# Vancomycin-Concentrations in Synovial Fluid Do Not Reach Chondrotoxic Thresholds Following Anterior-Cruciate Ligament Reconstruction with Vancomycin-Soaked Autologous Soft Tissue Grafts: An In-Vivo Prospective Observational Study in Humans.

Thomas Pfeiffer [1], Arne Althoff [1], Sophia Krombholz [2], Max Dautert [3], Jan-Hendrik Naendrup [1], Daniel Günther [1], Bertil Bouillon [1], Mario Thevis [2]

[1] Cologne Merheim Medical Center, Department of Orthopedic surgery and Sporttraumatology, University Witten/Herdecke, Cologne, Germany

[2] Institute of Biochemistry/Center for Preventive Doping Research, German Sport University Cologne, Cologne, Germany.

Investigation performed at the Cologne Merheim Medical Center,

Cologne, Germany

Source of Funding:

None

Competing Interests:

none declared

Ethical Approval Statement:

University Witten/Herdecke Ethics Committee (S-247/2021)

Address Correspondence to:

Thomas Pfeiffer, MD

Department of Orthopaedic Surgery and Sporttraumatology,

University of Witten/Herdecke, Ostmerheimerstr 200, 51109

Cologne Germany

E-mail: Pfeiffert@kliniken-koeln.de

**Objectives:**

The purpose of this study was to measure the Vancomycin-concentration in the synovial fluid following ACL reconstruction with Vancomycin-soaked autografts. It was hypothesized that intra-articular Vancomycin-concentrations in the synovial fluid do not reach chondrotoxic threshold of 1000 µg/ml following Vancomycin-soaking of autologous semitendinosus and soft tissue quadriceps grafts for ACL reconstruction.

**Methods:** 10 patients undergoing ACL reconstruction using four-strand semitendinosus tendon autografts and 10 patients undergoing ACL reconstruction using soft tissue quadriceps tendon autografts were enrolled. Each graft was intraoperatively wrapped in 5 mg/ml vancomycin-soaked gauze swabs prior to implantation. Following wound closure an aspirate of 5 ml synovial fluid was taken of each patient. The vancomycin-concentration of the aspirate was analyzed using high performance liquid chromatography – tandem mass spectrometry (HPLC-MS/MS). Spearman-Rho correlation coefficients were used to identify relationships between the parameters and t-test to test for differences between graft types. A p-value of $< 0.05$ was considered statistically significant.

**Results:** 20 patients (15 women; 5 men, 29.35 ± 11.3 years) were included in the study. The mean Vancomycin-concentration measured in the synovial fluid was 23.23 µg/ml (± 21.68 µg/ml) with a minimum concentration of 2.32 µg/ml and a maximum concentration of 71.56 µg/ml. There was no significant difference between the two graft types ($p=0.911$). Significant positive correlation ($r = .644$ $p < 0.05$) was observed only between the Vancomycin-concentration and the duration from initiation of Vancomycin-soaking of semitendinosus grafts to implantation (13.4min ± 6min). No correlations were observed between the Vancomycin-concentration and the duration from

implantation to fluid aspiration as well as the Vancomycin-concentration and the graft diameter (Median 8.5mm Range 6.0-10.0mm) for both graft types.

**Conclusion**: Chondrotoxic Vancomycin-concentrations of equal to or greater 1000 µg/ml were not reached in any aspiration of synovial fluid following ACL-reconstruction using soft tissue autografts that were intraoperatively soaked in a 5 mg/ml Vancomycin-solution. Against the backdrop of multiple studies, showing significantly reduced infection rates after ACL-reconstruction when using Vancomycin-soaking, this study suggests that the chondrotoxic properties of this method are negligible due to its submarginal intraarticular concentrations.

**Level of Evidence:** Prospective observational study (Level of Evidence 3)

**Keywords:** Anterior Cruciate Ligament (ACL); Vancomycin Soaking; Vancomycin; Infection; Chondrotoxicity.

**Text Box:**

- This study describes the short-term postoperative intraarticular Vancomycin-concentration following ACL-reconstruction with hamstring and quadriceps tendon autografts using intraoperative Vancomycin-soaking.
- No chondrotoxic thresholds were reached in the synovial fluid on average 15 minutes after graft implantation.
- No differences regarding the intraarticular Vancomycin-concentration following ACL-reconstruction using hamstring and quadriceps tendon autografts were observed.

