The Corneal Transplant Follow-up Study II (CTFS II) – a prospective clinical trial to determine the influence of HLA class II matching on corneal transplant rejection: baseline donor and recipient characteristics.

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drafting and review of paper.

Synopsis

The Corneal Transplant Follow-up Study II is a prospective trial that has accrued 1077 transplants to determine whether HLA class II matching influences the risk of rejection in high-risk penetrating keratoplasty.

Abstract

Purpose: To describe a study to determine the influence of HLA class II matching on allograft rejection of high-risk, full-thickness corneal transplants.

Methods: A prospective, longitudinal, clinical trial (ISRCTN 25094892) with a primary outcome measure of time to first clinically determined rejection episode. Tissue typing used DNA-based techniques. Corneas were allocated to patients with ≤2 HLA class I antigen mismatches by cohort minimization to achieve 0, 1 or 2 HLA class II (HLA-DR) antigen mismatches. Transplants were to be followed up at 6 months and then annually on the anniversary of surgery for 5 years. Power calculations estimated a sample size of 856 transplants to detect a 0.1 difference in probability of rejection at one year between HLA class II matched and mismatched transplants at the 5% level of significance with 80% power.

Results: To allow for loss to follow up, 1133 transplants in 980 patients were accrued to the study between 3 September 1998 and 2 June 2011. 17% of transplants had 0 HLA-DR mismatches. The most frequent indication was bullous keratopathy, accounting for 27% of transplants and 54% of the transplants were regrafts. Median waiting time for a matched graft was 3 months. Donor and recipient characteristics were distributed evenly across the study groups.

Conclusion: Recruitment to the CFS II has closed with 1077/1133 transplants meeting all the study criteria. Follow-up has been completed and final analysis of the data has started.

Despite the avascularity of the cornea in the healthy eye and the historical presumption of immune privilege in anterior chamber, allograft rejection remains a leading cause of penetrating keratoplasty (PK) failure.[1-3] Even when rejection episodes are successfully reversed, there is still a negative impact on long-term graft survival.[4] Penetrating keratoplasty is being increasingly superseded by endothelial keratoplasty (EK) and deep anterior lamellar keratoplasty (DALK) but is still required for many patients. In 2015/16, 35% of corneal transplants recorded in the UK Transplant Registry were PK (Mark Jones, *personal communication*) and a global survey of corneal transplantation based on 2012 data showed that PK accounted for 70% of transplants.[5] While the risk of rejection appears to be reduced in DALK and EK, especially for Descemet Membrane endothelial keratoplasty (DMEK), it is not eliminated.[6 7] Therefore, there remains a need to increase our understanding of the immunobiology of corneal transplantation in order to improve strategies for the prevention of rejection, especially for those types of allograft, such as PK, that are more prone to this serious postoperative complication.

In organ transplantation there is a clear benefit from human leukocyte antigen (HLA) matching between donors and recipients.[8] Moreover, the role of donor-specific anti-HLA antibodies and antibody-mediated rejection in late renal allograft failure is becoming clearer,[9] which may give insights into the mechanism of late endothelial failure in corneal transplantation.[10] There have been many reports over the years concerning the influence of HLA matching on the risk of cell-mediated corneal transplant rejection. These include retrospective single- and multi-centre registry based studies as well as multi-centre prospective studies; but there is still no firm consensus on the value of HLA matching.[3 11-18] The Combined Cornea Transplant Study (CCTS) carried out in the USA is one of the few randomized, prospective studies on HLA matching in corneal transplantation.[15] This carefully designed study found no effect of HLA matching in high risk patients; however, a significant error rate in serological tissue typing, especially for HLA class II, was subsequently discovered.[19] Simulations by Völker-Dieben et al.[11] suggested that errors

in as few as 5% of tissue types were sufficient to reduce the impact of HLA class II (HLA-DR) matching and that higher numbers of errors completely nullified the benefit of the intended matching. Errors in HLA typing could therefore explain both the CCTS results showing no influence of HLA matching and the apparent negative effect of HLA-DR matching found in a retrospective UK study.[13 15 16 19] While corneal allograft rejection is typically considered to be cell-mediated, studies in rodents have shown the extent of redundancy in the immune response and have confirmed that there are several different immunological pathways that could all lead to rejection.[20] This may provide an alternative immunological explanation for the lack of consistency in the outcomes of HLA matching studies in corneal transplantation. Whatever the reasons for these seemingly discordant outcomes for HLA-DR matching, there appears to be a degree of consensus, with the exception of the CCTS, for a beneficial effect of HLA class I matching, at least in high risk grafts.[3 12 14 18 21] However, any potential benefit of matching for a given patient may be offset by having to wait an unacceptably long time for a suitably matched transplant.[22]

