$).

No significant differences in the comparison between the proximal and distal tendon ends could be found for either acute or chronic Achilles tendon rupture in terms of collagens, MMPs and TIMPs. The relationship between the tendon ends showed almost no difference. The only gene with a significant difference between the tendon ends was CD163 that had a significantly lower expression at the distal tendon end compared with the proximal end ($p<0.001$).

Conclusion: The mRNA levels of matrix metalloproteinases (MMPs) and inflammatory markers are significantly lower in Achilles tendon ruptures with delayed treatment. This could be a plausible explanation of why chronic Achilles tendon ruptures require surgical repair with reinforcements to regain ankle function and avoid postoperative tendon elongation. There is no indication of differences in gene expression between the proximal and distal tendon ends except for CD163. Although there is a temporal variation in mRNA levels, this does not explain the full complexity of Achilles tendon ruptures.

06

DISCUSSION

General discussion

The aim of this thesis was to summarise and discuss various aspects of chronic Achilles tendon ruptures and the way the injury affects patients on different levels. The main findings in this thesis show that there are good treatment alternatives for chronic Achilles tendon ruptures with satisfactory results regarding functional outcomes (Studies II, III and IV) and patient-reported outcomes (Studies I, II, III and IV) at a reasonable cost (Study I). The main difficulty with chronic Achilles tendon ruptures appears to be the general elongation of the tendon and associated muscle hypotrophy. Study V was able to detect significant differences in the tendon in terms of genes related to degradation when acute ruptures were compared with short-term chronic and long-term chronic ruptures. This suggests possible early tissue/matrix degradation and indicates that the treatment of chronic Achilles tendon ruptures should not be delayed when surgical treatment is necessary.

The main difficulties in the research on chronic Achilles tendon ruptures are the limited number of patients, the age gap towards acute ruptures, with older patients more commonly affected, and the multiple surgical techniques that exist in the literature. Another challenging aspect is that outcome measurements of Achilles tendon ruptures differ between hospitals/institutions and countries. The median age of patients in this thesis is higher than that in other studies reporting the outcome of the surgical management of chronic Achilles tendon ruptures. One explanation for this might be that other hospitals/institutions may feel that the risks of surgical intervention outweigh the perceived benefits. This thesis shows that patient symptoms and function can be improved following reconstructive surgery.

Study I

The most important finding in this study was that the treatment of chronic Achilles tendon ruptures is more expensive and potentially has a poorer functional outcome than the treatment of acute Achilles tendon ruptures. The

healthcare costs of chronic Achilles tendon ruptures were higher than those of acute Achilles tendon ruptures, regardless of whether the acute ruptures were treated surgically or non-surgically. The main reason was the more complicated surgical technique required to treat chronic Achilles tendon ruptures. The technically more difficult procedure results in longer surgery times and higher costs. A longer surgery time can also result in more surgically related complications and thereby additional healthcare costs.

The central problem with explorations of production-loss costs is the high level of individuality that is often presented by patients. For instance, patients with physically demanding jobs tend to have a longer absence from work than patients with more sedentary professions. In this study, the demographic mean age of patients with chronic Achilles tendon ruptures was considerably higher than that of patients with acute Achilles tendon ruptures. As a result, more patients had retired and had no income to lose during the rehabilitation period postoperatively. This meant that the mean production-loss cost was lower, which resulted in a lower total cost for these ruptures. This was why a separate analysis was performed solely on working patients, which produced substantially higher total costs. Additionally, another limitation of the study was that the preoperative and postoperative ATRS were filled out retrospectively at the same time. The risk of recall bias was thereby high.

To our knowledge, no previous studies have evaluated the economic impact of chronic Achilles tendon ruptures. However, preceding this study, a few health-economic studies have evaluated whether surgical or non-surgical treatment is economically favourable when treating acute Achilles tendon ruptures. Truntzer et al. [217] reported that the non-surgical treatment of acute Achilles tendon ruptures resulted in significantly lower costs than surgical treatment when healthcare costs were analysed exclusively. Westin et al. [231] found that surgical treatment is more expensive, but that it could be considered equally cost effective if there is a willingness to pay 50,000 €/QALY. Moreover, Carmont et al. [29] found that the percutaneous repair of the Achilles tendon is a cost-effective alternative to open repair.

The present study showed that chronic Achilles tendon ruptures required considerable extra healthcare costs in comparison with acute Achilles tendon ruptures. These expenses could have been avoided if the ruptures had been detected at an earlier stage. On the other hand, there were no statistically significant differences between chronic and acute Achilles tendon ruptures in

terms of production-loss costs. The health-economic costs introduced in this study are based on the Swedish healthcare system and may not be transferable to other countries and healthcare structures. The limited number of included patients may also suggest that the study population does not represent the overall population. Nevertheless, the drop-out analysis revealed no obvious demographic differences between the two presented groups. Moreover, due to the limited number of females included in the study, no comparison between the sexes was possible. There was, however, a larger proportion of females in the group with chronic Achilles tendon ruptures than in the group with acute Achilles tendon ruptures.

The strength of the study is that it analyses the economic impact and functional outcome of chronic Achilles tendon ruptures exclusively. To our knowledge, this has never been done before and the condition is relatively unexplored. Hopefully, this study will result in a deeper understanding of chronic Achilles tendon ruptures and their economic impact on the healthcare system, patients and society. By analysing the economic cost, the patient-reported outcome and the functional outcome of chronic Achilles tendon ruptures, the healthcare system may be able to direct reasonable resources to find these ruptures at an earlier stage and develop new and more cost-effective treatment methods.

Study II

The results of this study showed that patients with chronic Achilles tendon ruptures treated with a gastrocnemius free flap performed less well on the injured side than the uninjured side despite surgical repair and one year of rehabilitation. At the same time, the patient-reported outcome scores were low in terms of the FAOS, the ATRS and the PAS. These facts imply that patients with chronic Achilles tendon ruptures are still significantly affected one year after surgery.