## Introduction

Despite being a rare complication, ranging from 0.14% to 4.4% in selected patient populations, postoperative septic knee arthritis following ACL reconstruction can individually have devastating long-term effects. [29,12,16,27] Therefore, any effort to minimize septic knee arthritis should be undertaken. Multiple meta-analyses showed that Vancomycin-soaking of the anterior cruciate ligament (ACL) graft can drastically reduce the incidence of postoperative septic knee arthritis following ACL reconstruction.[4,14,17,32]

However, reservation regarding this method still prevail and in 2021 two thirds of the members of the prestigious ACL Study Group denied soaking the ACL Graft in Vancomycin prior to implantation.[33] The most common concerns are Vancomycin induced weakening of the graft and chondrotoxicity. Regarding the weakening of the ACL graft, several in-vitro studies of human tenocytes suggest that there is a safe Vancomycin-concentration range, leaving tenocyte viability unaffected. Depending on the respective study, safe Vancomycin-concentrations varied between 2.5 mg/mL for up to 1 h of treatment and 12.8 mg/mL for up to 6 h of treatment.[20,31] Likewise, Vancomycin showed no effect on the biomechanical properties of ACL grafts.[11,15,26] Several studies did not report increased re-rupture rates, Offerhaus et al. even observed a reduced re-rupture rate after Vancomycin soaking.[5,6,8,10,19,21,23,25,32]

Regarding the chondrotoxicity, several in-vitro studies investigated the Vancomycin-concentration threshold in human and animal chondrocytes and synoviocytes, finding chondrotoxic effects in concentrations as low as 1000 µg/mL. [3,18,24,34] As the soaked ACL graft can act as Vancomycin-reservoir, the release and elution rate of human Vancomycin-soaked grafts in the synovial fluid in the knee joint is of pivotal importance. Grayson et al. investigated the in vitro elution rate of Vancomycin-soaked animal tendons at different concentrations. The

experiment showed that elution rates declined rapidly in a 1-hour interval without reaching chondrotoxic concentrations while at the same time being above the minimum inhibitory concentration for Staphylococcus.[9]

Currently, there is no in vivo or in vitro study, showing adverse effects with respect to Vancomycin-soaking. However, the preventive approach of Vanocmycin soaking of ACL Grafts has been first established in 2012 and no long-term outcome data is at hand.[30] Especially chondrotoxic effects would only become apparent in a longer observation period. Therefore, it is pivotal to know whether this technique puts the cartilage at risk by reaching chondrotoxic thresholds. In vitro data showed that Vancomycin concentrations approximate the maximum concentration within the first ten minutes due to the rapid decrease of the elution rate.[9] However, in-vivo data within this decisive time period immediately following the implantation of vancomycin-soaked tendons are not available.

Therefore, the purpose of this study was to measure the Vancomycin-concentration in the synovial fluid immediately following ACL-reconstruction with Vancomycin-soaked autografts. It was hypothesized that intra-articular Vancomycin-concentrations do not reach the chondrotoxic threshold of 1000 µg/ml following ACL-reconstruction using Vancomycin-soaked autologous semitendinosus and soft tissue quadriceps grafts.

## Materials and Methods

After approval was obtained from the local ethics committee (S-247/2021), patients undergoing primary isolated ACL-reconstruction with or without meniscal surgery by one of two Orthopaedic surgeons (PT and GD) at our tertiary care hospital were recruited. Patients were excluded if they had prior ipsilateral or contralateral ACL-injuries, prior knee surgeries, and

intravenous or oral vancomycin intake. Two groups were formed, one with 4-folded semitendinosus tendon grafts and the other with soft tissue quadriceps tendon grafts.

