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In vitro biomechanical comparison of clamped suture knot techniques as a model of extra-capsular stabilization of canine cruciate ligament repair

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Original Article

In vitro biomechanical comparison of clamped suture knot techniques as a model of extra-capsular stabilization of canine cruciate ligament repair

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Abstract

Six clamped suture knot techniques were compared to identify the effects on the biomechanic properties used as a model of extra-capsular stabilization. Six clamped suture knot techniques included square knot formed by clamping the first throw with toothed mosquito forceps (SQ-TM), non-toothed mosquito forceps (SQ-NTM), or needle holder (SQ-NH) and surgeon's knot formed by clamping the first throw with toothed mosquito forceps (SG-TM), non-toothed mosquito forceps (SG-NTM), or needle holder (SG-NH). Monotonic loading test was performed on each suture-loop. Initial loop tension, ultimate load, load at 3 mm elongation, elongation at failure, stiffness, and failure mode were compared among techniques. Clamping with NTM or NH achieved greater initial loop tension than clamping with TM when tying SQ ($P < 0.05$), whereas clamping with NH led to more loosening of the loops than clamping with NTM when tying SG ($P < 0.05$). SG-NTM had the highest ultimate load ($P < 0.05$). In failure mode, the possibility of knot slippage was six times more likely to occur in SQ than SG ($P < 0.05$). In conclusion, SG resulted in less knot slippage and NTM was able to maintain initial loop tension more effectively. SG-NTM technique is expected to show less risk of suture failure and better clinical outcomes.

Keywords: clamped suture knot techniques, surgeon's knot, non-toothed mosquito forceps, extra-capsular stabilization

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Introduction

Cranial cruciate ligament (CCL) injury is the most common orthopedic disorder encountered in dogs (Fischer *et al*., 2010; Dycus *et al*., 2013). Extra-capsular stabilization using suture materials is a commonly performed surgical procedure to stabilize CCL-deficient stifles (Tonks *et al*., 2011; Chang *et al*., 2013). Because premature slippage, broken constructs, or premature loosening can induce postoperative joint instability prior to formation of sufficient periarticular fibrosis, the optimal suture material and the fixation method have been investigated to reduce the occurrence of premature failure (Rose *et al*., 2012; Dycus *et al*., 2013; Chang *et al*., 2016).

Adequate mechanical properties such as high tensile strength, stiffness, and good knot security and fixation providing compact knots without premature slippage are to be considered for the ideal orthopedic suture materials and fixation techniques, respectively (Wong and Bronzino, 2007). Crimp clamp fixation of monofilament nylon provides stronger and stiffer mechanical properties compared to knot fixation; however, crimp clamp fixation of multifilament ultra-high-molecular-weight polyethylene (UHMWPE) has been shown to be weaker than knotted UHMWPE (Anderson *et al*., 1998; Burgess *et al*., 2010; Maritato *et al*., 2012). For this reason, the placement of knotted multifilament UHMWPE has recently been considered to be a reasonable choice for extra-capsular stabilization.

In vitro studies have indicated that the type of knot can affect the structural properties of suture loops (Huber *et al*., 1999; Tonks *et al*., 2011). Several types of knot have been compared in previous studies including square knot (SQ), surgeon's knot (SG), self-locking knot, and tube knot using nylon, polydioxanone, polyglactin, polyglyconate, and polyester (Fong *et al*., 2008; Mulon *et al*., 2010; Dycus *et al*., 2013; Chang *et al*., 2016). However, there were only few studies evaluating UHMWPE suture material even if multifilament UHMWPE sutures have been reported to be stronger, stiffer and produce more compact knots than monofilament nylon, polydioxanone, or polyester sutures (Lo *et al*., 2004; Barber *et al*., 2006; Wust *et al*., 2006; Burgess *et al*., 2010; Rose *et al*., 2012; Zhao *et al*., 2013). Among types of knot, SQ and SG have been the gold standard of surgical knots used in tissue repair, ligature, and wound closure (Zhao *et al*., 2013). However, when tying SQ or SG, the drawback which is the tendency of the first throw to slip before placing the second throw, can occur frequently (Turker *et al*., 2012). Fixing the first throw with a clamping instrument is necessary to prevent slippage of the first throw while the second throw is placed and various clamping instruments including hemostats and needle holders have been used based on surgeon's preference (Burgess *et al*., 2010; Mulon *et al*., 2010; Turker *et al*., 2012). To the authors' knowledge, there are no published data that indicate which of the commonly employed clamping techniques is the most advantageous for use in dogs with CCL rupture.