With reports in the literature suggesting a benefit,[12 14] no benefit,[15] or a negative effect of HLA-DR matching,[3 13 16] the present study was designed to test the hypothesis that HLA class II matching, against a background of HLA class I matching, reduces the risk of allograft rejection in full-thickness corneal transplants. Only transplants considered being at increased risk of rejection were included in the study. Limiting the study to high risk grafts is supported by observations in rats that suggested HLA class II matching was beneficial in regrafts but not first grafts.[23] The risk factors for rejection were based on a previous study.[3] To avoid the possibility of serological typing errors undermining the accuracy of HLA matching, all the tissue typing of donors and recipients used DNA-based rather than serological methodologies.

Methods

Study design. A multi-centre, prospective, longitudinal clinical trial registered with ISRCTN (ISRCTN25094892) and included in the UK National Institute for Health Research Clinical Research Network portfolio (NIHR CRN Study ID 9871). The study was approved by the National Health Service South West Multicentre Research Ethics Committee (MREC/97/6/8) and by the National Research Ethics Service (IRAS Project ID 11351). All transplants were matched for HLA class I, defined as not more than two antigen mismatches at the HLA-A and HLA–B loci combined. The study groups were defined by the number of mismatched HLA-DR antigens between donors and recipients; namely, 0, 1 or 2 mismatches.

HLA typing. To avoid potential serological tissue typing errors, especially for HLA class II,[19] DNA-based molecular techniques (PCR-SSO/SSP) were used for low resolution (i.e., antigen level) typing for all donors and recipients. For class II antigens (HLA-DR), the matching algorithm included the following splits (the broad antigen is given in brackets): DR15(2), DR16(2), DR11(5), DR12(5), DR13(6), DR14(6); however, for class I (HLA-A and HLA-B) donors and recipients were matched only for broad antigen specificities.

Donors. The great majority (87%) of corneas were from organ donors that had already been tissue typed. The remaining 13% of corneas were from non-organ donors and HLA-typed using DNA extracted from the neural retina.

Corneal storage. All corneas were stored by organ culture at 34°C in the Bristol and Manchester eye banks for up to 4 weeks.[24] The minimum endothelial cell density was 2200 cells/mm².[25]

Recipients. Individuals who agreed to participate in the study all gave written informed consent, which complied with the tenets of the Declaration of Helsinki. The inclusion and exclusion criteria for patient selection are listed in Table 1.

Table 1. CTFS II inclusion and exclusion criteria.

Inclusion criteria	
Donors	HLA typed by PCR-SSP or PCR-SSO
	Corneas stored for up to 4 weeks by organ culture at 34°C
	Endothelial cell density ≥2200 cells/mm²
Recipients	Penetrating keratoplasty (PK)
	Patients of either sex aged 16 years or older
	Patients competent to give informed consent
	Patients who have given consent to participate
	HLA type by PCR-SSP or PCR-SSO
	Patients with indications and risk factors that increase the risk of allograft rejection:[3] regraft, bullous keratopathy, vascularized cornea, active or past inflammatory/infectious disease (e.g., herpes infection, uveitis), glaucoma
Exclusion criteria	
Donors	HLA type by serology
	Corneas not stored by organ culture
	Endothelial cell density <2200 cells/mm²
Recipients	Corneal transplant procedures other than PK
	Patients under 16 years old
	Patients not competent to give consent
	Patients who have not given consent
	HLA type by serology
	Patients not at increased risk of allograft rejection

Patients at increased risk of rejection[3] were registered with NHS Blood and Transplant (NHSBT) and placed on the UK Transplant Registry waiting list for HLA matched corneal transplants. The only intervention was the allocation of patients to one of the study groups

determined by the level of HLA class II mismatching (i.e., 0, 1 or 2 HLA-DR mismatches): the surgical technique and postoperative management of the patients were according to surgeon preference.