*Procedure of intraoperative vancomycin soaking:*

After harvesting the quadriceps or semitendinosus tendons using respective harvesting instruments, tendons were wrapped in a Vancomycin-soaked gauze compress. The Vancomycin-concentration was 5mg/ml based on the standard protocol published by Vertullo et al. [30] Subsequently, the preparation of the grafts was performed. Before implantation, the Vancomycin was not rinsed or wiped off with a compress containing Ringer's solution. A soaking time of minimum 10 minutes was ensured.

*Sample collection procedure:*

The graft was inserted under arthroscopic view. After femoral and tibial fixation, only a short arthroscopy followed to check for graft positioning and to remove debris. Subsequently, the remaining fluid was sucked out of the joint and wound closure of the harvest site and medial portal was performed. Before suturing the anterolateral portal, sterile punction of the joint through the anterolateral portal was performed, aiming for the knee compartment in proximity to the soaked graft in anticipation of maximum concentrations. The aspirated fluid was placed in Falcon tube and frozen at -80°C. The time intervals between initiation of Vancomycin-soaking and implantation (t1) and between implantation and sampling (t2) were measured.

*Analysis technique:*

The Vancomycin-concentration of the collected synovial fluid was the analyzed using

high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) analogous to previous methods described for the determination of Vancomycin in serum. [7,13,28]

Samples were processed at room temperature. Briefly, 100 µL of the sample were transferred into an Eppendorf tube and fortified with the internal standard (Acebutolol) to a final concentration of 10 µg/mL. Proteins were precipitated by adding 400 µL of methanol, followed by thorough vortex-mixing and centrifugation at 17 000 x *g* for 10 minutes. The clear supernatant was subsequently diluted with 0.1 % formic acid in a LC vial to a ratio of 1:100, vortex-mixed and subjected to LC-MS/MS analysis. The samples were analyzed by reversed-phase HPLC coupled to an orbitrap mass spectrometer equipped with a heated electrospray ion source operating in positive mode. MS/MS experiments were performed, monitoring two diagnostic ion transitions each for Vancomycin (*m*/*z* 725 → 1305; 725→1143) and the internal standard (*m*/*z* 337→116; 337→319) as quantifier and qualifier ion pairs. Estimation of the analyte's concentration was achieved by means of calibration curves prepared in blank synovial fluid utilizing the peak area ratios of the quantifier ion transitions of Vancomycin and internal standard. The method was validated according to ICH criteria.[2] Before analyzing the Vancomycin-concentrations following ACL-reconstruction with Vancomycin soaked grafts, pre-test of synovial fluid following ten ACL-reconstructions without Vancomycin soaked grafts was used to validate the method by adding predetermined concentrations of Vancomycin in vitro. As expected, no Vancomycin was detected in synovial fluid following ACL-reconstruction without Vancomycin soaked grafts.

*Statistical Analysis*

Descriptive statistics were performed as follows. Means, standard deviations, minimums, and maximums were determined for all continuous variables. Spearman's rank correlation coefficient was used to analyze correlations. A Mann-Whitney-U-Test performed to compare Vancomycin-concentrations between groups based on graft choice and remnant preserving. A Kolmogorov-Smirnov Test was used to determine the distribution of the data and a goodness-of-fit test including Anderson-Darling statistic was performed to investigate if the data are well-described by the fitted distribution. To calculate the probability of a value reaching a certain threshold we used the cumulative distribution function (CDF). Significance was set at p-value < 0.05.

A post hoc power analysis was performed using G*Power 3.1.9.2 (Franz Paul, Kiel, Germany) to calculate the power of the present study. For a t-test comparison between n=10 Vancomycin-concentrations following ACL-reconstruction with Vancomycin soaked hamstring grafts and n=10 Vancomycin-concentrations following ACL-reconstruction without Vancomycin soaked hamstring grafts, a power of 0.9 was calculated. Based on the results of the Spearman's rank correlation coefficient regarding the Vancomycin-concentrations and the duration from Vancomycin-soaking of semitendinosus grafts to implantation, a power of 0.42 was calculated. Based on the results of the independent t-test comparing the Vancomycin-concentration in the synovial fluid of remnant preserving and non-remnant preserving ACL-reconstruction, an effect size of 0,97 ($\alpha$=0.05), and a study group of 20 patients, a power of 0.51 was calculated. For a t-test comparison between hamstring and quadriceps ACL-reconstruction, a power of 0.1 was calculated.