The purpose of this study was to compare mechanical properties of six different clamped suture knot techniques using two types of knots and three clamping instruments and to determine the optimal clamped suture knot technique when using multifilament UHMWPE suture.

Materials and Methods

Group design: Six groups were prepared for six different clamped suture knot techniques with 10 suture loop samples in each group. The six clamped suture knot techniques included SQ formed by clamping the first throw with toothed mosquito forceps (SQ-TM), non-toothed mosquito forceps (SQ-NTM), or needle holder (SQ-NH) and SG formed by clamping the first throw with toothed mosquito forceps (SG-TM), non-toothed mosquito forceps (SG-NTM), or needle holder (SG-NH).

Loop formation: All tests were performed with non-sterilized No. 5 multifilament UHMWPE (Force Fiber, Teleflex Medical OEM, USA). The suture material was cut into 300 mm lengths for making loop samples. A single investigator (J.J.L.) tied the knots in a random order. Sutures were tightened around a 35-mm-diameter rod and an adjacent 2-mm-diameter rod (Fig 1). Both tags of suture were tied to push-pull gauge and pulled until the knots were subjected to 50 N. The purpose of the additional smaller rod was to permit easy separation of the suture loops from the larger rod following removal of the smaller rod. Square knot and SG were formed by a single-wrap throw and double-wrap throw followed by another single-wrap throw, respectively. Both knots were formed by clamping the first throw with three different clamping instruments while the second throw was placed (Fig 1). Three additional single-wrap throws were then applied. The tags of suture knot were cut and remained 3 mm apart from the knot.

Mechanical testing: After separation of the suture loops from the rods, the initial loop tension was recorded to determine loop tightness. Loop tensions were measured using push-pull gauge (model NK-100, Graigar, China) and compared among groups. Each loop was pulled 5 cm indicating knotted loop length, and then the tension (N) displayed on the gauge was recorded (Fig 2A). Higher initial loop tension indicates tighter loop.

Monotonic loading test was performed to detect ultimate load, load at 3 mm elongation, and elongation at failure using the materials-testing machine (model QUASAR 5, Galdabini, Italy). Ten prepared suture loop samples of each group were mounted on a pair of square bracket-shaped jigs with the knot located in the middle of the loop (Fig 2B). Preload of 20 N was applied to the loops for 15 seconds to prevent any slack between throws before starting the monotonic loading test. The preload of 20 N (10 kg x 20% x 9.8 m/s2) was calculated by the vertical ground reaction force of a hind limb of a 10 kg dog with weight-bearing stance (1,20). Load was applied to the loops until failure at a constant distraction rate of 500 mm/min, which was the maximum speed of the machine.

The results of the monotonic loading tests were recorded with data acquisition software (model QUASAR 5, Galdabini, Italy) and imported to spread sheets (Microsoft, USA) for creating load-elongation curves of each test. Ultimate load, load at 3 mm elongation, and elongation at failure were measured using the load-elongation curves and compared among groups. Ultimate load was defined as the highest load applied to the suture loop before failure and was acquired from the peak point of load-elongation curve. Since 3 mm in length was the acceptable limit of cranial tibial thrust, the load required to elongate the suture loops by 3 mm was recorded (9,20).

Failure mode was defined and recorded as slippage (unraveling of all five throws) or breakage (breaking of the suture into two portions at knot site) for each of the tests.

