

## Vancomycin soaking of ACL reconstructions does not alter the mechanical strength

V. JAHIER, J. MAXIMEN, P. GUILLEMONT, Q. BIDARD, M. ROPARS, H. COMMON

*Orthopaedic and trauma surgery, Rennes University Hospital Pontchaillou, Rennes, France.*

Correspondence at: Julien Maximen, Orthopaedic and Trauma surgery, Rennes University Hospital Pontchaillou, 2 rue Henri le Guilloux, 35033 Rennes, France, Email [julien.maximen@chu-rennes.fr](mailto:julien.maximen@chu-rennes.fr)

**Reconstruction of the anterior cruciate ligament (ACL) using hamstring autograft presents a greater risk of surgical site infection than other transplants (0.5% to 1.5%). Various prophylactic methods have been evaluated, such as double antibiotic protection with intravenous Cefazolin and soaking of the transplant with vancomycin, which results in a near-zero infection rate. This soaking method has only been studied in vitro, confirming the absence of toxicity of vancomycin on tenocytes. The primary objective of our study was to assess the mechanical quality of vancomycin-soaked tendon transplants at 6 months post-operatively. The secondary objective was to assess the rate of new rupture at 1 year.**

**This is a retrospective, single-center study including patients who underwent ACL reconstruction using a vancomycin-soaked hamstring autograft at Rennes University Hospital between December 2018 and February 2021. An objective assessment of joint laxity by means of the GNRB® at 134N was performed at 6 months post-operatively and a clinical evaluation (Lysholm and subjective IKDC questionnaires) at 1 year were performed.**

**Fifty-three patients aged between 18 and 57 years were included. At 6 months, GNRB® of the operated knee joints was significantly different compared with the non-operated ones but was below the threshold for complete or partial rupture according to the manufacturer's clinical significance threshold (3 mm for a complete rupture and 1.5 mm for a partial rupture). At one year, 3.8% of our population had an early rupture, the reason for which was technical defects in the positioning of the graft.**

**The stability of knees reconstructed with a Vancomycine soaked ACL graft is comparable with that of the contralateral knee with an intact ACL.**

Level of evidence : IV, retrospective study.

**Keywords :** anterior cruciate, vancomycin, hamstring, strength, ligamentization.

### INTRODUCTION

Early post-operative infection is a serious complication of anterior cruciate ligament (ACL) reconstructions. Fortunately, it is a rare event, the frequency of which varies between 0.5 and 1.5% according to studies for hamstring autograft<sup>1,2</sup>. Perez-Prieto et al. concluded that the rate of contamination occurred mainly during harvesting and preparation of the graft<sup>3</sup>. In 2012, Vertullo et al.<sup>4</sup> proposed soaking of hamstring auto-grafts using vancomycin in addition to antibioprophyllaxis in order to reduce the rate of infectious complications. Their study confirmed that this method significantly reduced the rate of post-operative infection, which is active against the germs that cause the majority of septic complications, such as *Staphylococcus Aureus* and *Epidermidis*. Numerous authors have subsequently confirmed these results, but for the moment there are

no universally accepted recommendations<sup>5-8</sup>. In 2022, Carrozzo et al.<sup>9</sup> published a systematic review of this technique, demonstrating a significant reduction in the post-operative infection rate of ACL reconstructions for all grafts used. These conclusions led to in vitro studies evaluating the safety of vancomycin, confirming the absence of adverse effects on tenocytes<sup>10</sup>.

To date, no study has objectively assessed the residual joint laxity of vancomycin-soaked ACL reconstruction in vivo. Clinical assessment of anterior and rotatory knee laxity by clinical examination remains imprecise and subjective<sup>11</sup>. Numerous automated measurement systems have been developed and tested to compensate for this lack of reliability, such as the KT-1000®, GNRB® and Telos®<sup>12-14</sup>. The GNRB® system appears to have the best inter- and intra-observer reproducibility, with threshold values for a 134N thrust of 1.5mm of differential anterior laxity (sensitivity of 76.5% and

specificity of 94%)<sup>14-17</sup>. A joint laxity difference of 1.5 mm between the two knees is in favor of a partial ACL rupture in the laxer knee. A joint laxity of more than 3 mm is in favor of a complete ACL rupture. It enables joint laxity to be analyzed pre-operatively and then post-operatively<sup>14</sup>: this assessment is objective and reproducible.

The primary objective of our study was to evaluate the laximetric qualities of vancomycin-soaked hamstring autograft at 6 months post-operatively after ACL reconstruction by comparing them with the joint laxity of the contralateral healthy knee on the GNRB® at 134N.

