Recovery of spermatogenesis following bone marrow transplantation

Survival following bone marrow transplantation (BMT), with an estimated 1.5 million transplants performed globally between 1957 and 2019,[1] has improved with reductions in all-cause late mortality.[2] Recent Australian data suggests that survivors of BMT performed between 2002-2011 reaching 2 years post-transplant, have survival rates only mildly inferior to the wider population.[3] However, BMT conditioning causes severe testicular damage, with high rates of azoospermia,[4] such that pre-BMT fertility insurance counseling for sperm cryopreservation is crucial for enhancing post-BMT survivorship care.

There is a paucity of contemporary data to describe the time-course and determinants of reproductive function recovery following BMT in men. Many old studies[5,6] no longer accurately reflect the impact of modern BMT conditioning regimens, or are cross-sectional[5,7] and so cannot elucidate the timeline of recovery. Several also focus on specific sub-populations, such as pediatric BMT,[7] particular diagnoses[8] or reduced-intensity conditioning,[9] and thus have limited generalizability. Data on the temporality of recovery is limited to findings of no recovery following conditioning with total body irradiation (TBI) and cyclophosphamide within 4 years after BMT,[10] and recovery of sperm concentration over time in patients with longer follow-up periods.[4]

This study therefore aimed to characterize the natural history of spermatogenesis recovery after BMT using time-to-event analysis of baseline and follow-up data from a sperm cryostorage program at the Andrology Department, Concord Hospital, Sydney, Australia as detailed elsewhere.[11,12] Patients were selected from the prospective departmental sperm cryostorage database based on treatment coding, and all consecutive men who underwent sperm cryostorage prior to a BMT between 1978 and 2022 were included. The prospective departmental sperm cryostorage database included baseline andrology review of marital and fertility status, measurement of testicular volumes (orchidometry) and reproductive hormones (serum luteinizing hormone [LH], follicle-stimulating hormone [FSH], testosterone, sex hormone-binding globulin [SHBG], immunoassay). Semen samples were collected by masturbation at the Clinical Andrology laboratory after a recommended abstinence interval of 2-3 days. Semen volume, sperm concentration, motility, and forward progression (before and post-thaw after freezing) and morphology were recorded according to recommendations of the extant World Health Organization (WHO) semen analysis manuals. Annual follow-up included clinical review of fertility, semen analysis, and assessment of testicular volumes and reproductive hormone levels. Data regarding BMT, including transplant date, type and conditioning regimen, were sourced from electronic patient records where available. The data collected over 44 years available within the clinic database was anonymized for analysis, consistent with local institutional policies and ethical standards for retrospective audits of completed medical care.

The primary outcome of this study was sperm output (per ejaculate) analyzed as a continuous variable after cube-root transformation to approximate a normal distribution according to the optimal power function from a Shapiro-Wilks analysis.[13] As a categorical variable, sperm output was defined as normozoospermia (>20 million), oligozoospermia (0.1-20 million) and azoospermia (<0.1 million), adopting the thresholds of the WHO reference range study for unselected men.[14] Categorical data were summarized by frequency and percentage and analyzed by χ^2 or Fisher's test. Continuous data was reported as mean and standard deviation, if normally distributed, and by median and interquartile range (IQR) otherwise. For longitudinal data, time was defined as years post-BMT, or date of first sperm cryopreservation where the transplant date was not available. Outcome associations were assessed by an ordinal logistic regression with outcomes at final follow-up categorized into azoospermia, oligozoospermia and normozoospermia. As sperm output over time since pre-treatment baseline was non-monotonic, plots of sperm output over time were fitted to a locally weighted non-linear (LOESS) curve fit. Time to outcomes was depicted as a Kaplan-Meier survival plot and assessed by univariate and multivariate Cox model regression survival analyses using sperm output for all semen data after 3 months from cryostorage to allow for the time to reach azoospermia following BMT conditioning. In all cases, a two-tailed *P* value of <0.05 was deemed significant. Statistical analysis was conducted using SPSS Statistics 29 (IBM, 2023), and R version 4.1 (R Core Team, 2021) using survival and survminer packages and NCSS 2024 (Kaysville, UT, USA, ncss.com).

We investigated the time-course of recovery of sperm output following BMT of 102 men with underlying diseases of leukemia (59%), non-Hodgkin lymphoma (14%), Hodgkin lymphoma (7%), and other diagnoses comprising 5% or fewer patients. The men, with a mean age of 30 years at BMT, had prior paternity in 21% with few (N=3) having any history of testicular pathology, and were mostly never-smokers (75%) with minimal alcohol intake (99% nil or moderate drinking). Prior to treatment, the mean testicular volume, reproductive hormone levels and semen parameters for the patient cohort were all within the normal range.

At initial review for cryopreservation, constitutional symptoms were fever (40%) and weight loss (30.5%) and previous

therapies included chemotherapy (91%) and radiotherapy (13%). BMT was allogeneic in 80% of patients and 13% reported more than one BMT. A minority (40%) of BMT conditioning regimens involved TBI.

Sperm output was severely reduced after BMT with some recovery emerging beyond 4-6 years post BMT (Figure 1). At final follow-up after a median of 4.1 years (IQR, 2.5-7.4) and three semen samples (IQR, 2-5) per patient, 53% of patients had azoospermia, 31% oligozoospermia and 15% normozoospermia. On ordinal logistic regression, univariate factors associated with recovery were deleterious effects of TBI conditioning (P<0.001) and prior chemotherapy (P<0.001), alongside favorable effects of diagnosis (aplastic anemia favorable; P=0.05). Whereas on multivariate analysis adjusting for all other potential associations, only TBI and diagnosis remained significant (Table 1).

