Complex Meniscus Tears Treated with Collagen Matrix Wrapping and Bone Marrow Blood Injection: A 2-Year Clinical Follow-Up

Tomasz Piontek¹, Kinga Ciemniewska-Gorzela¹, Jakub Naczk¹, Roland Jakob¹, Andrzej Szulc⁶, Monika Grygorowicz¹, and Michal Slomczykowski²

Abstract

Objective. To collect and analyze the 2-year follow-up clinical and MRI results of patients treated with an arthroscopic technique of collagen membrane-based meniscus repair. Design. 53 consecutive patients with combined (horizontal and radial or longitudinal component) and complex meniscal tears (tear extended through avascular zones or/and composed with two or more morphological tear pattern) were treated with an “all-inside” arthroscopic suture of meniscus and wrapping with a collagen membrane (Chondro-Gide) technique with bone marrow blood injection. The IKDC 2000 subjective score, IKDC 2000 clinical evaluation score, Lysholm score and Barret clinical criteria of meniscal healing were recorded. All patients were examined by MRI 2 years postoperatively, using modified WORMS criteria for meniscal integrity. Results. The 2 year follow-up was achieved in 50 cases. Of these, 2 patients were excluded from the evaluation due to incomplete data and 2 patients underwent partial meniscectomy and were classified as failures. In 46 patients (86.8% of the intended to treat cases), a statistically significant improvement in IKDC 2000 subjective, Lysholm scores and IKDC 2000 clinical assessment between preoperative and the 2-year follow-up time points were observed. Barret criteria demonstrated an improved clinical outcome between pre- and post-operative values. MRI revealed a non-homogeneous signal without meniscal tear (WORMS grade 1) in 76% of the operated menisci (13% WORMS grade 2). Conclusions. The 2-year follow-up data demonstrate that this technique is safe and can offer an additional tool to save the meniscus in the patients otherwise scheduled for meniscal removal. Level of evidence IV

Keywords
meniscus wrapping, collagen membrane, arthroscopic, complex, combined

Introduction

Meniscal injuries located in the low or nonvascularized zones (red-white and white-white zones) are often treated with partial or total meniscectomy. Although meniscectomy is a relatively simple and quick surgery with good immediate postoperative clinical outcomes, the long-term results are poor.¹-³ These poor outcomes are not surprising since many studies have demonstrated the importance of the meniscus for the knee function.⁴-¹⁰ It has also been accepted that surgeons should preserve as much meniscal tissue as possible. Both complete and partial meniscectomy are associated with early degenerative osteoarthritis.⁷⁻¹¹ To preserve function of the knee joint, it is now suggested that meniscal tears should be treated by meniscal repair instead of meniscectomy whenever possible.¹²⁻¹⁴

Various augmentation techniques for meniscal repair have been described. They include use of a fibrin clot, platelet-rich plasma, pro-inflammatory cytokines, and application of growth factors.¹⁴⁻¹⁹ Further progress in meniscus healing has been made possible by the innovative use of cells and scaffolds.²⁰ Improved methods to isolate and concentrate mesenchymal stem cells from autologous sources (e.g., bone marrow or adipose tissue) allows the evaluation of the role of cell-based techniques to augment healing. Furthermore, the development of advanced scaffold materials and associated techniques has contributed to creating an intracapsular biological environment for improved meniscus healing. Notably, Jakobi and

¹Rehasport Clinic, Poznan, Poland
²Geistlich Pharma AG, Wolhusen, Switzerland
³Clinic of Pediatric Orthopedic Surgery UM Poznań, Poland

Corresponding Author:
Kinga Ciemniewska-Gorzela, Rehasport Clinic, Górecka 30, Poznan, 60-201, Poland.
Email: kinga@rehasport.pl
Jakob tested 30 patients with the treatment of meniscal tears by suturing and wrapping the repair in a collagen matrix. The results obtained were encouraging, but the surgical technique, an inside-out suturing, presents some challenges since it can be time-consuming and requires an additional surgical approach. In a goat animal study, Jülke et al. showed that a transient, short-term guided tissue regeneration of avascular meniscal tears occurs when a collagen membrane is used. The addition of expanded autologous chondrocytes supports more sustainable long-term tear healing in the goat model.

Inspired by those advances in the treatment of injured menisci, in 2010 a fully arthroscopic technique has been developed to treat combined (possessing horizontal and radial or longitudinal component) and complex (tear extended through all vascular zones or and composed with 2 or more morphological tear pattern) meniscal tears. Conceptually, the surgical technique is a modification of the historical Henning’s and Jakob procedure, included suturing and wrapping the menisci in a collagen matrix, followed by the injection of liquid bone marrow collected from the tibial proximal epiphysis, into the area of the meniscal lesion. A detailed description of the surgical method had been published by Piontek et al. in 2012. By using this surgical technique in patients sustaining complex and/or combined meniscal injuries, meniscus resection could be avoided in these tear patterns cases that otherwise would have been treated with meniscectomy.

We hypothesized that complex and combined meniscal tears occurring in white-white and white-red zones can safely and successfully be treated arthroscopically by meniscal suturing and wrapping in the collagen matrix, instead of being partially or totally resected.

The purpose of this work is to present a 2-year follow-up of clinical and magnetic resonance imaging (MRI) results from a prospective consecutive case series of patients treated with a newly developed, fully arthroscopic technique of collagen matrix-based meniscus repair (AMMR) and the injection of bone marrow aspirate into the area of the meniscal lesion.

Materials and Methods

This clinical study has been approved by the Institutional Review Board committee of the Medical University of Poznan, Poland, and has been performed according to the ethical standards laid down in the 1964 Declaration of Helsinki. All participants provided their written informed consent to participate in this study.

Study Design

The study was designed as a case series, a level IV clinical study.

Inclusion criteria for the procedure comprised (1) full-thickness, combined meniscal tear greater than 20 mm in length, (2) horizontal and radial tear, (3) tear location reaching more than 6 mm from the meniscocapsular junction including the avascular zone, or (4) both degenerative and non-degenerative meniscus (i.e., horizontal and radial tears, involving the white-white and red-white zones, as well as extensive tears of the bucket-handle type). An example of meniscus injury treated as a part of this study is shown in Figure 1 and as a schematic drawing in Figure 2.

Patients were excluded from the procedure if (1) full-thickness combined meniscus tear was larger than 85% of the meniscus body or (2) meniscus tear with fibrillation extended in all area of tear which was not able to be stabilized with a suture (meniscus lesion beyond repair).