When compared with the functional outcomes of acute Achilles tendon ruptures presented in the randomised controlled studies by Olsson et al. [168] and Nilsson-Helander et al., [157] the functional result could be regarded as inferior. The largest differences between chronic Achilles tendon ruptures and acute Achilles tendon ruptures were shown in heel-rise repetitions and heel-rise work. The remaining problem with comparisons between chronic Achilles tendon ruptures and acute Achilles tendon ruptures is the large age difference that exists between the two groups. However, when considering the LSI percentage, which presents the ratio between the injured and the healthy side, this will be partially compensated for.

The overall results of muscle function tests indicate that jumping performance is recovered to a greater extent than heel-rise capacity. However, it should be emphasised that only 50% of the patients that participated in the heel-rise test (20 patients) performed the jump tests (10 patients), which may have resulted in misleading results. Those who chose to abstain from the jumping tests stated that they were not comfortable with jumping because they were not used to that type of activity or because of other lower extremity conditions that posed problems, such as knee osteoarthritis. In contrast to the present study, Nilsson-Helander et al. [158] found no differences between chronic Achilles tendon rupture and re-rupture patients in terms of muscle function or age (mean 46 years, range 26-71). The younger and more age-homogeneous cohort in their study may be one reason for their results being better in terms of muscle function and more equal between chronic Achilles tendon rupture and re-rupture groups.

A more recent study performed by Westin et al. [229] showed that patients affected by re-ruptures performed better on functional tests but reported an inferior patient-reported outcome (ATRS) than patients who sustained an acute Achilles tendon rupture. The main reason that was suggested was that patients with re-ruptures had a more severe injury with a greater psychological impact and a longer rehabilitation time. A similar psychological impact could be present for chronic Achilles tendon ruptures, as shown by a qualitative interview study performed by our research group. [161] In that study, the experiences of ten patients with a mean (SD) age of 65 (14) years were summarised in four main categories: (1) “The injury”, where the patients described immediate functional impairments, (2) “The diagnosis”, where the patients expressed relief about receiving the diagnosis, (3) “The treatment”, where the patients expressed high expectations and consistent satisfaction, and (4) “The outcomes”, where the patients expressed overall satisfaction with the long-term outcome and no obvious limitations in physical activity.

Moreover, large variations were present in the existing functional result for chronic Achilles tendon ruptures. This reveals that some patients recovered well, whereas others were afflicted for a long time after surgical repair. The wide spectrum of outcome is not unique to chronic Achilles tendon ruptures, but it is also present among acute Achilles tendon ruptures and Achilles tendon re-ruptures. The way the patient performance on functional tests corresponds to general well-being needs to be further explored.

The limitation of this study is the low number of patients. However, the incidence of chronic Achilles tendon rupture is relatively low and it is therefore difficult to include many patients in the studies. On the other hand, the strengths of the study are that, to date, it is the first study to use a comprehensive battery of validated outcome measurements and include patients treated with the same surgical technique and rehabilitation protocol.

In this study, as well as in Study III, the patients were evaluated one year postoperatively. In a previous study by Brorsson et al., [23] it was shown that patients treated for acute Achilles tendon ruptures improved significantly in terms of heel-rise height between the one-year and the two-year follow-ups. This may imply that the patients presented in Studies II and III would perform better in functional tests and report superior patient-reported outcomes if they were evaluated later in the rehabilitation process. In a subsequent seven-year follow-up of the same study cohort, no further significant improvements could be found. [23]

Study III

The study shows that the endoscopically assisted reconstruction of chronic Achilles tendon ruptures using a semitendinosus autograft is a valuable alternative to pre-existing techniques. The patient-reported outcome measurements and clinical evaluation show a satisfactory outcome. With endoscopic assistance, it was possible to minimise the size of the surgical incision and thereby reduce the risk of postoperative wound infections. In the literature, acute Achilles tendon ruptures treated with a minimally invasive technique have fewer wound healing complications. [46]

In a previously mentioned study by Nilsson-Helander et al., [158] an open reconstruction using a free gastrocnemius flap was performed and evaluated at the same hospital. The patients in that study reported a median ATRS of 83 points, 29 months postoperatively, compared with 74 points in the present study, 12 months postoperatively. The group in the previous study also performed better in functional tests, with higher concentric power and heel-rise work compared with the present study. Direct comparisons are, however, difficult to make between the two techniques due to different indications in terms of rupture site, rupture gap and patient characteristics. For example, the mean (SD) patient age in that study was 46 (10.8) compared with 60 (12) in the present study. The ATRS and functional results may also have been higher in the present study, with a longer follow-up time, as seen in previous studies of acute Achilles tendon ruptures. [167]

In Study II, the same surgical technique was used but with a slight alteration to the rehabilitation protocol. In that study, the ATRS one year postoperatively was 67 points, with functional outcomes and clinical measurements similar to those in the present study. This study had a more similar study cohort and the same follow-up period. For this reason, gastrocnemius aponeurosis free flaps and semitendinosus autografts can be regarded as equally effective in regaining push-off strength after a chronic Achilles tendon rupture.

In the literature, many other tendon grafts have been described in the repair of chronic Achilles tendon rupture and re-ruptures. Numerous grafts involve the local tendon transfer of the flexor hallucis longus, peroneus brevis or plantaris tendon. The transfer of these local grafts has the potential to affect a patient's balance and gait through weakening of the foot. [9, 67, 176] The semitendinosus and other distant grafts therefore have the advantage of not interfering with the foot and ankle. Another advantage of this technique is that the graft is attached directly to the calcaneus using an interference screw and not to the distal tendon end. This allows for the treatment of insertional ruptures and enables the bridging of large tendon defects.