**Results**

A total of 20 patients were included in the study. Demographic characteristics for the cohort are outlined in Table 1. The mean Vancomycin-concentration measured in the synovial fluid was 23.23 µg/ml (± 21.68 µg/ml) with a minimum concentration of 2.32 µg/ml and a maximum concentration of 71.56 µg/ml. With respect to Vancomycin-concentrations, no statistically significant differences were identified between semitendinosus (21.45 ± 22.37 µg/ml) and quadriceps tendon autografts (25.01 ± 22.02 µg/ml; p = 0.91). (Figure 1A)

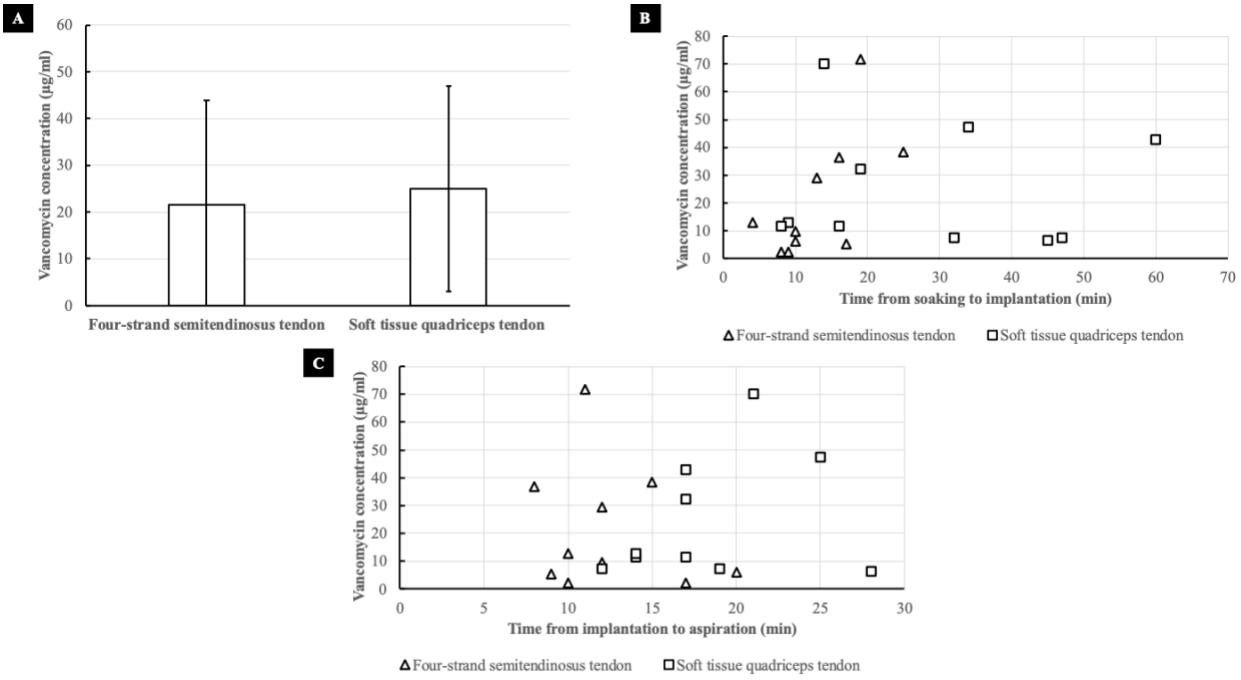


Figure 1 (A) Bar graph of vancomycin concentration in synovial fluid (µg/ml). There is no significant difference between semitendinosus and quadriceps tendon autografts. (B) Time between soaking and implantation of the ACL Graft in minutes (min) and vancomycin concentration in synovial fluid after implantation correlates only in semitendinosus tendon autografts (triangles) (r = .644 p <0.05). (C) There is no correlation between time from graft implantation to sample collection in minutes (min) with vancomycin concentration (µg/ml) in either quadriceps tendon autografts (squares) or semitendinosus grafts (triangles).