Time to surgery was defined as a time elapsed between injury or patient decision to seek orthopaedic treatment to the AMMR surgical intervention. The length of the meniscal tear was measured during arthroscopic procedure by using arthroscopic ruler. Degenerative meniscus was in most cases defined as a adipose (fat) degeneration or significant fibrillation not exceeding 85% body of the meniscus.

The primary outcome of the study defined as a result of a clinical of the knee joint and meniscus related symptoms before and after treatment (at 2-year follow-up). The safety
of the procedure was assessed based on pass-fail criteria’s and adverse events related to the procedure or medical device used (Chondro-Gide collagen matrix).

The clinical results were assessed and evaluated by the number of different scoring systems based on patient reported outcomes and clinical findings. The following information were recorded at each assessment point: International Knee Documentation Committee (IKDC) 2000 subjective score, IKDC 2000 clinical evaluation score, Lysholm score, and Barrett clinical criteria of meniscal healing.

Based on original Barrett criteria score, we have developed a converted method (described in details in Appendix A) to allow for statistical analysis. The aim of this calculation is to convert the four clinically meaningful signs (pain, effusion, clicking/locking, McMurray test), which are coded binary (0 = absence and 1 = presence) into a numerical notation. The final values may vary from 0 to 15 and each value represents the discreet stage of the knee joint with respect to the initial Barrett’s evaluation criteria.

In an attempt to lower the confounding influence of the concomitant knee diseases in the patients included in the study, the analysis of the subgroup of patients with isolated meniscus injury was performed. Data from 14 patients were available for the analysis.

Secondary outcome included an MRI evaluation at 24 months postoperatively.

Magnetic resonance imaging scans were acquired with a 1.5-tesla whole-body scanner using a commercial circumferential knee coil. Imaging sequences included, axial T1-weighted spin-echo (SE: 700/11 [TR ms/TE ms], 20 cm field of view (FOV), 5 mm/1 mm [slice thickness/inter-slice gap], 256 × 192 matrix, frequency encoding [FE] anterior-posterior, one excitation), coronal T1-weighted SE (600/11, 16 cm FOV, 4 mm/0.5 mm, 256 × 192, FE superior-inferior, 2 excitations averaged), sagittal T1-weighted SE (600/11, 16 cm FOV, 4 mm/0.5 mm, 256 × 192, FE anterior-posterior, 2 excitations averaged), sagittal T2-weighted fast spin echo (FSE: 2500/90; echo train length (ETL) of 8; 14 cm FOV, 4 mm/0 mm, 256 × 192, FE superior-inferior, 2 excitations averaged) with fat suppression (frequency-selective presaturation), and sagittal fat-suppressed T1-weighted 3-dimensional (3D) spoiled gradient echo (FS-3DSPGR: 58/6, 40° flip angle, 14 cm FOV, 256 × 128 matrix, 60 contiguous 2-mm slices covering all articular cartilage plates in the knee, FE, superior-inferior, one excitation, frequency-selective fat saturation, superior-inferior saturation bands to minimize pulsation artifacts).

All images were transferred to the Osirix v.5.8.5 DICOM viewer software (Pixmeo SARL, Bern, Switzerland). Images were scored with respect to modified Whole Organ MRI Score (WORMS) criteria for medial and lateral meniscal integrity. Image assessments were performed independently by 2 orthopedic surgeons (TP, KC-G). Readers used all images to evaluate each feature. Problematic cases were assessed by both readers at the same time to achieve consensus.

The anterior horn, meniscal body segment, and posterior horn of the medial and lateral menisci were scored separately on the scale from 0 to 4 based on both the sagittal and coronal images: 0 = intact; 1 = nonhomogeneous signal but not a meniscal tear (in original WORMS scale minor radial tear or parrot-beak tear); 2 = nondisplaced tear or prior surgical repair; 3 = displaced tear or partial resection; 4 = complete maceration/ destruction or complete resection. A cumulative grade for each meniscus was determined using the following scheme: 0 = all 0; 1 = at least one 1, but no >1; 2 = 2 in only one region; 3 = 2 in more than one region; 4 = 3 in one or more regions; 5 = 4 in only one region; 6 = 4 in more than one region. These modifications were introduced in order to accurately describe MRI scans.

In this study, the following pass-fail criteria were used:

1. Failure if at the follow-up process patient underwent partial/complete meniscectomy or knee replacement after AMMR procedure.
2. Pass was defined as an overall grade of A or B at final follow-up with clinical IKDC 2000 assessment.
3. Pass was also defined if the patient exhibited no pain at rest or with activity and a negative McMurray’s test.

Patients who did not met the aforementioned criteria were characterized as “poor” with regard to their latest clinical follow-up.

Additionally, a WORMS cumulative grade 2 and more classified patients in the poor MRI results group.

**Collagen Matrix**

The Chondro-Gide Collagen Matrix (Geistlich Pharma AG, Wolhusen, Switzerland) was used for meniscal wrapping. This is a non–cross-linked collagen type I/III matrix, with a 2-layer (bilayer) structure. The porous layer (placed facing the defect) allows the in-growth of cells and a newly formed tissue. The compact layer acts as a scaffold to prevent cells being flushed out of the meniscal site defect.

**Surgical Technique**

A diagnostic knee arthroscopy was performed to identify other pathologies, such as ligament or cartilage lesions. All cartilage and ligament lesions were repaired during surgery. The extent and type of meniscal lesion was evaluated to determine whether or not the meniscal lesion meets the study inclusion criteria.

Lesions were reduced and meniscal fragments were fixed with a meniscal fixing device (Fast-Fix, Smith & Nephew, Andover, MA, USA).

The matrix, usually of the size 30 × 20 mm, was prepared by addition of nonabsorbable suture loops (Ethibond
2, Ethicon Inc), passing through the surface of the matrix on both sides. The matrix was then inserted into an applicator.

Using direct arthroscopic vision, the loops of threads running through the meniscal posterior horn and the meniscal body were passed with a special suture shuttle (Accupass, Smith & Nephew, Andover, MA, USA) at the level of the anterior border of the tear.

Ethibond 2, matrix-passing sutures, were inserted to the meniscal posterior horn and to the anterior part of the lesion. The applicator with the collagen matrix was introduced into the knee joint. The matrix was inserted into the knee joint with smooth surface facing cartilaginous surfaces of the tibia and femur and with porous part directed to the meniscal surface.