Sample size. Sample size was estimated using data from a previous retrospective study, which reported freedom from rejection probability estimates at 1 year for HLA-DR matched (0 mismatched antigens) and HLA-DR mismatched transplants of, respectively, 0.7 and 0.6.[3] Assuming a ratio of 1:2 matched to mismatched transplants, the sample size required to detect a difference of 0.1 in probability of rejection at 1 year between the groups at the 5% level of significance with 80% power was calculated to be 856.

Allocation to study groups. The allocation of corneas to patients was a two-step process. First, patients were identified who were matched at HLA class I with the cornea donor. Corneas were then allocated by cohort minimization to these patients to achieve 0, 1 or 2 HLA-DR mismatches with the donor.[26 27] If there were sufficient HLA class I-matched patients on the waiting list such that a cornea could be assigned to more than one of the study groups, a weighting factor was used to increase the probability (0.95) that the cornea would be allocated to the group with the fewest accrued transplants and decrease the probability of being allocated at random to one of the other groups.

Clinical follow-up data. Data were submitted by surgeons completing NHSBT Ocular Tissue Transplant Audit forms used for routine data collection for corneal transplants in the UK. Data were submitted at the time of patient registration with NHSBT, at the time of transplantation, at 6 months postoperatively and then every 12 months on the anniversary of the transplant for up to 5 years.

Outcome. The primary outcome measure for the study was time to first clinically determined allograft rejection episode regardless of whether it led directly to graft failure or was treated successfully. The criteria for determining allograft rejection included one or more of the following: red eye, photophobia, loss of vision, cells in the anterior chamber, keratic

precipitates, endothelial or epithelial rejection line, subepithelial infiltrates, or an area of localized graft oedema.

Statistical methods. A range of donor, recipient and cornea characteristics are described. Continuous measures are summarised using mean and standard deviation (SD) or median if the distribution was skewed, and categorical variables are summarised as number and percentage.

Results

Accrual of transplants to the study began on 3 September 1998 and closed on 2 June 2011. To allow for loss to follow up, a total of 1133 transplants in 980 recipients carried out in 31 hospitals were accrued to the study (see Acknowledgements for list of participating surgeons). Figure 1 shows the numbers of transplants accrued, excluded and randomized to the study groups.

Donor characteristics

Mean donor age was 50 (SD 14) years and 54% of donors were male. The mean intervals between death and enucleation and between enucleation and corneoscleral disc excision were, respectively, 19.6 (SD 7.1) hours and 17.7 (SD 6.7) hours. Corneas were stored in organ culture for 20.9 (SD 4.1) days. Mean endothelial cell density was 2684 (SD 231) cells/mm². Descriptive statistics for the individual study groups are shown in Table 2.

Table 2. Donor variables by study group (0, 1 or 2 HLA-DR mismatches). Mean (SD) given for continuous variables.

	Number of HLA-DR mismatches		
Donor variables	0	1	2
n (%)	182 (17)	482 (44)	413 (39)
Age (y)	51.1 (14.1)	49.9 (13.1)	49.5 (14.2)
Sex (% male)	52	53	56
Death-Enucleation (h)	20.3 (6.9)	19.6 (7.1)	19.3 (7.2)
Enucleation-excision (h)	17.8 (6.7)	17.3 (6.9)	18.2 (6.4)
Storage time (d)	20.8 (4.1)	21.0 (4.1)	21.0 (4.1)
ECD (cells/mm²)	2681 (219)	2676 (225)	2694 (241)

Recipient characteristics

As expected, recipient age (p<0.0001) but not donor age (p=0.7) varied with indication for transplantation (Table 3). High risk was defined by both indication and the presence of other preoperative risk factors. The overall distributions of indications and other risk factors are shown, respectively, in Table 3 and Figure 2. Of the 1077 transplants that met all the study criteria and were allocated to the study groups, 136 had none of the other risk factors (i.e., the indication by itself was considered to increase the risk of rejection), 293 transplants had 1 of the other risk factors and 646 transplants had >1.

Table 3. Distribution of indications and mean (SD) recipient and donor ages.