The secondary objective was to assess the rate of rupture at 1 year.

Our hypothesis was that intraoperative soaking of the transplant with vancomycin did not affect the biochemical and histologic remodeling of the graft tissue from tendon to ligament and consequently the residual joint laxity of ACL reconstructions using hamstring autograft.

## MATERIALS AND METHODS

This is a single-operator retrospective monocentric cohort study (H.C.) of patients operated on for ACL reconstruction using hamstring autograft, with arthroscopic guidance, between December 2018 and February 2021, at Rennes University Hospital. All participants gave their consent to take part in the study with the authorization of the institution's ethics committee issued a favourable opinion (opinion 22.97).

All patients underwent ACL reconstruction using hamstring autograft with or without a Lateral Extra-articular Tenodesis (LET) with a minimum follow-up of 1 year.

Exclusion criteria were the use of a tendon transplant other than hamstring, multi-ligament lesions, revision ACL reconstruction, patients of legal age under guardianship or curatorship, contralateral rupture not allowing reliable laximetry, and patients with a vancomycin allergy.

### *Preoperative protocol :*

All patients received a pre-operative shower with mild soap but no antiseptics within 24 hours prior to surgery. In the operating theatre, alcoholic chlorhexidine was used before sterile drapes were applied. Antibiotic prophylaxis with a 2g bolus of intravenous cefazolin was carried out at the time of induction, approximately 30 min before the incision. A tourniquet was used

during the arthroscopic procedure. Harvesting of the hamstring tendons was realized without tourniquet.

During the operation, the harvested hamstrings were soaked with a solution of vancomycin (1g/20mL) using a compress which was positioned around the graft throughout the reconstruction procedure. Soaking ended when the transplant was placed in the tunnels. If a LET was performed using hamstring autograft, it was soaked the same way as described above. The transplant fixation system depended on the surgical technique used: adjustable endobutton (Tightrope® RT and ABS; Arthrex, Naples, FL) or interference screws (Crosslig®; FH Orthopedics).

Every ACL reconstruction patient was monitored jointly by the orthopaedic department and the sports medicine department. Pre-operative evaluation of laximetry was performed using GNRB® at 134N, with the pathological knee compared with the healthy knee. Post-operative clinical assessments were performed at 1 month, 4 months, 6 months and 1 year, and a laximetry test was systematically performed at four and a half months and six months postoperatively. During the test, 3 measurements were taken in order to obtain a median. The outpatient follow-up session at one year was used to assess the patient's return to work and sport, and to collect functional scores (Lysholm scale and subjective IKDC).

A rehabilitation care pathway was carried out on an outpatient basis by state-qualified physiotherapists following a standardized protocol.

The secondary endpoint was assessed by clinical examination at 1 year, supplemented by questionnaires validated in ACL reconstruction follow-up (Lysholm and subjective IKDC<sup>18</sup>).

Quantitative variables were expressed as mean, standard deviation, minimum and maximum, and qualitative variables as percentages.

For the analysis of our primary endpoint, a univariate analysis using Student's t-test on a matched population was performed on the absolute values of joint laxity measured with GNRB® at 6 months at 134N, matching healthy and operated knees.

For the secondary endpoint, the rupture rate at 1 year in our cohort was reported.

A Mann-Whitney Wilcoxon test was performed to identify any difference in laxity of the knee operated on at 134N on the GNRB® at 6 months, depending on whether or not a meniscus suture had been performed. Similarly, a second Mann-Whitney Wilcoxon test was performed to identify any difference in laxity between the healthy knee and the knee operated on at 134N on

the GNRB® at 6 months, depending on whether or not a LET had been performed.

Statistical analyses were performed using SAS software (SAS Institute, Cary, USA) version 9.4 by a biostatistician. All p-values less than 0.05 were considered statistically significant.

**RESULTS**

Eighty-five patients underwent surgery between December 2018 and February 2021. A total of 32 patients were excluded. Fifty-three patients were included, 39 men and 14 women. The mean age was 28 years (minimum 18 years, maximum 57 years). The mean follow-up time from surgery at the time of data collection was 35 months (±7). The mean Tegner score was 5 (± 1). Figure 1 shows the flow chart of our study. The characteristics of our population are detailed in Table I.

At one year, no patient had developed a post-operative infection. No adverse effects related to vancomycin soaking was observed.