After assuring that the matrix adhered to the meniscus from the tibial side the scaffold was fixed to the meniscus, with arthroscopic simple knotted sutures. As a result, the meniscus was wrapped by the collagen matrix on both sides, and the matrix was fixed into the meniscus in a stable way. Additionally, 1 to 4 (3 on average, depending on the extension of the tear) Fast-Fix sutures were inserted into the meniscus wrapped with the matrix for a better stabilization of the meniscal tear and for an increased tightness between the meniscus and the matrix.

An example of suture placement and meniscus wrapping with collagen matrix is shown in Figure 3 and as a schematic drawing in Figure 4.

The bone marrow aspirate was obtained from the tibial proximal epiphysis of the operated knee. Approximately 5 mL of liquid bone marrow was aspirated. The bone marrow aspirate was injected between the Chondro-Gide matrix and the meniscus, under direct arthroscopic control and carried out in ‘dry arthroscopy’ conditions.

The surgery was completed by closing the wounds without a drainage the knee joint. No knee-stabilizing orthosis was used. An extensive surgical description of the technique has been published by Piontek et al.23 in 2012.

Postoperative Physiotherapy Protocol

Patients began postoperative treatment from toe touch crutch walking and a range of motion exercises on the first postoperative day, for a period of 4 weeks. Over the next 2 to 4 weeks the patients were allowed to walk with partial weight-bearing. The patients were encouraged to return to their daily activity by 12 weeks. Sports activity was possible after 6 months postsurgery. Detailed rehabilitation guidelines used in our center have been presented in Appendix B.

Statistical Evaluation

Statistical evaluation included descriptive statistics to compare the pre- and postoperative indexed values.

The comparison between preoperative (time 0) and postoperative (24 months follow-up) values of IKDC 2000 clinical, IKDC 2000 subjective, and Lysholm separately had been calculated by nonparametric Wilcoxon test.

Results

The comprehensive data, including demographics, pre- and postoperative data of the patients participating in the study, are summarized in Table 1.

Between April 2010 and November 2011, 53 consecutive patients (40 male and 13 female, age 18-59 years) meeting
## Table 1. Summary of Patients and Outcomes Data.

| Notes          | Count | Sex | Age (Years) | TTS (Months) | BMI (kg/m²) | Length (mm) | Barrett Pre/2-year FU Value | IKDC Clinical Pre/2-year FU | Lysholm score Pre/2-year FU | IKDC Subjective Pre/2-y FU | MRI Cumulative Scoring |
|----------------|-------|-----|-------------|--------------|-------------|-------------|-----------------------------|-----------------------------|------------------------------|---------------------------|------------------------|
| Failure        | 30    | M   | 30          | 18           | 46          | 32          | 15/0                        | D/A                         | 89/90                       | 26.4/37.6                 | 1                       |
| Failure        | 31    | F   | 57          | 60           | 27          | 30          | 15/1                        | C/N/A                       | N/A                         | N/A                       | N/A                     |
| Failure        | 32    | M   | 57          | 27           | 30          | 15/0        | 11/0                        | C/A                         | 70/95                       | 52.9/100                  | 1                       |
| Failure        | 33    | M   | 37          | 60           | 18          | 30          | 15/0                        | D/A                         | 66/94                       | 57.5/92                   | 1                       |
| Failure        | 34    | F   | 41          | 12           | 27          | 35          | 13/0                        | D/A                         | 64/100                      | 48.3/95                   | 1                       |
| Failure        | 35    | M   | 18          | 2            | 26          | 35          | 15/0                        | D/A                         | 56/73                       | 51.7/50.6                 | 0                       |
| Data partly missing | 41    | M   | 47          | 60           | 30          | 35          | 15/0                        | D/A                         | Missing/92                  | Missing/92                | 1                       |
| Failure        | 42    | M   | 40          | 60           | 27          | 30          | 15/0                        | C/A                         | 90/85                       | 62.5/95                   | 2                       |
| Failure        | 43    | F   | 49          | 24           | 23          | 30          | 15/0                        | D/A                         | 71/89                       | 29.9/70.1                 | 0                       |
| Failure        | 44    | M   | 19          | 4            | 27          | 30          | 15/0                        | D/A                         | 35/94                       | 32.2/87.4                 | 0                       |
| MRI missing    | 45    | M   | 33          | 3            | 27          | 30          | 15/0                        | D/B                         | 52/57                       | 39/54                     | NA                      |
| MRI missing    | 46    | M   | 19          | 4            | 29          | 30          | 15/0                        | C/A                         | 57/100                      | 32.2/87.4                 | 2                       |
| MRI missing    | 47    | M   | 39          | 2            | 24          | 30          | 15/0                        | D/A                         | 70/95                       | 58.6/88.5                 | Missing                 |
| MRI missing    | 48    | M   | 28          | 36           | 22          | 30          | 15/0                        | D/A                         | 70/77                       | 64.4/70.1                 | 1                       |

Abbreviations: BMI, body mass index; F, female; FU, follow-up; IKDC, International Knee Documentation Committee; M, male; N/A, not applicable; TTS, time to surgery; Barrett scores, binary value for pain, effusion, clicking/locking, McMurray test; Barrett values, converted Barrett scores (Att.1).

*MRI cumulative based on WORMS modified criteria.2*
Two-year follow-up period was achieved in 50 cases, 3 (5.7%) patients being lost to follow-up. In addition, 2 patients were completely excluded from the evaluation due to the incomplete pre- or postoperative data.

Two patients (4.2%) underwent partial meniscectomy and according to the pass-fail criteria were classified as failures. Based on pass-fail criteria used in this study, 46 patients were successfully treated. This value represents 95.8% of the cases entered the prospective evaluation and 86.8% of the intended to treat cases.

IKDC 2000 subjective and Lysholm scores pre and postop comparison (Table 3) indicated a statistically significant improvement ($P = 0.0001$, Wilcoxon test).

The analysis of the IKDC clinical scores after 2 years (Table 4) demonstrated that 96% patients achieved A or B scores, compared with none of the patients present in either category preoperatively. A statistically significant improvement in IKDC 2000 clinical assessment was also observed between preoperative and 2-year follow-up ($P = 0.0001$, Wilcoxon test).

The clinical assessment based on Barrett criteria (calculated using the method described in Appendix A) is presented in Table 5, panels A and B. Calculated values demonstrate a tendency for the patients to score low (good clinical outcome) at the 24-month follow-up. Significant improvement for Barrett’s clinical score assessment between pre and postoperative values was observed ($P = 0.0001$, Wilcoxon test).