Other techniques using a semitendinosus graft have previously been described in the literature. [82, 122, 126, 191, 204, 220] Those studies reported ATRS scores of between 86 and 99 points. This is superior to this study, but the mean age was generally lower and the follow-up period longer. Olsson et al. [167] showed that, for acute Achilles tendon ruptures, the ATRS increased between the 12-month follow-up and the 24-month follow-up, albeit not significantly for the surgically treated group. [167] As the surgical treatment for chronic Achilles tendon rupture is often more complicated than the treatment of acute Achilles tendon rupture, it is possible that the rehabilitation might also be more complicated and take longer.

In a previously mentioned study by Carmont et al., [29] it was also shown that the percutaneous repair of acute Achilles tendon ruptures could be an economically preferable alternative to open repair. This could also be the case for chronic Achilles tendon ruptures. However, this needs to be further assessed before any conclusions can be drawn.

Study IV

This systematic review has shown that both gastrocnemius aponeurosis flaps and semitendinosus grafts are effective in treating chronic Achilles tendon

ruptures, with similar patient-reported outcome scores, clinical measurements and functional outcomes. This corresponds well to the findings in Studies II and III, as well as previous systematic reviews [9, 66] where similar results were reported. The systematic review did, however, also show that studies of higher quality are needed fully to determine the superior treatment. All the included studies were case series without any matched control groups (Level IV). The studies were also heterogeneous in terms of outcome measurements, follow-up period, specific surgical technique and patient characteristics. This limited the comparisons between studies and prevented a quantitative meta-analysis from being performed.

The comparison of complications shows that patients treated with a gastrocnemius aponeurosis flap have a higher incidence of infections and wound-healing problems, while patients treated with a semitendinosus graft are more prone to sural nerve injuries. However, due to the large-scale heterogeneity between studies, no significant differences could be determined.

The MINORS assessment generally resulted in low quality with a median score averaging eight of 16 points. Moreover, until recently, many studies have used the non-validated AOFAS as the main PROM. A validated and more specific PROM such as the ATRS is therefore generally recommended.

Following this systematic review, the authors' recommendation is to individualise the treatment of chronic Achilles tendon ruptures, depending on factors such as functional demands, patient characteristics, the size of the tendon gap and the general experience of the orthopaedic surgeon treating the patient. The use of a gastrocnemius aponeurosis flap [51, 158] in tendon ruptures with a gap that is less than 5 cm is regarded as effective. In tendon ruptures with larger defects (>5cm), a semitendinosus tendon graft is needed to bridge the defect. [124, 178] This is in accordance with Studies II and III in this thesis.

Study V

The main finding in this study was that a prolonged delay in the treatment of Achilles tendon ruptures results in reduced mRNA levels of matrix metalloproteinases (MMPs) and inflammatory markers compared with acute Achilles tendon ruptures. The lower levels of these genes in chronic Achilles tendon ruptures demonstrate that the inflammatory response and the activity of MMPs are more short lived in Achilles tendons post-rupture compared with other regenerative and degenerative processes.

These temporal changes in gene expression could imply that patients with chronic ruptures have a reduced ability to regenerate new tissue within the extracellular matrix (ECM) of the tendon. This could be a plausible explanation of why chronic Achilles tendon ruptures require advanced tendon reconstruction using reinforcing tendon grafts to avoid tendon elongation and re-rupture. [1, 16, 57, 105, 109] However, the way the surgical repair affects these tendons is unknown. Study II showed that chronic Achilles tendon ruptures elongate approximately 2 cm, despite having been reinforced using a gastrocnemius aponeurosis flap, [158] compared with approximately 1.5 cm in patients with surgically treated acute Achilles tendon ruptures. [11] For acute Achilles tendon ruptures, no significant differences in elongation have been found between surgical and non-surgical treatment in multiple studies. [24, 192, 193] One exception is Heikkinen et al. [69] who showed at the 18-month follow-up that the tendon was 19 mm longer in the group of patients treated non-surgically. In previous studies, tendon elongation has been shown to affect gait pattern and the ability to perform heel rises. [3, 213]

Robertson et al. [185] have previously shown that the increased expression of MMP-1 and MMP-9 correlated with the failure of the supraspinatus tendon to heal. How this expression correlates to clinical outcomes in Achilles tendon ruptures is still unknown. Similar changes in gene expression have been shown for acute Achilles tendon ruptures and chronic Achilles tendinopathy. [182] The main strength of this study is that it evaluates tendon changes in later phases post-rupture. The number of patients was also high compared with previous studies.

07

LIMITATIONS

Patient-reported outcomes (PROMs) (Studies I, II, III and IV) have been a large part of the results in all the presented studies. The ATRS has been used in Studies I, II, III and IV. Other scales, such as the AOFAS, PAS, Leppilahti and FAOS, have been used in Studies II and IV. The only score that has been validated for Achilles tendon ruptures is the ATRS. However, it has not been validated for chronic Achilles tendon ruptures. To our knowledge, no PROM has been validated for these ruptures. Another problem with chronic Achilles tendon ruptures is that many of the scores listed above have been developed to evaluate functional impairments in young or middle-aged athletes. In an elderly population, it is generally thought to be better to use more general PROMs, such as the EQ-5D [48] and SF-36, [116] due to comorbidities and lower functional demands in this population. The reason why the ATRS and other scores have been used in these studies is to be able to refer to the impairments patients suffer. Previous literature has consequently used these scores to evaluate both chronic Achilles tendon ruptures and acute Achilles tendon ruptures. Another problem with these PROMs is that a floor/ceiling effect is unavoidable. This is especially present in a group of patients such as the elderly, who have been operated on. The ATRS has been reviewed with regard to this in a study performed by Kearney et al. [92] They showed that the ATRS only had a ceiling effect in the preoperative group and not postoperatively, where it has been used in the presented studies.