Post-operative differential joint laxity was improved in all patients (p<0.0001), with a mean of 1 ± 0.52mm from a mean pre-operative laxity of the ruptured ACL of 6.40 ± 1.04mm. The mean GNRB® laxity at 6 months at 134N did not differ between the healthy (3.49 ± 0.91mm) and the operated knee (4.50 ± 1.43mm).

Figure 2 shows the laximetric data measured at 6 months post-operatively.

At 12 months, two patients (3.8%) had a rupture of their ACL reconstruction. The diagnosis was initially clinical, after a new trauma, confirmed by MRI. Our population had a mean Lysholm score of 87 ± 13 and a mean subjective IKDC of 85.7 ± 4.3%.

**Table I.** — Clinical characteristics of the 53 ACL surgery patients included in the study

Characteristics	Value
Age (years) *	28 (±8)
Sex **	
Male	39 (74)
Female	14 (26)
Mean Tegner*	4.9 (±1)
Post-operative follow-up at inclusion date (months)*	35 (±7)
Type of transplant**	
Five-strand	40 (76)
Four-strand	5 (9)
Six-strand	5 (9)
Three-strand	3 (6)
Associated meniscus suture**	35 (66)
Associated Lateral extra-articular tenodesis**	45 (85)

\*N (±standard deviation); \*\*N (%).

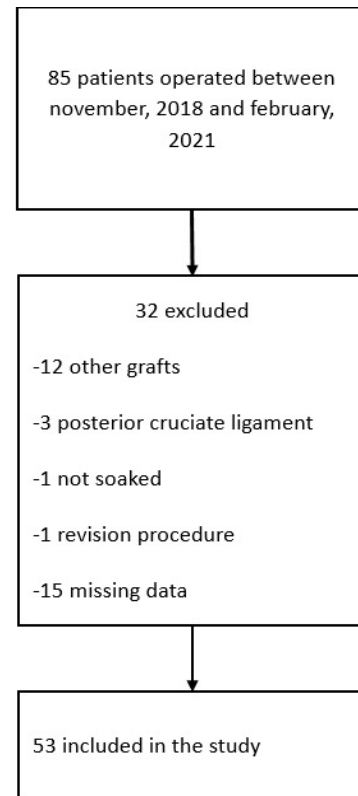


Figure 1 — Flow chart of the study.

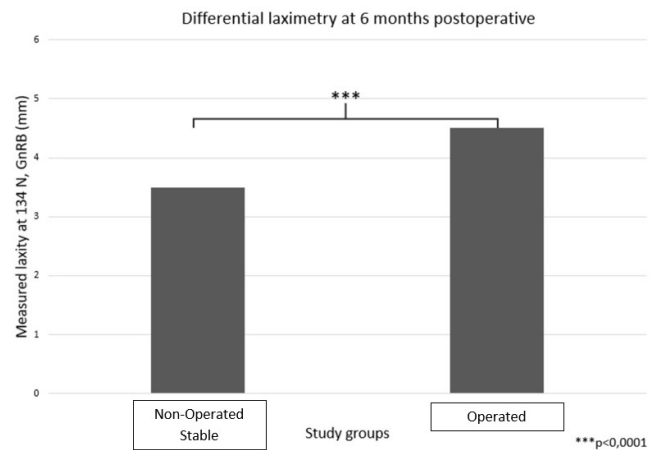


Figure 2 — Absolute values of transplant laxity at 6 months, in millimeters, on the GNRB® at 134N between the non-operated stable and the operated knee.

\*\*\*Mann-Whitney Wilcoxon test.

The results of the comparative analysis according to whether meniscus suture had been performed showed no significant difference in laximetric data at 6 months. There was also no significant difference at 6 months according to whether a lateral tenodesis had been performed. Table II presents the results of the comparisons made.

**Table II.** — GNRB® laximetric data at 134N at 6 months, depending on the associated procedure

Laximetric data	Mean	P**
Joint Laxity*		
<i>Meniscal suture</i>		
Yes	4,8 (1,6)	0,091
No	4,0 (0,9)	
<i>Lateral tenodesis</i>		
Yes	4,6 (1,5)	0,296
No	4,1 (1,2)	
Delta*		
<i>Meniscal suture</i>		
Yes	1,1 (1,7)	0,560
No	0,7 (1,1)	
<i>Lateral tenodesis</i>		
Yes	1,0 (1,5)	0,990
No	1,0 (1,6)	

\*Mean ( $\pm$  standard deviation); \*\* Mann-Whitney Wilcoxon test.