In the subgroup of isolated meniscal injuries, the IKDC 2000 subjective and Lysholm scores were slightly lower preoperatively than in the overall study population, but the results were in the range of the general study population after 2 years (Tables 6 and 7). An improvement in IKDC 2000 subjective and Lysholm scores in this subgroup of patients was observed on average and in patient by patient assessments (data not included in this article).

In 11 patients, an isolated horizontal cleavage tear of the menisci were observed (average age 41 years, range 20-59 years). In this group of patients, an average improvement of subjective IKDC (from 47 to 72) and Lysholm (from 79 to 89) outcomes were observed. None of those 11 cases had undergone any additional surgical knee treatment and had their meniscus saved. The worst results were obtained among patients suffering from cartilage damage (grade IV) irrespective of age. The remaining patients with additional damage to the knee (mainly posttraumatic), obtained very good and excellent results. No significant difference in the rate of meniscal repair failure was noted for anterior cruciate ligament–intact, compared with anterior cruciate ligament–deficient knees.

In the 2 failures, a second-look arthroscopy was performed. In 1 case, the meniscal healing in arthroscopic assessment was found but due to persisting pain symptoms, a partial meniscectomy was performed 12 months after initial treatment. Postoperatively the patient still complained about the pain localized in the region of the medial tibial condyle. Subsequent MRI evaluation demonstrated stress fracture of the medial tibial condyle. After appropriate treatment, the patient reported pain-free knee and full knee joint function. The second patient did not comply with the physiotherapy protocol and returned to full physical activity 3 months postoperatively, despite the doctor’s recommendations. As a result, he developed a second injury of the treated meniscus leading to partial meniscectomy 6 months after indexed procedure.

Analysis of the follow-up MRI images revealed 85% of good meniscus outcomes based on the WORMS classification (cumulative score $\leq 1$) (Table 1).

In the MRI examination at 2 years postoperatively, 76% of all operated menisci showed meniscal abnormalities corresponding to a nonhomogeneous signal without meniscal tear and were classified as grade 1 according to WORMS classification. Eleven percent of menisci had been classified as grade 0, with a fully regenerating meniscal cartilage with homogenous MRI signal. The WORMS cumulative grade 2 was established in 6 out of 45 patients (13%) and they were classified as a poor MRI results.

The analysis of the MRI results and clinical findings at 2 years FU demonstrated that in this case series the cumulative WORMS results did not correlate with either clinical (clinical IKDC 2000 and Barrett score) or subjective scores (IKDC 2000 subjective and Lysholm score) (Table 8).

Notably, in 29 (62%) of the cases, MRI revealed the presence of a cyst located in the vicinity of the T-fix anchor. The presence of the cyst had no correlation with clinical (IKDC 2000 clinical, $P = 0.47$; and Barrett criteria) or cumulative WORMS results. Additionally, no correlation was found between the number of used T-fix anchors and
Table 3. The Subjective IKDC and Lysholm Scores Preoperative and 24 Months Postoperative Follow-Up Results.

|                        | n  | Average | Minimal | Maximal | 95% CI | P Value (Wilcoxon Test) |
|------------------------|----|---------|---------|---------|--------|-------------------------|
| IKDC subjective preoperative | 46 | 44.5    | 16.0    | 79.3    | 5.2    | 0.0001                  |
| IKDC subjective 24-month FU | 46 | 79.1    | 37.9    | 100.0   | 9.1    |                         |
| Lysholm preoperative   | 46 | 66.1    | 29.0    | 94.0    | 6.2    | 0.0001                  |
| Lysholm, 24-month FU   | 46 | 86.4    | 33.0    | 100.0   | 5.1    |                         |

Table 4. The IKDC 2000 Clinical Results.

| IKDC 2000 Clinical Score | Preoperative | 24-Month FU |
|--------------------------|--------------|-------------|
| Number of Cases | % | Number of Cases | % | P Value (Wilcoxon Test) |
| A                     | 0  | 38 | 82.6 | 0.0001 |
| B                     | 0  | 6 | 13.0 | 0.0001 |
| C                     | 15 | 2 | 4.4 |        |
| D                     | 33 | 0 | 0.0 |        |

Figure 5. CONSORT (Consolidated Standards of Reporting Trials) case series diagram. SAE, severe adverse event (according to ISO 14155).

Table 3. The Subjective IKDC and Lysholm Scores Preoperative and 24 Months Postoperative Follow-Up Results.

| IKDC subjective preoperative | 46 | 44.5 | 16.0 | 79.3 | 5.2 | 0.0001 |
| IKDC subjective 24-month FU | 46 | 79.1 | 37.9 | 100.0 | 9.1 |        |
| Lysholm preoperative | 46 | 66.1 | 29.0 | 94.0 | 6.2 | 0.0001 |
| Lysholm, 24-month FU | 46 | 86.4 | 33.0 | 100.0 | 5.1 |        |

Abbreviations: CI, confidence interval; FU, follow-up; IKDC, International Knee Documentation Committee.

Figure 6A–D illustrates the MRI history of one representative case of a 50-year-old man who had undergone medial meniscus repair with AMMR technique and cartilage regeneration of medial femoral condyle with arthroscopic AMIC procedure in a one-stage knee arthroscopy.

In our series, 13 serious adverse events (including 2 failures) were registered. However, none of these events were related to either the indexed procedure or material used. The summary of adverse events is presented in Table 9.

Discussion

The data obtained demonstrate that combined and complex meniscal tears located in the white-white and white-red zones can be treated arthroscopically safely and with clinical success treated by meniscal suturing and wrapping in the collagen matrix. All patients participating in this study
would have had their menisci partially or totally resected otherwise.

The present results, demonstrate that the specially developed “all-inside” technique of suture wrapping of the lesion with a collagen membrane and in situ administration of blood bone marrow aspirate through an arthroscopic technique, is feasible. It presents, in our opinion, an alternative to total or partial meniscectomies in cases of combined tears located in the red-white and white-white zones. As previously suggested, the collagen scaffold may facilitate healing by providing an appropriate biological environment for cells and bone marrow blood to facilitate the process of natural healing and regeneration.

The primary outcome results of our 2-year follow-up data indicate not only possibility for meniscus preservation but also increase in the IKDC subjective score from 44 to 79 and in the Lysholm score from 66 to 86 on average preoperatively and at 2-year follow-up, respectively. IKDC 2000 clinical and Barrett scores were also improved at 24 months postsurgery.