Preoperative data are missing (Studies I, II, III and IV) in all the studies for practical reasons. This is a strong limitation in all the studies. However, comparing preoperative and postoperative data can imply a great deal of bias in PROMs. The functional tests are therefore a preferable option. Missing preoperative data and a retrospective study design were also some of the main limitations with several studies in the systematic review.

Age differences (Studies I, II, III, IV and V) make it difficult to compare chronic Achilles tendon ruptures and acute Achilles tendon ruptures. The median age of patients suffering acute Achilles tendon ruptures is 40-50 years.

Meanwhile, the median age of patients in the presented studies was 60-62 years. This obviously makes results difficult to compare for clinical relevance. Many of the patients were not able to perform heel rises or jumps due to other musculoskeletal conditions, such as osteoarthritis in the knees and hips, or chronic back pain. In a study by Westin et al., [230] it was shown that older patients reported poorer function than younger patients one year postoperatively.

The limited number of patients (Studies I, II and III) in all the studies affects the generalisability of the studies and the thesis. It is, however, difficult to conduct studies on a larger scale with a population as small as the one comprising patients affected by chronic Achilles tendon ruptures.

No matched control group (Studies I, II, III and V) was used in any of the five presented original studies or in any of the presented studies from the systematic review. The reason for this is the small cohort sizes, as well as the strongly individualised treatment plan for all the affected individuals. In the future, control groups would be needed if the field wishes to reach any consensus on which treatment strategy is preferable and when.

Exclusion criteria, low quality and heterogeneity in the systematic review (Study IV). The heterogeneity in the systematic review made comparisons between articles difficult and necessitated a more descriptive analysis of the results. Another limitation was the poor quality of the included studies. The included studies had MINORS with a median of 8 (range 2-13). Most of the studies on the subject were retrospective case series with a high risk of bias. The exclusion criteria could have affected the results. This study excluded all non-English studies and all studies with fewer than 10 patients. This exclusion removed 101 studies because of language and 47 studies for their small size. It is possible to argue that these 148 studies could produce a broader, more nuanced result. However, of the 47 studies excluded for their small size, most were case reports reporting only one or two patients. Their inclusion could make the results even more heterogeneous and make comparisons more difficult. Additionally, the 101 studies that were excluded because of language are studies none of the authors could have read, making result extraction difficult.

Low RNA quality and no histopathological method or immune-histochemistry (Study V). The limitation was that human tendon samples are difficult to manage logistically, which can have a negative impact on subsequent RNA

concentration and integrity following RNA extraction. This can be exemplified by the small number of acceptable RIN values reported in the present study. In order to fully determine the differences in tendon structure between the two tendon ends, it is, nevertheless, also important to compare them using histopathological methods and immunohistochemistry. Before this is possible, RNA sequencing or RNA microarray should be performed to determine the gene signature present in acute and chronic Achilles tendon ruptures.

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CONCLUSIONS

The treatment of chronic Achilles tendon ruptures implies high, yet reasonable costs, acceptable functional outcomes with multiple surgical techniques and satisfactory patient experience. The chronic Achilles tendon ruptures also show significantly more degradation and lower gene activity when compared molecularly with acute Achilles tendon ruptures using the RT-PCR of mRNA levels. In spite of this, the results of this thesis show good clinical outcomes and patient satisfaction following chronic Achilles tendon ruptures. However, none of the surgical techniques presented was shown to be superior to another. These chronic Achilles tendon ruptures need to be further explored with larger cohort sizes to draw wider and more clinically relevant conclusions for the future.

Study I: This study showed that chronic Achilles tendon ruptures impose considerable extra healthcare costs in comparison with acute Achilles tendon ruptures. These expenses could have been avoided if the ruptures had been detected at an earlier time. The patients did, however, improve significantly in terms of the ATRS and the treatment can therefore be considered to be cost effective.

Study II: The results of this study showed that patients with chronic Achilles tendon ruptures performed less well on the injured side versus the healthy side despite surgical repair using a free gastrocnemius aponeurosis flap and one year of rehabilitation.

Study III: The main finding in this study was that the endoscopically assisted reconstruction of chronic Achilles tendon ruptures and re-ruptures using a semitendinosus graft is a viable alternative to pre-existing surgical techniques. The patients did, however, still have persistent limitations similar to those presented in Study II.

Study IV: Surgical reconstruction using either semitendinosus tendon grafts or gastrocnemius aponeurosis flaps is regarded as effective in treating chronic

Achilles tendon ruptures with good patient-reported outcomes and few re-ruptures. There is a continued need for more prospective controlled trials using established outcome measurements, tested for validity and reliability.

Study V: The expression of matrix metalloproteinases (MMPs) and inflammatory markers is predominantly high after an acute Achilles tendon rupture and lower with delayed treatment. Chronic Achilles tendon ruptures are therefore subject not only to elongation but also to changes in gene expression with potentially more loose tissue with poorer suture hold. This is important to consider when developing new surgical reinforcement techniques, as well as rehabilitation programmes.

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FUTURE PERSPECTIVES

The remaining challenge with chronic Achilles tendon ruptures is to assemble a large number of patients in studies of a higher quality. All the studies included in this thesis, as well as studies identified in the systematic review, only included 20-30 patients with Level IV evidence. More national or international registers for Achilles tendon ruptures and chronic Achilles tendon ruptures are needed to increase our knowledge and understanding of these ruptures. This would enable researchers to study a large cohort of patients and draw more comprehensive conclusions in terms of incidence and the outcome of different surgical techniques and different patient characteristics related to tendon length, tendon gap and functional demands.

It would also be important to evaluate the ATRS and functional outcome measurements in terms of reliability and validity for chronic Achilles tendon ruptures to make them the first validated injury-specific outcome measurements for chronic Achilles tendon ruptures. In the meantime, the ATRS, AOFAS and functional outcome measurements for acute Achilles tendon ruptures can be used. They should, however, be used and analysed together with a more generic outcome measurement such as the EQ5D or SF-36 that has been validated for patients of higher age with different comorbidities.