Indication	n (%)	Recipient age (y)	Donor age (y)
Ectasias	75 (7)	41.6 (13.9)	50.1 (13.6)
Dystrophies	235 (21)	67.3 (13.3)	50.7 (12.2)
Previous ocular surgery	310 (27)	69.8 (13.9)	50.2 (14.0)
Infection	200 (18)	58.5 (17.3)	48.3 (14.7)
Injury	75 (7)	48.9 (16.3)	49.8 (14.1)
Ulcerative keratitis	21 (2)	57.1 (17.6)	49.6 (13.4)
Opacification	61 (5)	58.7 (18.4)	51.7 (12.9)
Miscellaneous	156 (14)	58.7 (16.9)	49.9 (14.7)
Total	1133	61.7 (17.3)	50.0 (13.7)
		$p<0.0001$	$p=0.7$

The distribution of HLA-DR mismatches is shown in Table 2. There was a preponderance of transplants with 1 or 2 HLA-DR mismatches and 182 transplants (17%) had 0 HLA-DR mismatches. Sixty-two percent of patients waited 3 months or less for an HLA matched transplant: 78% waited ≤6 months and 87% received a matched transplant within 12 months of being registered for the study (Figure 3).

Discussion

Allograft rejection remains a serious postoperative complication following corneal transplantation, especially for PK. Even with the increasing preference for EK, which has reportedly lower rates of rejection than for PK,[6 7] it is still important to better understand corneal transplant rejection and to explore ways to reduce the risk especially since globally just 30% of corneal transplants are lamellar procedures.[5]

The varying results from studies on HLA matching in corneal transplantation could be due to several factors, including study design (typically retrospective), low numbers of transplants (i.e., low statistical power), HLA serological typing errors, and different HLA matching strategies (e.g., class I only, class II only, both class I and II, or overall number of matched HLA antigens ignoring whether class I or class II). In view of these equivocal results, the CTFS II was designed to investigate the influence of HLA class II matching, against a background of HLA class I matching, in a large, well-defined cohort of high risk PKs in a prospective clinical trial. Class II matching was targeted because of the results from different studies suggesting a benefit of matching,[11 28] no influence of matching[29] or a detrimental effect of matching.[3 13]

So far as the inclusion criteria are concerned, some consider pseudophakic bullous keratoplasty (PBK) to be a moderate- rather than a high-risk graft; however, in a previous study we found that the relative risk of rejection at one year for PBK was similar to that for regrafts and therefore believe the inclusion of PBK in the CTFS II to be justified.[3] Moreover, it is likely that the level of risk will vary between the different factors included in the selection criteria for defining grafts at risk of rejection. Regression coefficients for each of the risk factors in the final Cox proportional hazards model will help quantify the level of risk for each factor and, potentially, allow a prognostic index to be generated to determine the overall level of risk for different combinations of factors.

The most widely accepted way to avoid bias in prospective clinical trials is to use random allocation of patients to treatment groups. With sufficient numbers, there should be no systematic differences between the study groups in patient characteristics and other variables, other than the allocated treatment, likely to influence the trial outcome. For the CTFS II, a classic randomized trial would have involved allocating each patient to receive a specific level of HLA class II match (i.e., 0, 1 or 2 HLA-DR mismatches) at the time of recruitment to the study, with each patient having an equal chance of being allocated to one

of the three study groups. Finding a specific level of HLA mismatch for a cornea depends on the donor HLA type and the distribution of HLA types of recipients on the waiting list – the larger the waiting list, the more likely it is to achieve the required level of matching for the cornea. However, the level of matching that can be achieved for a given patient depends purely on their own HLA type and the HLA type of the donor cornea available at any one time. The genes of the human major histocompatibility complex (HLA) are highly polymorphic, resulting in hundreds of different alleles at each HLA locus. Therefore, random allocation could result in a patient being allocated to a study group where the likelihood of achieving the required level of matching would be wholly unrealistic within a reasonable time. This would be unethical and could result in highly unbalanced study groups.