In the subgroup of 11 cases of horizontal tear we were able to save the meniscus in all cases with 82% of good to excellent clinical results, and 64% of very good and excellent subjective score assessment.

In our case series, we have used an “all inside” surgical technique. It provides good access to the operating site, is simple and was preferred by the operating surgeon. There is, however, no reason to restrict the surgical technique to the one approach only. The goal is to fix the torn meniscus and wrap it in to the collagen membrane.

| Table 6. Results of IKDC 2000 Subjective Scores for the Patients with Isolated Meniscal Injuries. |
|---------------------------------------------------------------|
| **n** | **Average** | **Minimal** | **Maximal** | **95% CI** |
| Preoperative | 14 | 37.6 | 16.0 | 64 (85.9 to 74.61) |
| 24 months | 14 | 79 | 41 | 96.5 (79.42 to 89.58) |

| Table 7. Results of Lysholm Scores for Patients with Isolated Meniscal Injuries. |
|---------------------------------------------------------------|
| **n** | **Average** | **Minimal** | **Maximal** | **95% CI** |
| Preoperative | 14 | 58 | 29 | 90 (73.88 to 85.92) |
| 24 months | 14 | 88 | 64 | 100 (86.52 to 94.48) |

| Table 8. Correlation Analysis Between MRI Findings and Clinical Results. |
|---------------------------------------------------------------|
| **MRI Cumulative Value vs.** | **n** | **Correlation Coefficient r** | **P Value** |
|-------------------------------|-----|-----------------------------|-------------|
| IKDC 2000 at 24 months postoperative | 47 | −0.0348 | 0.8165 |
| IKDC subjective at 24 months postoperative | 47 | 0.07 | 0.6401 |
| Lysholm at 24 months postoperative | 47 | 0.0114 | 0.9336 |
| Barrett at 24 months postoperative | 47 | −0.0294 | 0.8444 |

Abbreviations: IKDC, International Knee Documentation Committee; MRI, magnetic resonance imaging.
Collagen membrane use to protect the cartilage healing after micro fracture is an established procedure since almost 10 years. Recently, Jülke et al. described encouraging results of collagen membrane use for short-term guided tissue regeneration of avascular meniscal tears on the goat model, especially if the autologous chondrocytes were applied.

Autologous bone marrow aspirate can be obtained in a relatively simple and inexpensive method, and is known as a source of stem cells as well as growth factors. Although alternative sources of stem cells are described in the literature, there is currently no evidence of superiority one over the other source or method of preparation.

After reviewing the clinical results of the series, we found no reason to limit the method to the patients presented with absent signs of an advanced arthrosis of the knee, neither to the patients of the young age. Based on our experience great care needs to be taken in patients with grade IV cartilage lesions which scored lowest in our case series. Additional research on a larger population may help in finalizing the indications for the proposed method of treatment.

Our study protocol includes MRI assessment as a secondary outcome. While there are number of publications indicating MRI as the imaging modality of choice for diagnosis of primary meniscal injuries, to date there is no suitable scoring system offering the assessment classification and allowing for an objective analysis of the MRI data after meniscus repair. Conventional MR accuracy for detection of meniscal recurrent tears status post-surgery has been previously reported as 66% to 82%.

In the absence of established MRI scoring system for assessment of meniscal repair, we have chosen the WORMS score as the classification best suited for the purpose of this study.

We found a higher prevalence of increased signal in the posterior horn of the treated meniscus in 35 cases (73%) in which degeneration and tears are most frequently found, and
Table 9. Details of Serious Adverse Events Not Related to the AMMR Procedure.

| No. | Patient ID | Serious Adverse Events                          | Treatment |
|-----|------------|-------------------------------------------------|-----------|
| 1   | 46         | Acute compartment syndrome ipsilateral leg      | Fasciotomy|
| 2   | 11         | Medial meniscus injury ipsilateral leg (indexed meniscus—lateral) | Second-look arthroscopy—partial medial meniscectomy |
| 3   | 9          | Medial meniscus injury contralateral leg        | None      |
| 4   | 24         | Brain aneurysm                                  | Embolization |
| 5   | 12         | Medial meniscal injury contralateral leg        | Arthroscopy—meniscus regeneration with Chondro-Gide and 2× Fast-Fix (AMMR) |
| 6   | 23         | Acute medial meniscus injury contralateral leg  | Arthroscopy—medial meniscus suture |
| 7   | 8          | Arthrofibrosis ipsilateral leg                  | Second-look arthroscopy—soft tissue release |
| 8   | 13         | ACL graft rupture, medial meniscus injury (indexed meniscus—medial) | Revision ACL reconstruction, medial meniscus suture |
| 9   | 22         | Arthrofibrosis ipsilateral leg                  | Second-look arthroscopy—soft tissue release |
| 10  | 52         | Medial meniscus injury contralateral leg        | Medial meniscectomy |
| 11  | 32         | ACL graft failure                               | Revision ACL reconstruction |
| 12  | 34 F       | Undetected fatigue fracture of left medial tibial condyle before AMMR procedure. Persistent pain after meniscus wrapping | Partial medial meniscectomy—histologically signs of regenerated meniscal tissue |
| 13  | 50 F       | Re-injury of ipsilateral leg; medial meniscus tear (indexed meniscus—medial). Patient did not comply with postoperative physiotherapy regime and returned to full professional activity as a firefighter | Arthroscopy—partial medial meniscectomy |

Abbreviations: ACL, anterior cruciate ligament; AMMR, arthroscopic technique of collagen matrix-based meniscus repair; MPFL, medial patellofemoral ligament.

*F indicates failure according to the pass-fail criteria for this study.

the differentiation of a high signal intensity from a meniscal tear at this site is of great clinical importance. The reason and meaning of this finding remains unclear, but it may be due to the scar formation and increased vascularity after regeneration. In 7 (15%) cases, we had found nonunion in posterior region of meniscus. Interestingly, we had observed a homogenous signal in 5 (10%) cases, which is uncommon after meniscus suturing without biological healing enhancing methods. We also had observed changes in shape of meniscus; however, this finding has been reported as sensitive but not specific for the diagnosis of recurrent tears.28

Similarly to Popescu et al.,30 no correlation between MRI outcomes, healing outcomes and clinical scores, Barrett’s criteria, patient’s satisfaction, or the return to previous level of activity was found in the study. On the other hand, Morgan et al.31 reported 84% asymptomatic patients after meniscal repair. Of these, 65 % had healed completely and 19 % had healed incompletely, leaving a failure of 16%. All failures remained symptomatic, while all healed and incompletely healed menisci were asymptomatic. Similar observations were described by Cannon and Vittori32 and Pujol et al.33,34

Miao et al.35 have recently compared meniscal treatment techniques, and found that strict clinical evaluation resulted in lower estimates of the healing rate compared with MRI or second-look arthroscopy. Similarly to Miao and Pujol, we are of the opinion that a thorough clinical evaluation, including medical history and physical examination, seems to remain the “gold standard” in short-term follow-up, and this may be supplemented with imaging studies when needed.35,36

“All-inside” suture materials, such as Fast-Fix, are now widely available. The occurrence of meniscal cyst is rather frequently related to all known suture techniques and materials.37,38 In the present study, anchor cysts were noted in 29 (62%) menisci in follow-up MRI evaluation, without significant or clinically relevant correlation with clinical outcome.