The incidence of chronic Achilles tendon ruptures is still unknown. Many studies refer to an RCT that reported the incidence as 25%. Apart from this study, there is a lack of epidemiological studies on the subject. It would be interesting to compare the incidence of acute Achilles tendon ruptures with that of patients with chronic Achilles tendon ruptures.

In terms of the biomolecular changes in chronic Achilles tendon ruptures, further studies using histopathological methods and immunohistochemistry are needed to fully understand the underlying processes of chronic Achilles tendon ruptures. With these data, it will, hopefully, be easier to develop new surgical techniques that can subsequently be assessed using validated outcome

measurements in clinical studies with matched controls. In the end, the main goal is, recognisably, to develop new and effective surgical techniques, as well as established rehabilitation programmes, to benefit patients affected by chronic Achilles tendon ruptures in the future.

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12

APPENDIX

ACHILLES TENDON TOTAL RUPTURE SCORE (ATRS) – SWEDISH VERSION. (1/2)

Sundberglaboratoriet för Ortopedisk Forskning

ATRS

(Achilles tendon Total Rupture Score)

Alla frågor avser hur du upplever eventuella besvär på grund av din skadade hälsena

Markera med ett kryss i den ruta som bäst motsvarar din uppfattning!

1. Är du begränsad av minskad kraft i vaden/hälsenan/foten?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

2. Är du begränsad av att du blir trött i vaden/hälsenan/foten?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

3. Är du begränsad av stelhet i vaden/hälsenan/foten?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

4. Är du begränsad av smärta i vaden/hälsenan/foten?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

5. Är du begränsad i ditt dagliga liv?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

Achilles Tendon Rupture Score (ATRS)
Version 6, Katarina Nilsson-Helander 2006-01-12

ACHILLES TENDON TOTAL RUPTURE SCORE (ATRS) – SWEDISH VERSION. (2/2)

Sundberglaboratoriet för Ortopedisk Forskning

Alla frågor avser hur du upplever eventuella besvär på grund av din skadade hälsena

Markera med ett kryss i den ruta som bäst motsvarar din uppfattning!

6. Är du begränsad när du går på ojämnt underlag?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

7. Är du begränsad när du går raskt uppför en trappa/backe?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

8. Är du begränsad vid aktiviteter som innebär att springa?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

9. Är du begränsad vid aktiviteter som innebär att hoppa?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

10. Är du begränsad att utföra hårt fysiskt arbete?

mycket begränsad											inte alls begränsad	Poäng
	0	1	2	3	4	5	6	7	8	9	10	

Achilles Tendon Rupture Score (ATRS)
Version 6, Katarina Nilsson-Helander 2006-01-12

ACHILLES TENDON TOTAL RUPTURE SCORE (ATRS) – ENGLISH VERSION. (1/1)

ATRS

(Achilles Tendon Total Rupture Score)

Today's Date:____/____/_____ Date of Birth____/____/_____

Name:_____

All questions refer to your limitations/difficulties related to your injured Achilles tendon.
Answer every question by grading your limitations/symptoms from 0-10.
Remember (0= Major limitations and 10= No limitations).

Please circle the number that matches your level of limitation

1. Are you limited due to decreased strength in the calf/Achilles tendon/foot?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

2. Are you limited due to fatigue in the calf/Achilles tendon/foot?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

3. Are you limited due to stiffness in the calf/Achilles tendon/foot?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

4. Are you limited due to pain in the calf/Achilles tendon/foot?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

5. Are you limited during activities of daily living?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

6. Are you limited when walking on uneven surfaces?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

7. Are you limited when walking quickly up the stairs or up a hill?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

8. Are you limited during activities that include running?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

9. Are you limited during activities that include jumping?

0 1 2 3 4 5 6 7 8 9 10 (No limitations)

10. Are you limited in performing hard physical labour?

0 1 2 3 4 5 6 7 8 9 10 (No limitations

Thank you very much for completing all the questions in this questionnaire.

FOOT AND ANKLE OUTCOME SCORE (FAOS)– SWEDISH VERSION (1/4)

FAOS

Frågeformulär för patienter med fot- och fotledsbesvär

DATUM: _____ PERSONNUMMER: _____

NAMN: _____

INSTRUKTIONER: Detta formulär innehåller frågor om hur Du ser på din fot / fotled. Informationen ska hjälpa till att följa hur Du mår och fungerar i ditt dagliga liv. Besvara frågorna genom att kryssa för det alternativ Du tycker stämmer bäst in på dig (ett alternativ för varje fråga). Om Du är osäker, kryssa ändå för det alternativ som känns riktigast.

Symptom

Tänk på de **symptom** Du haft från din fot / fotled under den **senaste veckan** när Du besvarar dessa frågor.

S1. Har foten / fotleden varit svullen?

Aldrig	Sällan	Ibland	Ofta	Alltid
☐	☐	☐	☐	☐

S2. Har Du känt att det maler i foten / fotleden eller hör Du klickande eller andra ljud från foten / fotleden?

Aldrig	Sällan	Ibland	Ofta	Alltid
☐	☐	☐	☐	☐

S3. Har foten / fotleden hakat upp sig eller låst sig?

Aldrig	Sällan	Ibland	Ofta	Alltid
☐	☐	☐	☐	☐

S4. Har Du kunnat sträcka vristen / fotleden helt?

Alltid	Ofta	Ibland	Sällan	Aldrig
☐	☐	☐	☐	☐

S5. Har Du kunnat böja vristen / fotleden helt?

Alltid	Ofta	Ibland	Sällan	Aldrig
☐	☐	☐	☐	☐

Stelhet

Följande frågor rör **stelhet**. Stelhet innebär svårighet att komma igång eller ökat motstånd. Markera graden av stelhet Du har upplevt i din fot / fotled den **senaste veckan**.