The mean duration between initiation of Vancomycin-soaking and implantation was 20.75 min (± 15.26 min) and the mean duration from implantation to fluid aspiration was 15.40 min (± 5.33 min). The only significant correlation ($r = .644$ $p < 0.05$) was observed between the Vancomycin-concentration and the duration (13.4min ± 6min) from initiation of Vancomycin-soaking of semitendinosus grafts to implantation.(Figure 1C) No correlations were observed between the Vancomycin-concentration in the synovial fluid and the duration from implantation to fluid aspiration (r=-0.73 p=0,841) as well as the Vancomycin-concentration and the graft diameter (median 8.5mm, range 6.0-10.0mm, r=-0.026, p =0.914) for both grafts.(Figure 1D) In four patients all reconstructed with semitendinosus autografts a substantial remnant of the original ACL was left intact, and the ACL graft was covered by the ACL stump. There was a significant difference (p=0.019) between the mean Vancomycin-concentration in the synovial fluid for remnant preserved ACL-reconstructions (7.61 µg/ml ± 4.66 µg/ml) compared to ACL-reconstructions without preserved remnant (27.13 µg/ml ± 22.58 µg/ml). (Figure 1B)

The vancomycin concentration was exponentially distributed. The goodness-of-fit tests showed that the data are well-described by the fitted exponential distribution (Kolmogorov-Smirnov p=0.171 an Anderson Darling statistic 0.500). Using the CDF we calculated the probability of the vancomycin concentration to reaching 100 µg/ml, 200 µg/ml, 500 µg/ml and 1000 µg/ml. Based on the data distribution, the probability for a measurement being > 100µg/ml was 0.02%, for >200µg/ml $2.22 \times 10^{-18}$ and for both >500 and >1000µg/ml $<10^{-30}$.

**Table 1: Descriptive Statistics**

| | Quadriceps Autografts | Semitendinosus Autografts | Significance |
|---|---|---|---|
| N | 10 | 10 | |
| Age (yr) | 27.0 ± 10.1 | 31.7 ± 12.5 | n.s. |
| Gender (male / female) | 4 / 6 | 2 / 8 | n.s. |
| Graft Diameter (mm) | 8.7 ± 0.6 | 8.3 ± 0,9 | n.s. |
| Remnant Preserving ACL Reconstruction | 0 | 4 | 0.04 |
| Time between Vancomycin-soaking and Graft Implantation (min) | 28.4 ± 17.9 | 13.10 ± 6.2 | 0.02 |
| Time between Graft Implantation and Aspiration (min) | 18.4 ± 5.0 | 12.4 ± 3.8 | 0.008 |

Age and Graft Diameter is presented as mean ± standard deviation. Gender is presented as count.

## Discussion

The most important finding of this study is that chondrotoxic Vancomycin-concentrations of equal to or greater 1000 µg/ml were not exceeded in any aspiration of synovial fluid following ACL-reconstruction using soft tissue autografts that were intraoperatively soaked in a 5 mg/ml Vancomycin-solution. Thereby, this study adds relevant in-vivo data to the ongoing discussion, suggesting that chondrotoxic effects of Vancomycin-soaking on the cartilage seem to be

clinically insignificant and that the method constitutes a safe prophylaxis of infection in ACL-surgery.