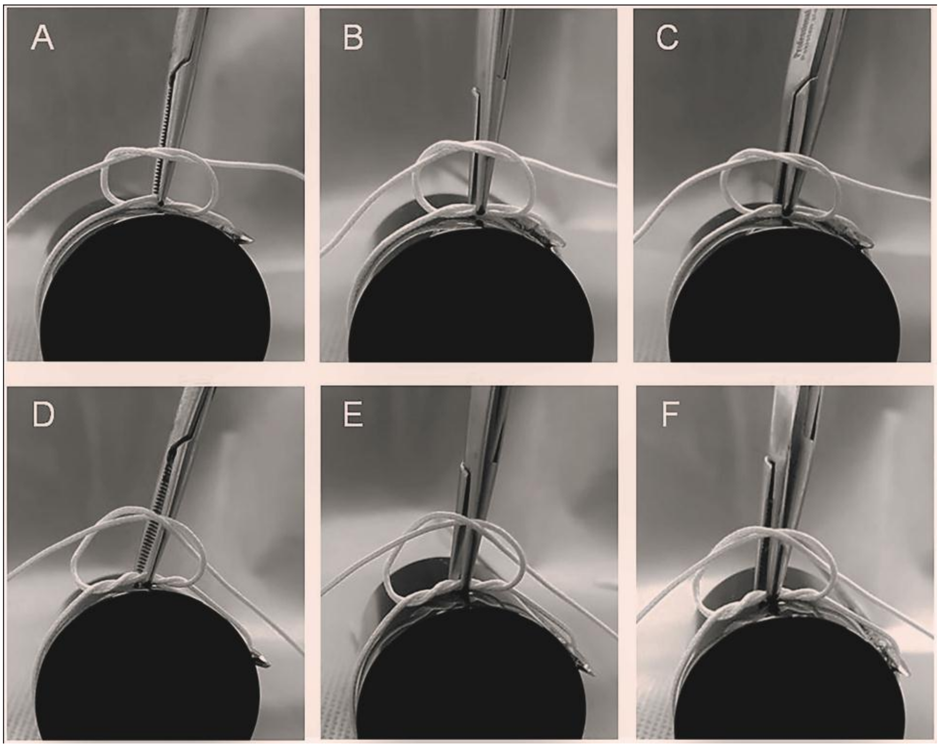

Figure 1 Loop formation using six clamped suture knot techniques: SQ-TM (A), SQ-NTM (B), SQ-NH (C), SG-TM (D), SG-NTM (E), and SG-NH (F). UHMWPE sutures are tightened around a 35-mm-diameter rod and an adjacent 2-mm-diameter rod. Square knots (A), (B), (C) are formed by clamping the first throw with toothed mosquito forceps, non-toothed mosquito forceps, or needle holder respectively while the second throw is placed. Surgeon's knots (D), (E), (F) are formed by clamping the first throw with toothed mosquito forceps, non-toothed mosquito forceps, or needle holder respectively while the second throw is placed.

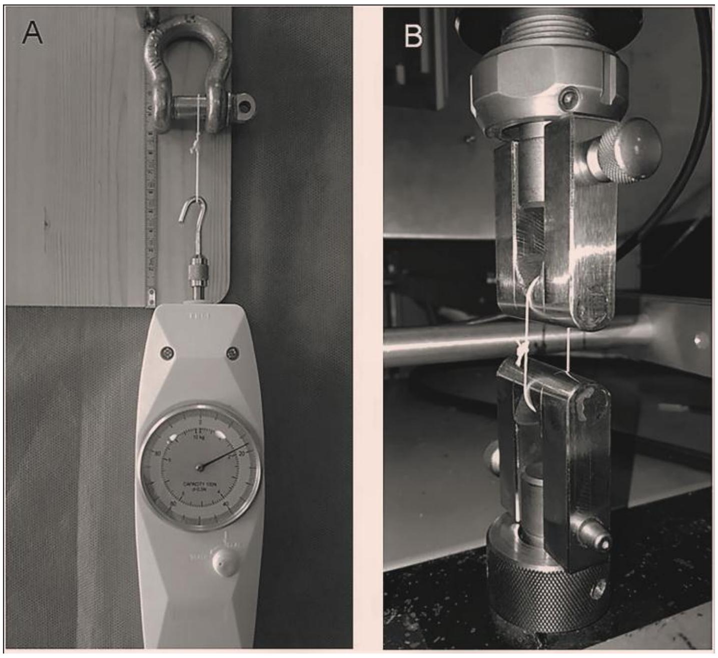

Figure 2 Initial loop tension is measured by push-pull gauge (A) immediately after completion of the knot. Initial loop tension is displayed on the gauge when the loops are pulled 5 cm. After measuring initial loop tension, suture loops are mounted onto the material testing machine (B) for monotonic loading test. Preload of 20 N is applied to the loops for 15 seconds, and then the loops are extended at a constant distraction rate of 500 mm/min until the loops fail.