S6. Hur stel har din fot / fotled varit när Du just har vaknat på morgonen?

Inte alls	Något	Måttligt	Mycket	Extremt
☐	☐	☐	☐	☐

S7. Hur stelt har din fot / fotled varit efter att Du har suttit eller legat och vilat **senare under dagen**?

Inte alls	Något	Måttligt	Mycket	Extremt
☐	☐	☐	☐	☐

FOOT AND ANKLE OUTCOME SCORE (FAOS) – SWEDISH VERSION (2/4)

Smärta

P1. Hur ofta har Du ont i foten / fotleden?

Aldrig	Varje månad	Varje vecka	Varje dag	Alltid
☐	☐	☐	☐	☐

Vilken grad av smärta har Du känt i din fot / fotled den **senaste veckan** under följande aktiviteter?

P2. Snurra/vrida på belastad fot

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

P3. Sträcka vristen / fotleden helt

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

P4. Böja vristen / fotleden helt

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

P5.Gå på jämnt underlag

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

P6. Gå upp eller ner för trappor

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

P7. Under natten i sängläge (smärta som stör sömnen)

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

P8. Sittande eller liggande

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

P9. Stående

Ingen	Lätt	Måttlig	Svår	Mycket svår
☐	☐	☐	☐	☐

Funktion, dagliga livet

Följande frågor rör Din fysiska förmåga. **Ange graden av svårighet Du upplevt den senaste veckan vid följande aktiviteter på grund av dina fot / fotledsbesvär.**

A1. Gå nerför trappor

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

A2. Gå uppför trappor

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

A3. Resa dig upp från sittande

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

FOOT AND ANKLE OUTCOME SCORE (FAOS) – SWEDISH VERSION (3/4)

Ange graden av **svårighet** Du upplevt med varje aktivitet den **senaste veckan**.

	Ingen	Lätt	Måttlig	Stor	Mycket stor
A4. Stå stilla	☐	☐	☐	☐	☐
A5. Böja Dig, t ex för att plocka upp ett föremål från golvet	☐	☐	☐	☐	☐
A6. Gå på jämnt underlag	☐	☐	☐	☐	☐
A7. Stiga i/ur bil	☐	☐	☐	☐	☐
A8. Handla/göra inköp	☐	☐	☐	☐	☐
A9. Ta på strumpor	☐	☐	☐	☐	☐
A10. Stiga ur sängen	☐	☐	☐	☐	☐
A11. Ta av strumpor	☐	☐	☐	☐	☐
A12. Ligga i sängen (vända dig, hålla foten i samma läge under lång tid)	☐	☐	☐	☐	☐
A13. Stiga i och ur badkar/dusch	☐	☐	☐	☐	☐
A14. Sitta	☐	☐	☐	☐	☐
A15. Sätta dig och resa dig från toalettstol	☐	☐	☐	☐	☐
A16. Utföra tungt hushållsarbete (snöskottning, golvtvätt, dammsugning etc)	☐	☐	☐	☐	☐
A17. Utföra lätt hushållsarbete (matlagning, damning etc)	☐	☐	☐	☐	☐

FOOT AND ANKLE OUTCOME SCORE (FAOS) – SWEDISH VERSION (4/4)

Funktion, fritid och idrott
Följande frågor rör Din fysiska förmåga. **Ange graden av svårighet Du upplevt den senaste veckan vid följande aktiviteter på grund av dina fot / fotledsbesvär.**

SP1. Sitta på huk

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

SP2. Springa

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

SP3. Hoppa

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

SP4. Vrida/snurra på belastad fot / fotled

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

SP5.Ligga på knä

Ingen	Lätt	Måttlig	Stor	Mycket stor
☐	☐	☐	☐	☐

Livskvalité

Q1. Hur ofta gör sig Din fot / fotled påmind?

Aldrig	Varje månad	Varje vecka	Varje dag	Alltid
☐	☐	☐	☐	☐

Q2. Har Du förändrat Ditt sätt att leva för att undvika att påfresta foten / fotleden?

Inte alls	Något	Måttligt	I stor utsträckning	Totalt
☐	☐	☐	☐	☐

Q3. I hur stor utsträckning kan Du lita på Din fot / fotled?

Helt och hållet	I stor utsträckning	Måttligt	Till viss del	Inte alls
☐	☐	☐	☐	☐

Q4. Hur stora problem har Du med foten / fotleden generellt sett?

Inga	Små	Måttliga	Stora	Mycket stora
☐	☐	☐	☐	☐

Tack för att Du tagit dig tid att besvara samtliga frågor!

Frågeformulär och användarguide kan hämtas från: www.koos.nu

FOOT AND ANKLE OUTCOME SCORE (FAOS) – ENGLISH VERSION (1/4)

FAOS FOOT & ANKLE SURVEY

Todays date: _____/_____/_____ Date of birth: _____/_____/_____

Name: _____

INSTRUCTIONS: This survey asks for your view about your foot/ankle. This information will help us keep track of how you feel about your foot/ankle and how well you are able to do your usual activities.
Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Symptoms
These questions should be answered thinking of your foot/ankle symptoms during the **last week**.

S1. Do you have swelling in your foot/ankle?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S2. Do you feel grinding, hear clicking or any other type of noise when your foot/ankle moves?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S3. Does your foot/ankle catch or hang up when moving?

Never	Rarely	Sometimes	Often	Always
☐	☐	☐	☐	☐

S4. Can you straighten your foot/ankle fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

S5. Can you bend your foot/ankle fully?

Always	Often	Sometimes	Rarely	Never
☐	☐	☐	☐	☐

Stiffness
The following questions concern the amount of joint stiffness you have experienced during the **last week** in your foot/ankle. Stiffness is a sensation of restriction or slowness in the ease with which you move your joints.