The lowest reported Vancomycin-concentration threshold regarding chondrotoxicity and toxicity of chondrocytes was 1000 µg/ml. The reported thresholds are solely based on in-vitro studies, investigating the effects of Vancomycin in direct contact with chondrocytes and describing the cellular sequelae with respect to cell viability, reproduction, and necrosis. Without the extracellular and cartilage matrix that functions as additional barrier in-vivo, however, Vancomycin- exposure to the cells presumably results in higher cell toxicity. Therefore, the in-vivo threshold for the chondrotoxicity of Vancomycin-concentrations might even be overestimated. [24] However, the true chondrotoxic Vancomycin concentration could only be identified by measuring Vancomycin concentrations in the chondral tissue in vivo. With a mean Vancomycin-concentration of 23.23 µg/ml and a maximum concentration of 71.56 µg/ml in the synovial fluid on average 15 min following graft implantation, the present results distinctly remain below the lowest reported Vancomycin-concentration threshold. Importantly, all Vancomycin concentrations are at the same time above the minimum inhibitory concentration of 2µg/ml for Staphylococcus aureus, reported by the European Committee on Antimicrobial Susceptibility Testing.[1] Furthermore, all but two measured concentrations were above the minimum inhibitory concentration of 4µg/ml for coagulase-negative staphylococci that include clinically relevant pathogens like Staphylococcus epidermidis. Hence, not only do harmful concentrations seem to be ruled out, but also sufficient antibiotic concentrations were reached. The corresponding in-vitro experiment by Grayson et al. showed three major findings: 1. The ACL grafts served as Vancomycin-reservoirs and the graft size influenced the amount of Vancomycin released. 2. The maximum elution rate occurred between 10 minutes and 1 hour

with a subsequent rapid decline. 3. The Vancomycin-concentration did not exceed osteoblast and chondroblast toxicity thresholds. In contrast to this in-vitro study, however, the postoperative knee joint is not a static environment. Due to postoperative hemorrhage, the intraarticular volume increases and further dilution is expectable, reducing the Vancomycin-concentration in the synovial fluid. In a synopsis of both studies, Vancomycin is unlikely to accumulate in the synovial fluid due to rapid elution rates and additional dilution despite the Vancomycin-reservoir in the graft.

Despite its larger surface, four-strand semitendinosus tendon grafts showed similar Vancomycin-concentrations compared to quadriceps tendon grafts. Regarding the relationship between Vancomycin-concentrations and duration of exposure, a significant correlation was only identified in semitendinosus tendons. Whether there is an influence of the absent tendovaginal sheath in quadriceps tendon grafts cannot be answered in the present study and should be investigated in-vitro, especially to ensure the viability of the tendocytes. Interestingly, preservation of the remnant ACL led to significantly lower Vancomycin-concentrations compared to ACL-reconstructions without remnant ACL, providing yet another argument for remnant preservation.

Regarding subgroup-analyses, this study does not have a sufficiently large sample size to answer all research questions of interest and the results are prone to being underpowered as shown by the post-hoc power analysis. However, even supposedly minor intraoperative interventions like in the present study always involves weighing up the risk for the individual patient and the potential increase in knowledge. In addition, no meaningful a-priori calculation of the sample size was possible, as there was no reliable data on the intra-articular Vancomycin-concentration. Therefore, a sample size of 20 was chosen as a balance between individual risk

and information gain. However, based on the calculations from the present distribution of data using the CDF, it was shown that it is very unlikely that a measured intra-articular concentration of vancomycin could be above the chondrotoxic threshold. Further, the good agreement of the fitted exponential function with the data distribution of the vancomycin concentration gives further evidence that the chosen number of cases is suitable to answer the main research question. Additionally, the time of sampling can be scrutinized. The mean duration from implantation to fluid aspiration was 15.40 min. Thereby, it was just in the time interval of maximum elution as shown by Grayson et al. and might miss the peak of intraarticular Vancomycin-concentration. Since patient safety was prioritized and the fluid aspiration was only reasonable under the sterile conditions in the operating room immediately following ACL-reconstruction, it was not possible to adjust the sampling time. Despite using a single-stranded and a multi-stranded graft, the results cannot be automatically transferred to bone - patellar tendon - bone grafts. Nevertheless, this study provides important in-vivo data on intraarticular Vancomycin-concentrations following ACL-reconstruction using Vancomycin-soaked grafts and adds to the growing body of evidence, suggesting that this is a safe method for prophylaxis of septic knee arthritis following ACL-reconstruction. Even though no general recommendation for vancomycin soaking can be given due to the lack of level I evidence, this method has the potential to become the future gold standard. Even now it appears to be difficult to withhold this method from high-risk patients in particular against the backdrop of multiple level II and II studies including more than 20,000 patients showing drastically reduced incidences of septic knee arthritis following ACL-recontruction.[22 32]

## Conclusion

Chondrotoxic Vancomycin-concentrations of equal to or greater 1000µg/ml were not reached in any aspiration of synovial fluid following ACL-reconstruction using soft tissue autografts that were intraoperatively soaked in a 5mg/ml Vancomycin-solution. Against the backdrop of multiple studies, showing significantly reduced infection rates after ACL-reconstruction when using Vancomycin-soaking, this study suggests that the chondrotoxic properties of this method are negligible due to its submarginal intraarticular concentrations.