Statistical analysis: Statistical analysis of the data was conducted using statistical software (IBM, USA). All data were tested for normality using Kolmogorov-Smirnov test. Two-way ANOVA was performed to assess the interaction effects, main effects, or simple effects of independent variables on measurement categories. Post hoc Tukey HSD and Tamhane multiple comparisons were used to identify significant differences between groups. Logistic regression was carried out to estimate the prevalence of knot slippage. *P* values of < 0.05 were considered statistically significant.

Results

Initial loop tension: Mean ± SD initial loop tensions for each technique group were recorded (Table 1). Knot type and clamping instrument had an interaction effect on the initial loop tension (p = 0.012). The initial loop tension was the greatest in SQ-NTM (p < 0.001), followed by SQ-NH; however, the difference was not statistically significant between SQ-NTM and SQ-NH (p = 0.695). SQ-NTM had significantly greater initial loop tension than SQ-TM (p = 0.015), SG-TM (p = 0.041), SG-NTM (p = 0.02), and SG-NH (p < 0.001). Simple effects of clamping instruments, the effect of clamping instrument within one type of knot, were analyzed and clamping with NTM or NH achieved greater initial loop tension than clamping with TM when tying SQ (p < 0.001 and p = 0.005, respectively), whereas clamping with NH led to more loosening of the loops than with NTM when tying SG (p = 0.014).

Ultimate load: Mean ± SD ultimate load for each technique group were recorded (Table 1). Knot type and clamping instrument had an interaction effect on the ultimate load (p = 0.009). The ultimate load was the greatest in SG-NTM (p < 0.001); however, SG-NTM was not significantly different from SG-TM (p = 0.976) and SG-NH (p = 0.814). SG-NTM withstood significantly greater ultimate load than SQ-TM (p = 0.002), SQ-NTM (p < 0.001), and SQ-NH (p = 0.009). SG-TM showed significantly greater ultimate load than SQ-TM (p = 0.021) and SQ-NTM (p < 0.001). Greater ultimate load was identified in SG-NH than SQ-NTM (p < 0.001).

Elongation: Mean ± SD load at 3 mm elongation and elongation at failure for each technique group were shown in table 1. The differences in the load required to elongate the suture loop more than 3 mm were statistically indistinct among the six groups (p = 0.096). Overall mean load at 3 mm elongation of the six groups was 169 ± 20 N. On elongation at failure, knot type had a main effect (p < 0.001) and SG tended to elongate more than SQ when sutures failed regardless of clamping instruments (p < 0.001).

Failure mode: Sixty percent of SQ groups (18 of 30) and 20% of SG groups (6 of 30) failed by knot slippage (Table 2). Logistic regression analysis showed that while clamping instrument had no significant effect on failure mode, knot type had significant effect on failure mode (p = 0.002). The possibility of knot slippage was six times more likely to occur in SQ than SG (p = 0.002). Breakage occurred at higher load than slippage in both knots regardless of clamping instruments (p < 0.001), and the ultimate load of SG was higher than that of SQ when loops failed by same failure mode (p < 0.013).

Table 1 Initial loop tension, ultimate load, load at 3 mm elongation, and elongation at failure in six groups under mechanical testing. (Mean ± SD)

Group	Initial Loop Tension (N)	Ultimate Load (N)	Load at 3 mm Elongation* (N)	Elongation at Failure (mm)
SQ-TM	17.77 ± 1.7^{bc}	581.8 ± 27.7^{cd}	154.7 ± 20.1	8.02 ± .44^{ab}
SQ-NTM	**20.22 ± 0.8^{a}**	536.2 ± 49.2^{d}	174.3 ± 19.7	7.89 ± .56^{ab}
SQ-NH	19.40 ± 1.1^{ab}	589.1 ± 45.3^{bc}	180.3 ± 15.4	7.52 ± .42^{b}
SG-TM	17.84 ± 1.8^{bc}	638.3 ± 48.3^{ab}	170.0 ± 16.3	**8.66 ± 1.06^{a}**
SG-NTM	18.54 ± 1.1^{b}	**651.0 ± 24.5^{a}**	168.4 ± 29.2	8.47 ± .95^{a}
SG-NH	17.11 ± 0.4^{c}	629.6 ± 27.1^{abc}	166.4 ± 9.3	8.56 ± .61^{a}