S6. How severe is your foot/ankle stiffness after first wakening in the morning?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

S7. How severe is your foot/ankle stiffness after sitting, lying or resting **later in the day**?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

FOOT AND ANKLE OUTCOME SCORE (FAOS) – ENGLISH VERSION (2/4)

Pain

P1. How often do you experience foot/ankle pain?

Never	Monthly	Weekly	Daily	Always
☐	☐	☐	☐	☐

What amount of foot/ankle pain have you experienced the **last week** during the following activities?

P2. Twisting/pivoting on your foot/ankle

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P3. Straightening foot/ankle fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P4. Bending foot/ankle fully

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P5. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P6. Going up or down stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P7. At night while in bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P8. Sitting or lying

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

P9. Standing upright

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, daily living

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your foot/ankle.

A1. Descending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A2. Ascending stairs

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

FOOT AND ANKLE OUTCOME SCORE (FAOS) – ENGLISH VERSION (3/4)

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your foot/ankle.

A3. Rising from sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A4. Standing

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A5. Bending to floor/pick up an object

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A6. Walking on flat surface

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A7. Getting in/out of car

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A8. Going shopping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A9. Putting on socks/stockings

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A10. Rising from bed

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A11. Taking off socks/stockings

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A12. Lying in bed (turning over, maintaining foot/ankle position)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A13. Getting in/out of bath

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A14. Sitting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A15. Getting on/off toilet

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

FOOT AND ANKLE OUTCOME SCORE (FAOS) – ENGLISH VERSION (4/4)

For each of the following activities please indicate the degree of difficulty you have experienced in the **last week** due to your foot/ankle.

A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

A17. Light domestic duties (cooking, dusting, etc)

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Function, sports and recreational activities

The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the **last week** due to your foot/ankle.

SP1. Squatting

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP2. Running

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP3. Jumping

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP4. Twisting/pivoting on your injured foot/ankle

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

SP5. Kneeling

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Quality of Life

Q1. How often are you aware of your foot/ankle problem?

Never	Monthly	Weekly	Daily	Constantly
☐	☐	☐	☐	☐

Q2. Have you modified your life style to avoid potentially damaging activities to your foot/ankle?

Not at all	Mildly	Moderatly	Severely	Totally
☐	☐	☐	☐	☐

Q3. How much are you troubled with lack of confidence in your foot/ankle?

Not at all	Mildly	Moderately	Severely	Extremely
☐	☐	☐	☐	☐

Q4. In general, how much difficulty do you have with your foot/ankle?

None	Mild	Moderate	Severe	Extreme
☐	☐	☐	☐	☐

Thank you very much for completing all the questions in this questionnaire.

Questionnaire and User's Guide can be downloaded from: www.koos.nu

THE PHYSICAL ACTIVITY SCALE WITH SIX LEVELS (PAS) – SWEDISH VERSION (1/1)

Lundberglaboratoriet för Ortopedisk Forskning
Laboratoriet för muskelfunktion och rörelseanalys

Ringa in det alternativ som bäst överensstämmer med din nivå just nu.

Fysisk aktivitetsnivå JUST NU

Ta hänsyn till vad du arbetar med, samt din fritid, motion och idrott

1 **Knappast någon fysisk aktivitet alls.**

2 **Mest stillasittande, ibland promenad, lättare trädgårdsarbete, eller liknande.**

3 **Lättare fysisk ansträngning omkring 2-4 timmar per vecka, t.ex. promenader, cykling, dans, ordinärt trädgårdsarbete, eller liknande.**

4 **Mer ansträngande motion 1-2 timmar per vecka t.ex. tennis, simning, löpning, motionsgymnastik, cykling (spinning), dans, fotboll, innebandy, tyngre trädgårdsarbete, byggarbete, eller liknande**
***ELLER* lättare fysisk aktivitet (enligt nivå 3) mer än 4 timmar per vecka**

5 **Mer ansträngande motion minst 3 timmar per vecka t.ex. tennis, simning, löpning, motionsgymnastik, cykling (spinning), dans, fotboll, innebandy, tyngre trädgårdsarbete, byggarbete, eller liknande**

6 **Hård träning regelbundet och flera gånger i veckan, där den fysiska ansträngningen är stor**

THE PHYSICAL ACTIVITY SCALE WITH SIX LEVELS (PAS) – ENGLISH VERSION (1/1)

Level	Description
1	Hardly any physical activity at all.
2	Mostly sitting, sometimes a walk, easier gardening or similar tasks.
3	Light physical exercise around 2-4 hours a week, for example walks, cycling, dancing and gardening.
4	Moderate physical exercise 1-2 hours a week, for example tennis, swimming, running, gymnastics, cycling, dancing, football, floorball, heavier gardening and construction work. **OR** Light physical exercise as Level 3 more than 4 hours a week.
5	Moderate exercise for at least 3 hours a week, for example tennis, swimming, running, gymnastics, cycling, dancing, football, floorball, heavier gardening and construction work.
6	Hard exercise regularly and multiple times a week, where the physical exertion is great.