Type of lesion, type of surgery, timing of biological healing, and the patient’s symptoms determine the various types of rehabilitation protocol available for a full recovery. The optimal physiotherapy program after meniscus repair has not been established.39-41 Specific physiotherapy treatment applicable after AMMR procedure has been designed and used for the patients of this study (see Appendix B).

The results presented in this study were collected on a consecutive case series from a single center. The present study has several limitations. By the virtue of the case series model, the study design did not include a control or comparison group. The alternative of treatment for the participants of current study would be total or partial meniscectomy. From the literature and clinical experience it is known that such treatment leads to biomechanical suboptimal condition and increases the risk of development of the arthritic changes in the knee joint.7,41 Possibility of control group containing simple suturing of menisci was also contemplated; however, the clinical experience of menisci sutured in white-white zone were not satisfactory. It has proven to be ethically unjustified to propose the clinical study with the control group, which will evidently not be benefiting from the treatment and such proposal was in fact not considered by our institutional review board. The goal of this study was to evaluate the safety of the procedure and early clinical outcome of the preserved menisci treated with the collagen wrapping technique.

Since majority of patients in the study had additional lesions, including cartilage defects and anterior cruciate ligament deficiency. It cannot be excluded that those
concomitant defects affected the final clinical outcome due to potential differences in the biology of the intrasynovial fluid.

Furthermore, neither calculation of the sample size nor power analysis was performed. Such a study design may be confounded by selection bias, which limits statements on the causality of correlations observed. The inclusion criteria based on the extent and type of meniscal lesion were assessed and operated by one surgeon. Video and photographic documentation have been however obtained for each case documenting meniscal lesion and the justification for using the collagen wrapping surgical technique according to the selection criteria of the study design.

For the recruitment for this study, the lower limb axis was not a limiting factor. Patients underwent full clinical orthopedic examination at each time point. The study objective was to assess the clinical state after applying the new technology regardless of the axis of the leg. No patient underwent any leg axis correction.

A Barrett score used for meniscus assessment is a well-established tool used for many years for research and clinical purposes.\(^\text{15}\) The numerical representation of the Barrett score, described in detail in Appendix A was simple conversion of the single variables into the one summarized value.

Safety of the procedure was evaluated based on pass-fail criteria and adverse events recording. None of the recorded adverse events were related to the procedure or material used in the procedure. There were 2 cases of arthrofibrosis occurring postoperatively (4%). Both patients have been successfully treated and achieved full recovery and full range of motion in the knee joint. Based on the published data the frequency of arthrofibrosis of the knee joint after arthroscopic interventions is reported between 8% and 10%.\(^\text{37,42}\)

The compartment syndrome can occur after arthroscopic procedure. This has been described in the literature.\(^\text{43-49}\) We agree that an accumulation of irrigation fluid passing through a popliteal cyst into the superficial flexor compartment can be potential cause at least should be suspected. In summary, even with an optimal perioperative management the subsequent compartment syndrome due to knee arthroscopy cannot be completely avoided.

The results of this study can be considered for “salvage” treatment of complex meniscal tears in patients who otherwise would have undergone total or partial meniscectomies. The 2-year follow-up is generally considered as a minimum time span to assess the results of the orthopedic intervention in the knee joint. In fact, the current understanding is that meniscal healing is reported to be achieved at 6 months.\(^\text{50,51}\)

The surgical technique applied in this study will be further validated through an extended follow-up for the next 3 years.

**Conclusions**

A clinical results at the 2-year follow-up of the patients operated by AMMR technique due to the meniscal lesions localized in the white-white and white-red zones demonstrated that presented surgical technique can offer a safe and promising alternative for surgeons who are willing to make an effort to save the meniscus in the patients otherwise scheduled for meniscal removal.

**Appendix A**

Converted Barrett score: Method of calculation of the digital representation of the Barrett’s clinical criteria of meniscal healing. This scoring system applies the concept of numerical notation used in the computations.

The aim of this calculation is to convert the 4 clinically meaningful signs (pain, effusion, clicking/locking, McMurray test) that are coded binary (0 = absence, 1 = presence) into the numerical notation enabled for further processing. The final values may vary from 0 to 15 and each value represents the discrete stage of the knee joint with respect to the initial Barrett’s evaluation criteria.

The following notations were adopted for the description of the Barrett’s criteria:

| Score | Description |
|-------|-------------|
| 0 | No symptoms in any of all four criteria |
| 1 | Pain or joint-line tenderness |
| 2 | Effusion |
| 3 | 1 + 2; pain or joint-line tenderness + effusion |
| 4 | Clicking or locking |
| 5 | 1 + 4; pain or joint-line tenderness + clicking or locking |
| 6 | 2 + 4; effusion + clicking or locking |
| 7 | 1 + 2 + 4; pain or joint-line tenderness + effusion + clicking or locking |
| 8 | Positive McMurray’s test |
| 9 | 1 + 8; pain or joint-line tenderness + positive McMurray’s test |
| 10 | 2 + 8; effusion + positive McMurray’s test |
| 11 | 1 + 2 + 8; 1 + 8; pain or joint-line tenderness + effusion + positive McMurray’s test |
| 12 | 4 + 8; clicking or locking + positive McMurray’s test |
| 13 | 1 + 4 + 8; pain or joint-line tenderness + clicking or locking + positive McMurray’s test |
| 14 | 2 + 4 + 8; effusion + clicking or locking + positive McMurray’s test |
| 15 | 1 + 2 + 3 + 8; pain or joint-line tenderness + effusion + clicking or locking + positive McMurray’s test |
## Appendix B