## DISCUSSION

The clinical and demographic characteristics of our 53 patients are comparable to those of previously published ACL series<sup>19,20</sup>. Regarding the tool used to assess joint laxity at 6 months, several studies have shown good reliability of GNRB® measurement, compared with the clinical Lachman or other laximetry devices<sup>11,15,17</sup>. In vitro studies have shown that vancomycin is not toxic to tenocytes<sup>21</sup> and that the tendon has a storage effect allowing vancomycin to be released in the first 24 hours post-operatively<sup>22</sup>. Studies carried out on animal models found no change in the mechanical properties of tendons after soaking with vancomycin<sup>23,24</sup>. Furthermore, a study by Falconiero et al. in 1998<sup>25</sup> found no significant change in the histological properties of the transplant after 6 months, particularly in terms of cellularity and vascularization, compared with the same transplant at 1 year, in reconstructions performed exclusively on hamstrings, suggesting that the ligamentization process is already well advanced at 6 months and is only slightly modified over time, making our analysis at 6 months relevant. Scranton et al.<sup>26</sup> in 1998 also corroborated these findings. The rate of post-operative sepsis reported in the literature ranged from 0.14 to 2.2% for transplants not soaked with vancomycin<sup>1,2,27</sup>. In our study, no infection was reported. This is comparable with what has been found in the literature after vancomycin soaking, reinforcing the interest of this soaking in knee ligament surgery<sup>4,9</sup>. In our series of 53 patients who underwent ACL reconstruction with a vancomycin-soaked hamstring autograft, we found a difference in joint stability that was statistically significant but not clinically significant, as

it was below the manufacturer's threshold values<sup>14</sup>. The mean Lysholm score is considered good to excellent<sup>18</sup>, and our Tegner score is also comparable to the normal post-ligamentoplasty progression. In view of these results, our hypothesis was confirmed: intraoperative soaking of the transplant with vancomycin does not alter its biomechanical qualities at 6 months post-operatively.

Regarding the secondary endpoint, our rupture rate was higher than in the literature at 1 year, with 3.8% of our cohort having an iterative ACL rupture. After reviewing the files, the cause of these ruptures was due to a technical error. One of the patients had a tibial tunnel that was too anterior and in conflict with the notch. The second patient had a femoral fixation defect. These early iterative ruptures do not therefore appear to be attributable to the use of vancomycin but resulting from surgical technical errors.

The presence of damage to the knee's secondary stabilizers (menisci and anterolateral ligament) may influence the result of GNRB® laximetry, which tests not only the ACL but also the overall anterior drawer. Subgroup analyses comparing joint laxity according to the presence of a meniscus suture or a LET did not find any significant difference between the groups. These results suggest that these characteristics did not influence our primary endpoint.

Our research has demonstrated the absence of deleterious effects of vanco soaking of ACL in vivo. First, to our knowledge and following a literature search, there is no other study looking at the in vivo impact of vancomycin soaking on ligamentoplasty transplants. Only one study investigated the ligamentization process using GNRB®, but did not take vancomycin soaking into account<sup>28</sup>. A study by Bohu et al.<sup>27</sup> in 2020 found no significant difference in return to sport (running) between patients with and without vancomycin-soaked transplants but did not assess the ligamentization process. Secondly, reconstructive ACL surgery has been consistent in terms of similar procedural set up in light of the fact that the single surgeon (HC) in this monocentric study did not alter the method of vancomycin soaking of the ACL tendon implant during the study period.

However, our study has certain limitations. Four types of transplants systems were used, which would possibly constitute a significant bias in the assessment of postoperative joint laxity. However, the literature, based on clinical and laximetric assessment, does not find any significant difference in ligamentization between the different hamstring transplants<sup>29-32</sup>. In addition, a 2014 study by Renata Vauhnik et al.<sup>33</sup> found

low interobserver reliability for GNRB®. The data collected for our study were measured by several study collaborators over 3 years, so this is a bias in our study. Finally, a major bias in our study was the absence of a control group who had undergone ligamentoplasty without vancomycin soaking. All knee reconstructive surgeons in our centre continue to soak ACL tendon implants with vancomycin.

## CONCLUSION

Vancomycin soaking of hamstring autograft for ACL reconstruction did not compromise the mechanical quality of the transplants at 6 months post-operatively. It did not appear to alter the post-operative laximetric results of the reconstructions or the rate of early rupture.

*Credit author statement:* VJ: data collection, statistical analysis, literature review and manuscript writing; JM: manuscript writing and editing; PG: study design, data collection; QB: literature review and manuscript writing; MR: study design; HC: study design, supervision, literature review and manuscript editing. All authors read and approved the final manuscript.