TISSUE HOMOGENISATION PROTOCOL (1/1)

Experiment name…… IDs…… Date ……

1. Thaw samples at room temperature
2. Add 100ul BCP per 1000ul Trizol
3. Vortex for 15 seconds, max speed, do this step as fast as possible
4. Leave for 2-15 minutes at R.T. …… min
5. Spin at 12000 g for 15 minutes at 4 degrees. Mark the lids on which side is up in the centrifuge. The mixture will now separate into 3 phases. Top = aqueus phase that contain the RNA, the mid phase contains DNA and the lower red phase contains proteins, including the RNAses.
6. Label Eppendorf tubes and add 4ul of glycogen.
7. Carefully transfer the top phase (set the pipette on 450 ul. Make sure not to touch any other phase. The remaining phases can be saved for later. Transfer this phase to an Eppendorf tube containing 4ul of glycogen.
8. Add an exactly equal volume (important) volume of isopropanol to the Eppendorf tube to precipitate the RNA. Mix by inversion.
9. Leave for 5-10 min in room temperature. …… min
10. Spin at 12000 g at 4-25 degrees for 8 minutes.
11. Carefully remove the supernatant with a pipette
12. Add 1 ml 75% ethanol (RNAse free). Wash sides by inversions.
13. Spin at 7500 g 4-25 degrees for 5 minutes.
14. Carefully remove the supernatant with a pipette
15. Spin at 7500 g 4-25 degrees for 1 minutes.
16. Remove the remaining supernatant
17. Add 100 ul RNAse free water on top of the pellet.
18. Leave for 5-10 minutes at room temperature …… min
19. Vortex carefully to dissolve pellet
20. Add 10 ul of 3M NaAc pH5.5 (RNAse free) and mix (no vortex but vigorously shaking).
21. Add 200ul 96% ethanol (RNAse free). Wash sides by inversions.
22. Leave for 2-15 min in room temperature. …… min
23. Spin at 12000 g at 4-25 degrees for 8 minutes.
24. Carefully remove the supernatant with a pipette
25. Add 1 ml 75% ethanol (RNAse free). Wash sides by inversions.
26. Spin at 7500 g 4-25 degrees for 5 minutes.
27. Carefully remove the supernatant with a pipette
28. Spin at 7500 g 4-25 degrees for 1 minutes.
29. Remove the remaining supernatant
30. Air-dry the pellet for 5 min, exactly! Place the tube lying with the lids open on the bench. Be careful to not completely dry the pellet as it will be difficult to resuspend.
31. Add 20 ul RNAse free water on top of the pellet.
32. Leave for 5 minutes at room temperature
33. Vortex
34. Leave for 1 minute at room temperature
35. Vortex and spin
36. Take out 3 ul to a new tube for quantification and quality measurement.

RNA QUALITY AND QUANTITY PROTOCOL (1/1)

Agilent RNA 6000 Nano Kit
(Bioanalyzer 2100, Agilent Technologies)

General

- *Make sure syringe works*
- *Check base plate in position C (nano)*
- *Work clean*
- *Pipette down at bottom of wells, avoid bubbles*
- *Store all kit vials in fridge 4°C when not in use, stable for 3 months*

A) Preparations

Prepare: *Thaw denatured Ladder (yellow) aliquots on ice*
Denature chip samples (same conditions as ladder)
Allow Dye concentrate (blue) reach RT for 30 min
Cleaning electrodes of Agilent
1. *Add 350 µl RNase AWAY in wells of RNase chip (page 12)*
2. *Place chip in holder, incubate 1 min*
3. *Add RNase free water, incubate 10 sec (page 27)*

B) Quick run (Step I to II at own lab, III to VI at core facility**)**

I - Gel

1. Pipette 550 µl gel matrix (red) into spin filter
2. Centrifuge @ 1500g for 10 min at RT
3. Aliquot 65 µl filtered gel into 0.5 ml RNase-free tubes.
   Use within 4 weeks.

II - Gel-Dye mix

4. Vortex Dye concentrate (blue) for 10 sec, spin down.
5. Add 1 µl of dye into a 65 µl aliquot of filtered gel (step 3)
6. Vortex well, centrifuge @ 13 000 g for 10 min

III - Loading Gel-Dye mix

7. Put a new chip on chip priming station
8. Pipette 9 µl gel-dye mix in well D3

	A	*B*	*C*	*D*
1	o	o	o	o
2	o	o	o	o
3	o	o	o	o
4	o	o	o	o

12 well Nano chip

9. Close chip priming station
10. Press plunger until it is held by the clip
11. Wait for 30 sec, then release clip
12. Pipette 9 µl gel-dye mix in well D1 and D2
13. Discard remaining gel-dye mix.

IV - Loading Nano Marker

14. Load 5 µl of Nano marker (green) to wells A-B-C, and D4
    (in total 13 wells, sample 1-12 and ladder)

V - Loading Ladder and Samples

15. Pipette 1 µl of prepared ladder in well D4

16. Pipette 1 µl of each sample in wells A-B-C
    (or 1 µl of Nano marker to un-used wells)

17. Place in adapter and vortex for 1 min @ 2000 rpm

18. Run the chip within 5 min (to avoid evaporation)

VI - Analysis

19. For class 5 to 10: RNA is good for realtime-PCR
    (Class 1 to 4: discard!)

RNA-EXTRACTION PROTOCOL (1/1)

A) Powdering tissue and TRIzol addition

Prepare: Liquid nitrogen
Calculate amount of TRIzol (1 ml for up to 100 mg tissue)
Soak vials (custom made), caps, wolfram balls and forceps in NaOH 2% over night, or in 20% for few minutes, rinse in water
Let TRIzol reach RT in hood
Prelabel PP tubes with o-ring, PolyPropylene – resists TRIzol)
Harvested tendons in -80 freezer

1. Use kimwipes to carefully **dry all water!**
2. Cool clean & rinsed units in liquid N_2 for at least 5 min (stops bubbling)
3. Homogenize in dismembranator for approximately 45 sec @ 2600 rpm (max speed), leave a little bit of liquid nitrogen in the vial during homogenization to keep the sample cool all the time.
   Check powderized, otherwise repeat step 1 and 2

4. In hood: **Quickly** add 1 ml TRIzol, invert and shake vial

5. In hood: Defrost at RT for 45 to 90 min. ……. *min*

6. In hood: Add extra TRIzol if weight > 100 mg

7. In hood: Transfer samples to PP tubes, be careful to get everything on walls

8. Incubate TRIzol tubes for 30 min @ RT ….*min*
   (meanwhile clean up, rinse)

9. Optional to continue (or freeze tubes in -80°C freezer) *freeze*