### Physiotherapy Guidelines for Autologous Membrane Meniscus Repair.

| Phase                        | Weeks  | Sessions | Suggested Therapeutic Interventions                                                                 |
|------------------------------|--------|----------|----------------------------------------------------------------------------------------------------|
| Protection and joint activation | 0-2    | Home-based supervised rehabilitation                                                                 |
|                              |        |          | - Exercise instruction                                                                          |
|                              |        |          | - Improved biomechanics patient education                                                        |
|                              |        |          | - Cryotherapy, elevation, and compression                                                         |
|                              |        |          | - Calf elongation                                                                               |
|                              |        |          | - Quadriceps isometric                                                                         |
|                              |        |          | - Gluteal isometric                                                                            |
|                              |        |          | - Antithrombotic exercises                                                                      |
|                              |        |          | - 0-2 Home-based supervised rehabilitation                                                       |
|                              |        |          | - Exercise instruction                                                                          |
|                              |        |          | - Improved biomechanics patient education                                                        |
|                              |        |          | - Cryotherapy, elevation, and compression                                                         |
|                              |        |          | - Calf elongation                                                                               |
|                              |        |          | - Quadriceps isometric                                                                         |
|                              |        |          | - Gluteal isometric                                                                            |
|                              |        |          | - Antithrombotic exercises                                                                      |
|                              |        |          | - 2 weeks after surgery                                                                          |
|                              |        |          | - No CPM exercises due to outside-facility rehabilitation                                        |
|                              |        |          | - Quadriceps NMES for 15-20 minutes                                                              |
|                              |        |          | - Foot-to-ground contact                                                                         |
|                              |        |          | - Recommendation for patient:                                                                    |
|                              |        |          | - To exercise 2-3 times a day                                                                    |
|                              |        |          | - To perform from 3 x 15 to 3 x 25 repetitions                                                   |
|                              |        |          | - If necessary antithrombotic stocking is applied                                               |
|                              |        |          | - Range of motion limited to 60°                                                                  |
|                              |        |          | - 2 weeks after surgery                                                                          |
|                              |        |          | - No CPM exercises due to outside-facility rehabilitation                                        |
|                              |        |          | - Quadriceps NMES for 15-20 minutes                                                              |
|                              |        |          | - Foot-to-ground contact                                                                         |
|                              |        |          | - Recommendation for patient:                                                                    |
|                              |        |          | - To exercise 2-3 times a day                                                                    |
|                              |        |          | - To perform from 3 x 15 to 3 x 25 repetitions                                                   |
|                              |        |          | - If necessary antithrombotic stocking is applied                                               |
|                              |        |          | - Range of motion limited to 60°                                                                  |

| Phase                        | Weeks  | Sessions | Suggested Therapeutic Interventions                                                                 |
|------------------------------|--------|----------|----------------------------------------------------------------------------------------------------|
|                              | 3-6    | 2-3 times a week 50-60 minutes per session | Aquatic therapy session                                                                                      |
|                              |        |          | - Weightbearing ROM exercise                                                                      |
|                              |        |          | - Hamstring flexibility exercises                                                                 |
|                              |        |          | - Triceps sure flexibility exercises                                                                |
|                              |        |          | - Isometric gluteal, hamstring, quadriceps, triceps sure muscle exercises                           |
|                              |        |          | - Active hip exercises in lying position                                                           |
|                              |        |          | - Weightbearing proprioceptive exercises                                                           |
|                              |        |          | - Active strengthening exercises with elastic resistance performed on healthy limb                |
|                              |        |          | - Active strengthening exercises with elastic resistance performed on operated limb               |
|                              |        |          | - Patellar mobilizations in all directions                                                         |
|                              |        |          | - Knee post–isometric relaxation exercises                                                         |
|                              |        |          | - Functional massage                                                                             |
|                              |        |          | - Deep massage                                                                                   |
|                              |        |          | - Fascia techniques                                                                               |
|                              |        |          | - Manual mobilization                                                                             |
|                              |        |          | - Knee extension exercise with external load                                                       |
|                              |        |          | - Stationary cycling with minimal external load                                                    |
|                              |        |          | - Double limb squat to 1/3 flexion ROM                                                              |
|                              |        |          | - Core stability exercises                                                                        |
|                              |        |          | - Quadriceps flexibility exercises                                                                  |
|                              |        |          | - Learning the proper limb loading on ground reaction force platform                                |
|                              |        |          | - Gait training according to weightbearing restrictions                                              |
|                              |        |          | - Forward step-down and forward step-ups from an 8-inch step                                       |
|                              |        |          | - Lateral step-ups within safe range of knee flexion with full weightbearing                       |
|                              |        |          | - Aquatic therapy added as soon as surgical incision is healed                                     |
|                              |        |          | - Walking, jogging in aqua jogging belts and with aquatics training kickboards                     |
|                              |        |          | - Swimming freestyle and backstroke                                                                |