In order to optimize the balance between the CTFS II study groups, we chose to use the method of minimization, which, according to Altman and Bland,[26 27] is a valid alternative to full randomization. Minimization includes a random element, which, for CTFS II, meant that a cornea would be allocated with a defined probability to a patient with whichever level of HLA class II mismatch would minimize the overall imbalance between numbers of transplants in the respective study groups. Despite this, there was a preponderance of HLA-DR mismatched transplants; however, the study groups were balanced in their respective distributions of donor factors (Table 2) and indication for transplantation (data not shown). While a degree of age matching between donors and recipients is practised in the UK to avoid corneas from very old donors being allocated for very young recipients, this was not the case for CTFS II where the primary allocation criterion was HLA match, ignoring donor age. Since the great majority of HLA-typed eye donors (87%) were also organ donors, the mean donor age of 50 (SD 14) years was lower than the overall mean of 61 (SD 18) years for UK eye donors and extreme donor-recipient age differences were not therefore a concern.

The CTFS II was designed to test the hypothesis that HLA class II matching reduces the risk of rejection in high-risk penetrating keratoplasty. Analysis of the data to establish whether this hypothesis is correct has started now that all the transplants have completed their follow up.

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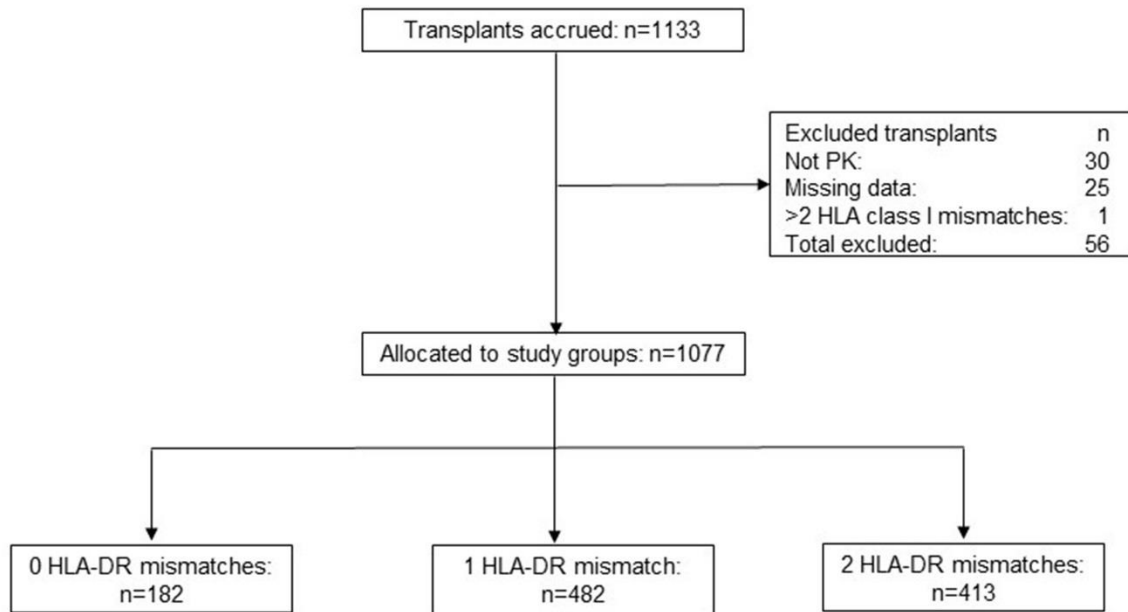
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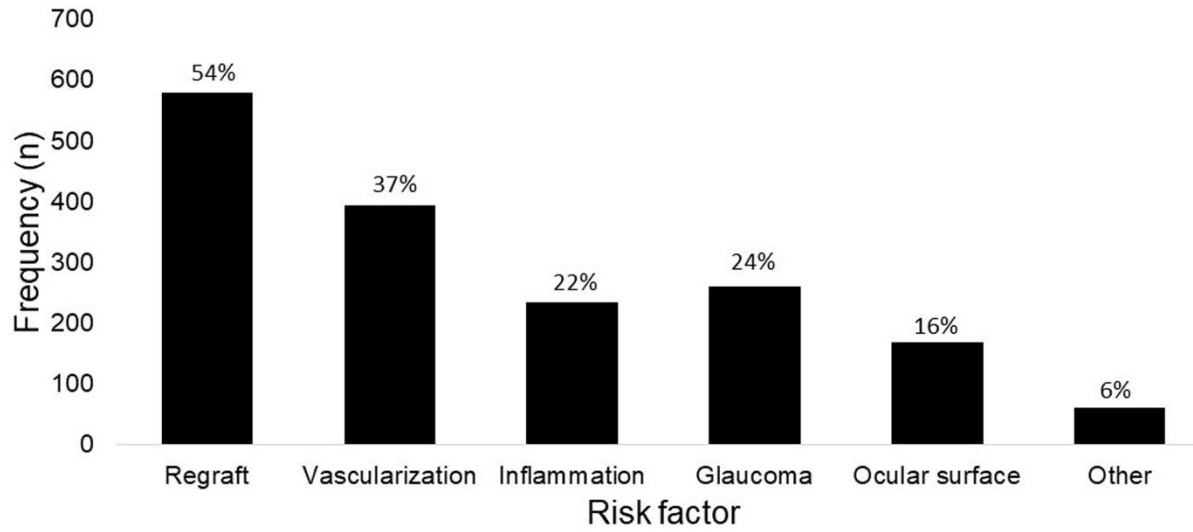Figure 1. Numbers of transplants accrued, excluded and randomized to the study groups.