In each measurement category, different letter superscripts indicate significant difference between groups (*P* < 0.05).
The mean values are high in alphabetical order. The highest mean value for each measurement category appears in bold.
*There were no significant differences among six groups (*P* = 0.096).
SQ-TM, square knot with toothed mosquito forceps; SQ-NTM, square knot with non-toothed mosquito forceps; SQ-NH, square knot with needle holder; SG-TM, surgeon's knot with toothed mosquito forceps; SG-NTM, surgeon's knot with non-toothed mosquito forceps; SG-NH, surgeon's knot with needle holder.

Table 2 Failure mode in six different clamped suture techniques

Group (n = 10 / each group)	Failure Mode	
	Breakage	Slippage
SQ-TM	6	4
SQ-NTM	2	8
SQ-NH	4	6
SG-TM	9	1
SG-NTM	8	2
SG-NH	7	3

SQ-TM, square knot with toothed mosquito forceps; SQ-NTM, square knot with non-toothed mosquito forceps; SQ-NH, square knot with needle holder; SG-TM, surgeon's knot with toothed mosquito forceps; SG-NTM, surgeon's knot with non-toothed mosquito forceps; SG-NH, surgeon's knot with needle holder.

Discussion

This study shows the different effects of clamped suture knot techniques on the biomechanics of extra-capsular stabilization. The important finding of this study was that NTM was the most suitable instrument to maintain initial loop tension. Initial loop tension has been considered an important clinical variable for the successful stabilization of the CCL-deficient stifles because the large loss in loop tension during knot formation could lead to premature failure (Vianna and Rose, 2006; Cabano *et al*., 2011). It was assumed that over 75% of initial loop tension is lost during the formation of a clamped square knot (Caporn and Roe, 1996). Although the loss in loop tension during knot formation is unavoidable, it can be minimized by using an adequate clamped suture knot technique. In this study, the interaction effect between clamping instrument and knot type was most evident in initial loop tension. When interpreting this interaction effect on initial loop tension, two concepts of drawback and knot rundown should be considered. First, the drawback is defined as the tendency of the first throw to slip before placing the second throw; it occurred differently depending on which clamping instruments were used in our results. Clamping with NTM resulted in the least drawback in both knot types while clamping with TM and NH resulted in the most severe drawback in SQ and SG, respectively. These results suggest that the instrument having smooth jaws (NTM) is more suitable for clamping the first throw than the instrument having serrated jaws (TM or NH), because the suture might be caught on the serrated jaws when removing the instrument from the first throw, resulting in severe drawback. The relatively thick jaws of NH might also form the large slack between the first and second throw and increase the drawback. Second, the knot rundown, the knot's ability to slip downward when force is applied to both tags of the knot, is related to the configuration of the first and second throw of the knot (Richard, 2008). Based on results of this study, SQ groups seemed to have greater initial loop tension than SG groups. This finding could be associated with a previous study which reported that the knot rundown of SQ can easily occur by slippage while the knot rundown of SG is physically demanding, because of the first double-wrap throw that causes high resistance to slippage (Zimmer *et al*., 1991). For these reasons, SQ-NTM or SG-NTM is recommended to minimize the drawback caused by clamping instruments; however, considering the knot rundown, SQ-NTM performed better than SG-NTM in initial loop tension.

SG-NTM had the greatest ultimate load and performed better than all SQ groups in ultimate load. The mean ultimate load of SG was higher than that of SQ when the loops failed by same failure mode. This superiority of SG in ultimate load seems to be related to the first double-wrap throw of SG. The interface between a loop and the first throw of a knot is the weakest point of a suture loop because stress concentrates at the point when throws are tightened (Anderson *et al*., 1998; Sicard *et al*., 2002). This result was verified with the finding that all breakages occurred at the level of knot in this study. The first single-wrap throw of SQ produced a small area for friction distribution resulting in much of a stress riser effect to the knot, compared with the first double-wrap throw of SG.