- Minimal or no pain Knee flexion-extension CPM movements for 15-20 minutes
- Minimal or no effusion
- Pain-free gait without crutches
- Knee flexion ROM ≥100°
- Muscular activation recovery
- Recovery of normal gait cycle
- 2-3 weeks—30% load
- 3-4 weeks—50% load
- 5-6 weeks—no crutches
- Range of motion limited to 90° for 4 weeks
- Load percentage is measured on ground reaction force platform
- In cases where the patient is able to walk with a locked knee one can give up crutches and walking with the knee extended
| Phase                  | Weeks | Sessions | Suggested Therapeutic Interventions                                                                 | Progression Criteria                                                                 | CPM          | NMES                      | Weightbearing Restrictions                                                                 | Additional Comments                                                                                     |
|-----------------------|-------|----------|----------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------|--------------|---------------------------|---------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|
| Functional joint recovery | 7-8   | 1-2 times a week | 50-60 minutes per session | Full load proprioceptive exercises  
Continuous flexibility exercises  
Gait training on treadmill  
Double limb squat to 90° of knee flexion on stable and unstable ground  
Progressing core stability training  
Gluteal, quadriceps, hamstring, hip muscles strengthening exercises progressing from concentric to eccentric load  
Closed kinetic chain exercises on stable and unstable ground  
Deadlift exercises  
Continue stationary cycling with progressing external load  
One-leg squat on stable and unstable ground  
Forward lunges  
Progressing forward step-down and step-ups, and lateral step-ups  
Continuous aquatic therapy with hand bars, thigh weights, aquatic ankle cuffs  
Continuous freestyle and backstroke with swimming fins  
Continuous flexibility exercises  
Progressing proprioception exercises from unidirectional to multidirectional, from double leg to single leg patterns  
Continuous cycle and orbitrek ergometer with increasing duration  
Progressing one-leg squat on stable and unstable ground with additional external load  
Progressing closed kinetic chain exercises on stable and unstable ground  
Progressing deadlift exercises  
Forward lunges, forward step-ups, and lateral step-ups within safe range of knee flexion after full weight bearing  
Progressing aquatic therapy with hand bars, thigh weights, aquatic ankle cuffs  
Progressing freestyle and backstroke with swimming fins | Pain-free 90° of knee flexion on stable and unstable ground  
No pain after high intensive exercises  
No effusion after high intensive exercises | If necessary knee flexion-extension CPM movements for 15-20 minutes | – 15-minute warm up on stationary cycle  
– If patient feels pain after high-intensity exercises we reduce the intensity and the rehabilitation process extends  
– Patients are recommended to practice at home |                                      |
| Aquatic therapy session (with strengthening program) | 9-16  | 1-2 times a week | 50-60 minutes per session | Continuous flexibility exercises  
Progressing proprioceptive exercises from unidirectional to multidirectional, from double leg to single leg patterns  
Continuous cycle and orbitrek ergometer with increasing duration  
Progressing one-leg squat on stable and unstable ground with additional external load  
Progressing closed kinetic chain exercises on stable and unstable ground  
Progressing deadlift exercises  
Forward lunges, forward step-ups, and lateral step-ups within safe range of knee flexion after full weight bearing  
Progressing aquatic therapy with hand bars, thigh weights, aquatic ankle cuffs  
Progressing freestyle and backstroke with swimming fins | Side to side absolute strength difference between 15% and 20% in isokinetic test at 16th week  
Side to side absolute endurance difference between 15% and 20% in isokinetic test at 16th week  
Side to side load difference between 15% and 20% in ground reaction force test at 16th week  
Postural Priority values between 40% and 60% in proprioceptive test at 16th week  
Knee flexion ROM ≥140°  
Ability to run on a treadmill at 8 km/h for more than 10 minutes | – 15-minute warm up on stationary cycle or Orbitrek  
– During the ground reaction force test patients perform “easier” movement patterns such as: upright position test, 90° knee squat, double leg jumps, jogging  
– Patients are recommended to practice at home |                                      |
At 16 weeks, the functional biomechanical evaluation in every (eligible) patient. The assessment consists of (1) isokinetic knee flexion—extension tests performed at 60 deg/s (5 repetitions) and 240 deg/s (30 repetitions) angular velocity to examine strength and endurance deficits, respectively; (2) proprioception and vestibular control strategy on Postural Proprioceptive System DPPS (on stable and unstable ground with eyes open and closed, depending on testing conditions); (3) ground reaction force investigation in 4 different movement patterns.

This functional biomechanical evaluation is performed to verify if the patient is prepared for dynamic exercises in the next rehabilitation phase.

| Phase   | Weeks | Sessions | Suggested Therapeutic Interventions | Progression Criteria | CPM | NMES | Weightbearing Restrictions | Additional Comments |
|---------|-------|----------|------------------------------------|----------------------|-----|------|---------------------------|---------------------|
| Sport-specific activity | 17-20 | 1-2 times a week | - Progressing exercises from previous phase | - Side to side absolute strength difference between 10% and 15% in isokinetic test at 20th week | | | | |
| Aqua therapy session | 50-60 minutes per session | | - Single-leg jump | - Side to side absolute endurance difference between 10% and 15% in isokinetic test at 20th week | | | | |
| | | | - Single-leg hop | | | | | |
| | | | - Double leg bounds | - Side to side load difference between 5% and 10% in ground reaction force test at 20th week | | | | |
| | | | - Single leg bounds | - Postural priority values ≥60% in proprioceptive test at 20th week | | | | |
| | | | - Plyometric exercises | - FMS test values ≥14 at 20th week | | | | |
| | | | - Aerobic exercises | - No pain during hell sitting exercises | | | | |
| | | | - Treadmill running exercises | | | | | |

At 20 weeks, the functional biomechanical evaluation in every patient is performed. The assessment consists of (1) isokinetic knee flexion—extension tests performed at 60 deg/s (5 repetitions) and 240 deg/s (30 repetitions) angular velocity to examine strength and endurance deficits, respectively; (2) proprioception and vestibular control strategy on Postural Proprioceptive System DPPS (on stable and unstable ground with eyes open and closed, depending on testing conditions); (3) ground reaction force investigation in 6 different movement patterns; (4) Functional Movement Screen test battery.

Functional biomechanical evaluation performed at this stage is to allow sport active patients for participating in high intensity sport-specific rehabilitation phase.
| Phase | Weeks | Sessions | Suggested Therapeutic Interventions                                      | Progression Criteria                                                                 | CPM       | NMES       | Weightbearing Restrictions | Additional Comments                                                                 |
|-------|-------|----------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------|-----------|------------|----------------------------|--------------------------------------------------------------------------------------|
| 21-24 | 1-2 times a week | 50-60 minutes per session | - Continue strengthening and flexibility exercises from previous phase  
- Aerobic running exercises  
- Progressive running  
- Acceleration-deceleration exercises  
- High-intensity exercises  
- Running with quick changes of direction  
- Coordination exercises  
- Functional sport-specific agility training  
- Presport conditioning training  
- Education and preparation for return to sport | - Side to side absolute strength difference  
- Side to side endurance difference  
- Side to side load difference  
- Postural priority values ≥60% in proprioceptive test  
- FMS test values ≥17 at 24th week  
- Lack of apprehension with sport specific movements  
- Dynamic neuromuscular control with multi-plane activities without pain or swelling | -  
-  
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At 24 weeks, the functional biomechanical evaluation in every active patients/athletes is performed. If the tested subject meets the results obtained at 20th week, he or she is allowed to return to sport activity.

Abbreviations: CPM, continuous passive motion; FMS, Functional Movement Screen; NMES, neuromuscular electrical stimulation; ROM, range of motion.
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Declaration of Conflicting Interests
Prof. Jakob serves as a paid consultant to Geistlich Pharma AG. Dr Slomczykowski serves as a Medical Director to Geistlich Pharma AG. The other authors do not report any conflicts of interest.

Ethical Approval
Ethical approval for this study was obtained from the IRB Committee of the Medical University of Poznan/Poland, and it has been performed according to the ethical standards laid down in the 1964 Declaration of Helsinki.

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