## REFERENCES

- Maletis GB, Inacio MCS, Reynolds S, Desmond JL, Maletis MM, Funahashi TT. Incidence of Postoperative Anterior Cruciate Ligament Reconstruction Infections: Graft Choice Makes a Difference. *Am J Sports Med.* 2013;41:1780-5.
- Barker JU, Drakos MC, Maak TG, Warren RF, Williams RJ, Allen AA. Effect of Graft Selection on the Incidence of Postoperative Infection in Anterior Cruciate Ligament Reconstruction. *Am J Sports Med.* 2010;38:281-6.
- Pérez-Prieto D, Portillo ME, Torres-Claramunt R, Pelfort X, Hinarejos P, Monllau JC. Contamination occurs during ACL graft harvesting and manipulation, but it can be easily eradicated. *Knee Surg Sports Traumatol Arthrosc.* 2018;26:558-62.
- Vertullo CJ, Quick M, Jones A, Grayson JE. A Surgical Technique Using Presoaked Vancomycin Hamstring Grafts to Decrease the Risk of Infection After Anterior Cruciate Ligament Reconstruction. *Arthrosc J Arthrosc Relat Surg.* 2012;28:337-42.
- Naendrup JH, Marche B, de SA D, Koenen P, Otchwemah R, Wafaisade A, et al. Vancomycin-soaking of the graft reduces the incidence of septic arthritis following ACL reconstruction: results of a systematic review and meta-analysis. *Knee Surg Sports Traumatol Arthrosc.* 2020;28:1005-13.
- Schuster P, Schlumberger M, Mayer P, Eichinger M, Geblein M, Reddemann F, et al. Soaking of the graft in vancomycin dramatically reduces the incidence of postoperative septic arthritis after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2020;28:2587-91.
- Pérez-Prieto D, Torres-Claramunt R, Gelber PE, Shehata TMA, Pelfort X, Monllau JC. Autograft soaking in vancomycin reduces the risk of infection after anterior cruciate ligament reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2016;24:2724-8.
- Phegan M, Grayson JE, Vertullo CJ. No infections in 1300 anterior cruciate ligament reconstructions with vancomycin pre-soaking of hamstring grafts. *Knee Surg Sports Traumatol Arthrosc.* 2016;24:2729-35.
- Sonnery-Cottet B, Carrozzo A, Saithna A, Ferreira A, Guy S, Chadli L, et al. Le prétrempage à la vancomycine des greffes de ligament croisé antérieur diminue la fréquence de l'arthrite septique postopératoire. *Revue systématique et méta-analyse de 26 183 patients. Rev Chir Orthopédique Traumatol.* 2021;107:S276.
- Xiao M, Leonardi EA, Sharpe O, Sherman SL, Safran MR, Robinson WH, et al. Soaking of Autologous Tendon Grafts in Vancomycin Before Implantation Does Not Lead to Tenocyte Cytotoxicity. *Am J Sports Med.* oct 2020;48:3081-6.
- Benvenuti JF, Vallotton JA, Meystre JL, Leyvraz PF. Objective assessment of the anterior tibial translation in Lachman test position. Comparison between three types of measurement. *Knee Surg Sports Traumatol Arthrosc Off J ESSKA.* 1998;6:215-9.
- Daniel DM, Stone ML, Sachs R, Malcom L. Instrumented measurement of anterior knee laxity in patients with acute anterior cruciate ligament disruption. *Am J Sports Med.* 1985;13:401-7.
- Zehi K, Ben Hamida F, Belkadh A, Naanaa T, Ben Zid M, Douik M, et al. Intérêt du Télors dans l'évaluation de la laxité chronique antérieure du genou en pré- et en postopératoire. *J Traumatol Sport.* 2007;24:67-72.
- Robert H, Nouveau S, Gageot S, Gagnière B. A new knee arthrometer, the GNRB: experience in ACL complete and partial tears. *Orthop Traumatol Surg Res OTSR.* 2009;95:171-6.
- Jenny JY, Puliero B, Schockmel G, Harnois S, Clavert P. Validation expérimentale de l'appareil GNRB pour la mesure de la translation antérieure du tibia. *Rev Chir Orthopédique Traumatol.* 2016;102:S302.
- Ryu SM, Na HD, Shon OJ. Diagnostic Tools for Acute Anterior Cruciate Ligament Injury: GNRB, Lachman Test, and Telors. *Knee Surg Relat Res.* 2018;30:121-7.
- Collette M, Courville J, Forton M, Gagnière B. Objective evaluation of anterior knee laxity: comparison of the KT-1000 and GNRB® arthrometers. *Knee Surg Sports Traumatol Arthrosc Off J ESSKA.* 2012;20:2233-8.
- Chaory K, Poiradeau S. Les grilles d'évaluations dans la ligamentoplastie du LCA. *Ann Réadapt Médecine Phys.* 2004;47:309-16.
- Sanders TL, Maradit Kremers H, Bryan AJ, Larson DR, Dahm DL, Levy BA, et al. Incidence of Anterior Cruciate Ligament Tears and Reconstruction: A 21-Year Population Based Study. *Am J Sports Med.* 2016;44:1502-7.
- Ardern CL, Webster KE, Taylor NF, Feller JA. Return to sport following anterior cruciate ligament reconstruction surgery: a systematic review and meta-analysis of the state of play. *Br J Sports Med.* 2011;45(7):596-606.
- Xiao M, Leonardi EA, Sharpe O, Sherman SL, Safran MR, Robinson WH, et al. Soaking of Autologous Tendon Grafts in Vancomycin Before Implantation Does Not Lead to Tenocyte Cytotoxicity. *Am J Sports Med.* 2020;48:3081-6.
- Grayson JE, Grant GD, Dukie S, Vertullo CJ. The In Vitro Elution Characteristics of Vancomycin from Tendons. *Clin Orthop.* 2011;469:2948-52.
- Schüttler KF, Scharm A, Stein T, Heyse TJ, Lohoff M, Sommer F, et al. Biomechanical and microbiological effects of local vancomycin in anterior cruciate ligament (ACL) reconstruction: a porcine tendon model. *Arch Orthop Trauma Surg.* 2019;139:73-8.
- Lamplot JD, Liu JN, Hutchinson ID, Chen T, Wang H, Wach A, et al. Effect of vancomycin Soaking on Anterior Cruciate Ligament Graft Biomechanics. *Arthrosc J Arthrosc Relat Surg.* 2021;37:953-60.
- Falconiero R, DiStefano V, Cook T. Revascularization and ligamentization of autogenous anterior cruciate ligament