Figure 2. Distribution of preoperative risk factors (n=1701) in the 1077 transplants allocated

to the study groups and percentages of transplants with each risk factor.

Figure 3. Distribution of patient waiting times for HLA matched transplants.

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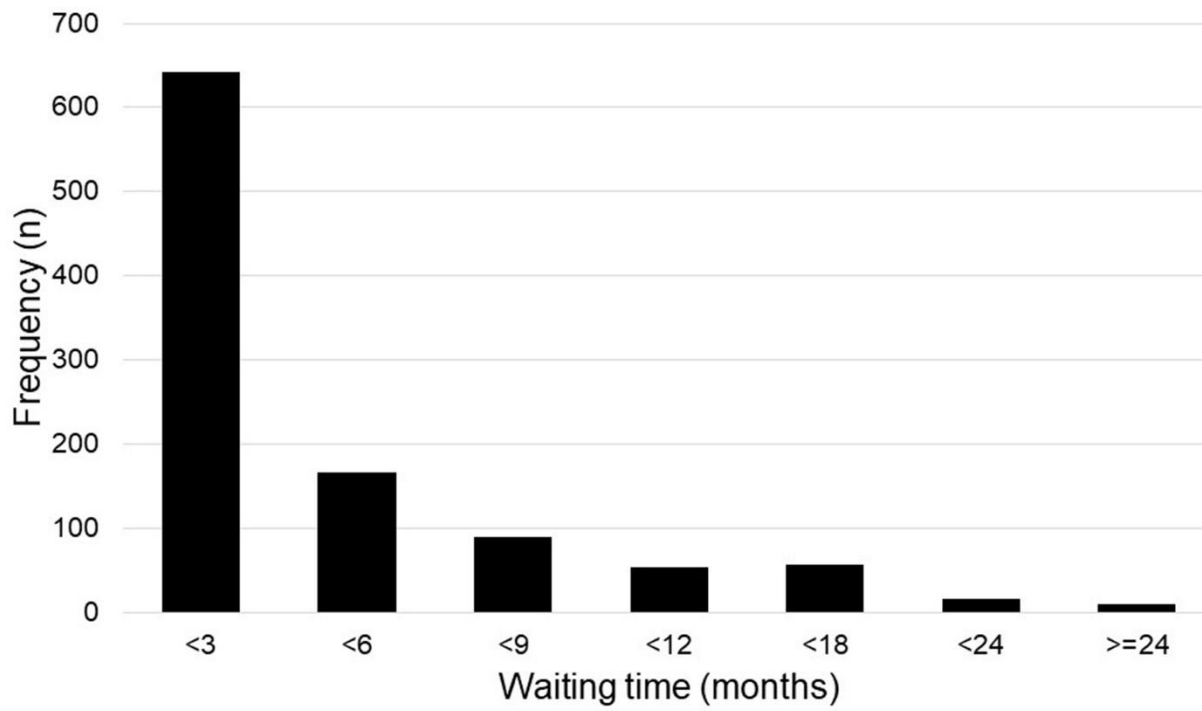
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