## References


1. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters version 13.0 2023 http://www.eucast.org/clinical_breakpoints/.

2. International Conference On Harmonisation Of Technical Requirements For Registration Of Pharmaceuticals For Human Use. ICH Harmonised Tripartite Guideline: Validation of Analytical procedures.

3. Antoci V, Jr., Adams CS, Hickok NJ, Shapiro IM, Parvizi J. Antibiotics for local delivery systems cause skeletal cell toxicity in vitro. *Clinical orthopaedics and related research.* 2007;462:200-206.

4. Bansal D, Khatri K, Dahuja A, Lakhani A, Malhotra N. Comparison Between Vancomycin and Gentamicin for Intraoperative Presoaking of Hamstring Graft in Primary Anterior Cruciate Ligament Reconstruction. *Cureus.* 2022;14(2):e22550.

5. Baron JE, Shamrock AG, Cates WT, et al. Graft Preparation with Intraoperative Vancomycin Decreases Infection After ACL Reconstruction: A Review of 1,640 Cases. *J Bone Joint Surg Am.* 2019;101(24):2187-2193.

6. Bohu Y, Klouche S, Sezer HB, 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(8):2578-2585.

7. Cass RT, Villa JS, Karr DE, Schmidt DE, Jr. Rapid bioanalysis of vancomycin in serum and urine by high-performance liquid chromatography tandem mass spectrometry using on-line sample extraction and parallel analytical columns. *Rapid Commun Mass Spectrom.* 2001;15(6):406-412.

8. Figueroa F, Figueroa D, Calvo R, et al. Vancomycin Presoaking of Hamstring Autografts in Anterior Cruciate Ligament Reconstruction Is Associated With Higher Magnetic Resonance Imaging Graft Signal Without Influencing Clinical Outcome. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association.* 2022;38(5):1528-1534.

9. Grayson JE, Grant GD, Dukie S, Vertullo CJ. The in vitro elution characteristics of vancomycin from tendons. *Clinical orthopaedics and related research.* 2011;469(10):2948-2952.

10. Hees T, Abdelatif Y, Karpinski K, et al. Soaking ACL grafts in vancomycin solution (1 mg/ml) reduces the infection rate without increasing the risk for re-rupture and arthrofibrosis. *Arch Orthop Trauma Surg.* 2022;142(6):1141-1146.

11. Jacquet C, Jaubert M, Pioger C, et al. Presoaking of Semitendinosus Graft With Vancomycin Does Not Alter Its Biomechanical Properties: A Biomechanical In Vitro-Controlled Study Using Graft From Living Donors. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association.* 2020;36(8):2231-2236.

12. Judd D, Bottoni C, Kim D, Burke M, Hooker S. Infections following arthroscopic anterior cruciate ligament reconstruction. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association.* 2006;22(4):375-384.

13. Konig K, Kobold U, Fink G, et al. Quantification of vancomycin in human serum by LC-MS/MS. *Clin Chem Lab Med.* 2013;51(9):1761-1769.

14. Kursumovic K, Charalambous CP. Relationship of Graft Type and Vancomycin Presoaking to Rate of Infection in Anterior Cruciate Ligament Reconstruction: A Meta-Analysis of 198 Studies with 68,453 Grafts. *JBJS Rev.* 2020;8(7):e1900156.

15. Lamplot JD, Liu JN, Hutchinson ID, et al. Effect of Vancomycin Soaking on Anterior Cruciate Ligament Graft Biomechanics. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association.* 2021;37(3):953-960.