Mechanical trauma induced by surgical instruments to the suture loops can also result in premature failure (Turker *et al*., 2012). According to previous studies, some of monofilament suture materials such as polypropylene and nylon were damaged and weakened by clamping the first throw with surgical instruments (Stamp *et al*., 1988; Turker *et al*., 2012). The ultimate load of multifilament polyglactic acid suture was reported not to be affected by clamping the first throw because of the compressibility of multifilament suture ma-terial (Turker *et al*., 2012). It was assumed that the partial damage of multifilament suture clamped with an instrument was not enough to provide ultimate load alteration (Mulon *et al*., 2010). In our evaluation for ultimate load of multifilament UHMWPE suture, three clamping instruments did not show significant differences. Whether suture materials were damaged by clamping or not cannot be concluded; however, these results showed that there were no significant differences in the degree of suture damage between the three clamping instruments.

It has been suggested that loop elongation should be a primary consideration for the selection of suture material and knot type when performing an extra-capsular stabilization because the loop elongation contributes to instability of the CCL-deficient canine stifle after surgery (Sicard *et al*., 2002; Vianna and Roe, 2006; Dycus *et al*., 2013). Many surgeons have believed restriction of cranial drawer to 2-3 mm is reasonable post-operatively for stabilization of the CCL-deficient canine stifle (Cabano *et al*., 2011; Rose *et al*., 2012; Choate *et al*., 2013). Therefore, the load required to elongate the suture loops by 3 mm was compared among groups in this study, and there were no significant differences among groups. The SQ groups had significantly less elongation at failure than the SG groups in this study; however, this result should be interpreted cautiously because the SG groups failed at higher load than the SQ groups. It is necessary to evaluate the stiffness for an accurate interpretation of the result that SG elongated more than SQ at failure. Stiffness is the constructs' rigidity to resist deformation under applied force (Burgess *et al*., 2010). Although the elongation at failure was different between SG and SQ, the stiffness did not differ between SG and SQ.

When assessing failure mode, breakage occurred at higher load than slippage in both knot types in this study. This result indicates that the knot which is easy to slip is more likely to cause premature failure of extra-capsular stabilization; therefore, it is important to minimize knot slippage in knot configuration. Based on the result of this study, knot slippage was six times more likely to occur in SQ than SG. The knot slippage can be influenced by factors affecting knot security: knot type, suture material, number of throws, and suture gauge (Marturello *et al*., 2014). Among these factors, knot type has an influence on internal interference related to how threads are woven (Burgess *et al*., 2010). The first double-warp throw of SG has more increased internal interference than the first single-wrap throw of SQ. Besides, it has been reported that 80% of SQ can change into a sliding half-hitch while the forces are applied (Avoine *et al*., 2016). For these reasons, it could be suggested that knot slippage is counteracted by SG more effectively than SQ.

There are some limitations in this study. The suture material used for extra-capsular stabilization in dogs with CCL injury is exposed to internal rotational and tibial shear forces; however, in this study, the mechanical tests was only performed under tensile loading. Monotonic loading tests performed for measuring ultimate load did not reflect the real clinical situation, in which continuous cyclic loading during locomotion was applied on extracapsular suture material. Biological factor, such as susceptibility to infection, was not considered. Therefore, the results from this study cannot be directly applied to clinical practice. Further studies with diverse mechanical, cadaveric, and biological tests using various suture materials are required to determine clinical utility of this suture knot technique.

Based on the results of the present study comparing the effects of six clamped suture knot techniques on the biomechanic properties used as a model of extra-capsular stabilization, SG-NTM was able to sustain significantly higher load than all of the SQ groups before failure; moreover, it had the greatest initial loop tension in the SG groups. Although SQ-NTM had greater initial loop tension than SG-NTM, the weak strength and frequent knot slippage of SQ-NTM can have a detrimental effect on extra-capsular stabilization in clinical situations.

Consequentially, it is reasonable to assume that the application of SG-NTM on extra-capsular stabilization of canine CCL injured stifles or other orthopedic problems requiring artificial ligament reconstruction with large gauge UHMWPE suture shows less risk of premature suture failure and better clinical outcome.

Conflicts of Interest: All authors declare that there are no conflicts of interest in relation to this study.

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