- grafts in humans. *Arthrosc J Arthrosc Relat Surg.* 1998;14:197-205.
26. Scranton P, Lanzer W, Ferguson M, Kirkman T, Pflaster D. Mechanisms of anterior cruciate ligament neovascularization and ligamentization. *Arthrosc J Arthrosc Relat Surg.* 1998;14:702-16.
  27. Bohu Y, Klouche S, Sezer HB, Herman S, Grimaud O, Gerometta A, et al. Vancomycin-soaked autografts during ACL reconstruction reduce the risk of post-operative infection without affecting return to sport or knee function. *Knee Surg Sports Traumatol Arthrosc.* 2020;28:2578-85.
  28. Poudroux T, Pauvert A, Robert H. Suivi de laxité et de la compliance des greffes courtes aux ischio-jambiers après ligamentoplastie primaire du LCA. Évaluation de la ligamentisation pendant la 1<sup>re</sup> année?? *Rev Chir Orthopédique Traumatol.* 2018;104:S109.
  29. Kyung HS, Lee HJ, Oh CW, Hong HP. Comparison of results after anterior cruciate ligament reconstruction using a four-strand single semitendinosus or a semitendinosus and gracilis tendon. *Knee Surg Sports Traumatol Arthrosc.* 2015;23:3238-43.
  30. Kondo E, Kitamura N, Onodera J, Yasuda K, Inagaki Y, Tanaka Y, et al. Prospective clinical comparisons of semitendinosus versus semitendinosus and gracilis tendon autografts for anatomic double-bundle anterior cruciate ligament reconstruction. *J Orthop Sci.* 2013;18:754-61.
  31. Gobbi A, Domzalski M, Pascual J, Zanazzo M. Hamstring anterior cruciate ligament reconstruction: Is it necessary to sacrifice the gracilis? *Arthrosc J Arthrosc Relat Surg.* 2005;21:275-80.
  32. Barenius B, Webster WK, McClelland J, Feller J. Hamstring tendon anterior cruciate ligament reconstruction: does gracilis tendon harvest matter? *Int Orthop.* 2013;37:207-12.
  33. Vauhnik R, Morrissey MC, Perme MP, Sevsek F, Rugelj D. Inter-rater reliability of the GNRB® knee arthrometer. *The Knee.* 2014;21:541-3.