16. Mouzopoulos G, Fotopoulos VC, Tzurbakis M. Septic knee arthritis following ACL reconstruction: a systematic review. *Knee Surg Sports Traumatol Arthrosc.* 2009;17(9):1033-1042.

17. Naendrup JH, Marche B, de Sa D, 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(4):1005-1013.

18. Newman RJ, Chow L, Goodrich LR, et al. Susceptibility of canine chondrocytes and synoviocytes to antibiotic cytotoxicity in vitro. *Vet Surg.* 2021;50(3):650-658.

19. Offerhaus C, Balke M, Hente J, et al. Vancomycin pre-soaking of the graft reduces postoperative infection rate without increasing risk of graft failure and arthrofibrosis in ACL reconstruction. *Knee Surg Sports Traumatol Arthrosc.* 2019;27(9):3014-3021.

20. Papalia R, Cicione C, Russo F, et al. Does Vancomycin Wrapping in Anterior Cruciate Ligament Reconstruction Affect Tenocyte Activity In Vitro? *Antibiotics (Basel).* 2021;10(9).

21. Perez-Prieto D, Perelli S, Corcoll F, et al. The vancomycin soaking technique: no differences in autograft re-rupture rate. A comparative study. *Int Orthop.* 2021;45(6):1407-1411.

22. Pfeiffer TR. Editorial Commentary: Vancomycin Soaking of the Graft in Anterior Cruciate Ligament Reconstruction: A Concept on the Way to Becoming the New Gold Standard. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association.* 2021;37(3):961-963.

23. 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(9):2729-2735.

24. Rohner E, Zippelius T, Bohle S, et al. Vancomycin is toxic to human chondrocytes in vitro. *Arch Orthop Trauma Surg.* 2021;141(3):375-381.

25. Schuster P, Schlumberger M, Mayer P, 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(8):2587-2591.

26. Schuttler KF, Scharm A, Stein T, 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(1):73-78.

27. Sechriest VF, 2nd, Carney JR, Kuskowski MA, et al. Incidence of knee sepsis after ACL reconstruction at one institution: the impact of a clinical pathway. *J Bone Joint Surg Am.* 2013;95(9):843-849, S841-846.

28. Shi M, Zhao X, Wang T, Yin L, Li Y. A LC-MS-MS assay for simultaneous determination of two glycopeptides and two small molecule compounds in human plasma. *J Chromatogr Sci.* 2018;56(9):828-834.

29. Torres-Claramunt R, Pelfort X, Erquicia J, et al. Knee joint infection after ACL reconstruction: prevalence, management and functional outcomes. *Knee Surg Sports Traumatol Arthrosc.* 2013;21(12):2844-2849.

30. 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. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association.* 2012;28(3):337-342.

31. Xiao M, Leonardi EA, Sharpe O, et al. Soaking of Autologous Tendon Grafts in Vancomycin Before Implantation Does Not Lead to Tenocyte Cytotoxicity. *Am J Sports Med.* 2020;48(12):3081-3086.

32. Xiao M, Sherman SL, Safran MR, Abrams GD. Significantly Lower Infection Risk for Anterior Cruciate Ligament Grafts Presoaked in Vancomycin Compared With Unsoaked Grafts: A Systematic Review and Meta-analysis. *Arthroscopy : the journal of arthroscopic & related surgery : official publication of the Arthroscopy Association of North America and the International Arthroscopy Association.* 2021;37(5):1683-1690.

33. Xiao M, Sherman SL, Safran MR, Abrams GD. Surgeon practice patterns for pre-soaking ACL tendon grafts in vancomycin: a survey of the ACL study group. *Knee Surg Sports Traumatol Arthrosc.* 2021;29(6):1920-1926.

34. Yazdi HR, Jamei Moayedi R, Shokrgozar MA, Dehghan MM, Mokhtari T. Evaluation of Delayed Effect of Intra-Articular Injection of Cefazolin, Gentamicin and Vancomycin on Articular Cartilage: an Experimental Study in Rabbits. *Journal of Research in Orthopedic Science.* 2014;1(3):0-0.