On time-to-event analysis, for the whole cohort the median times to non-azoospermia (first appearance of sperm) and normozoospermia were 4.7 (IQR, 3.9-6.2) years and 11.5 (IQR, 6.8-not reached) years respectively (Figure 2). Cox model associations with normozoospermia at recovery were BMT conditioning including TBI (P<0.001), past smoking (P<0.001), and allogeneic (*vs*. autologous) BMT (P<0.001), whereas other variables (diagnosis, cryptorchidism, testis pathology, lower serum LH and FSH) which were significant on univariate analysis, were no longer significant after multivariate adjustment.

The present study is the first, to our knowledge, to use time-to-event analysis to investigate the natural history of spermatogenic recovery following BMT. Although primarily descriptive, significant associations with outcomes in this study comprised favorable pre-BMT diagnosis (aplastic anemia) and the detrimental impact of TBI-based conditioning regimens. These data provide practical counseling for men scheduled to undergo BMT regarding both the expected long-term effects on fertility as well as the importance of pre-BMT sperm cryopreservation for fertility insurance. Consistent with previous findings,[4] this study also highlights the importance of patient registries and longer systematic follow-up in future post-BMT fertility research.

The present cohort exhibited a lower rate of azoospermia (53%) at final follow-up compared to previous studies reporting over 69%[4,10,15] although the distribution of underlying diagnoses was comparable. This may reflect fewer patients

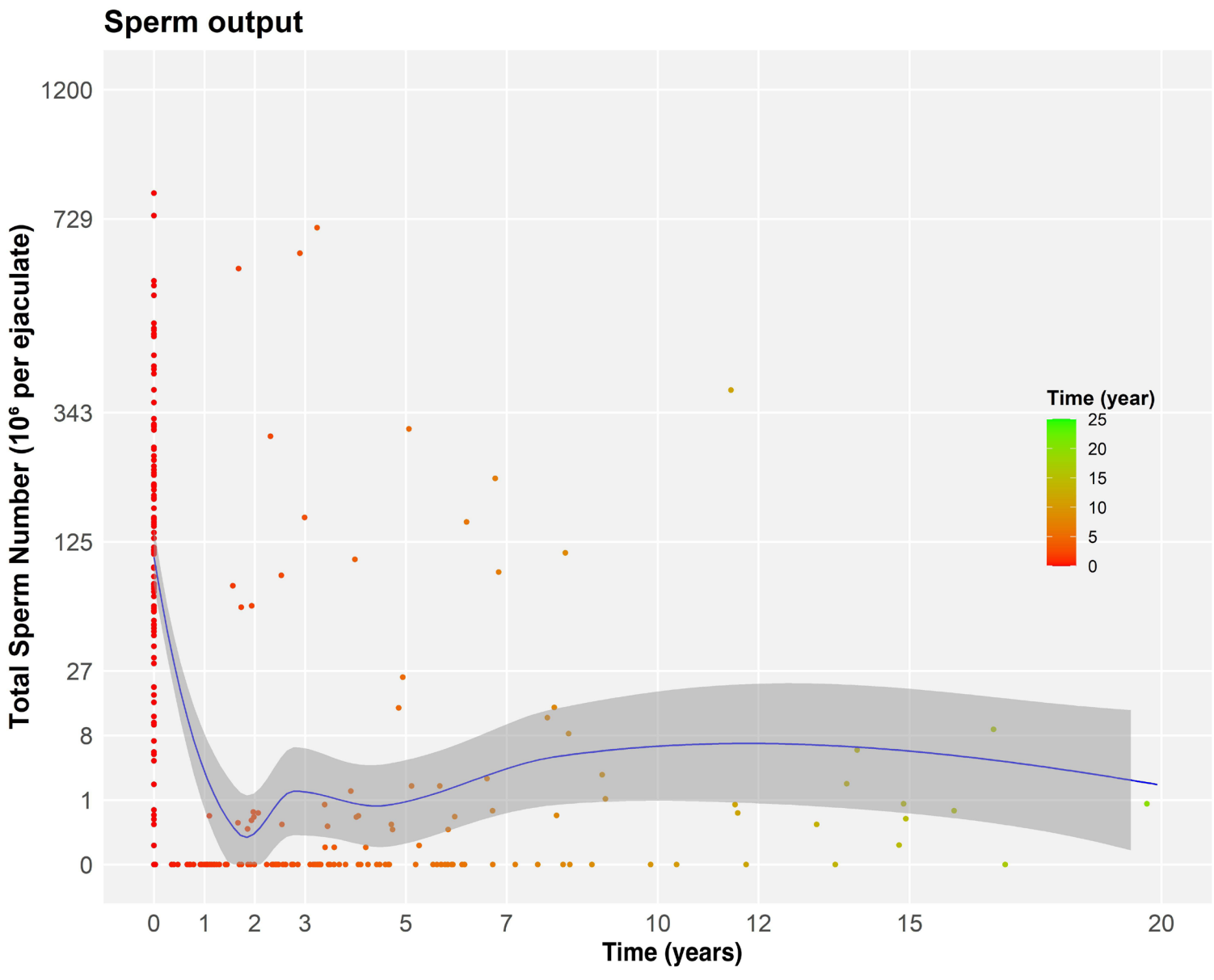

Figure 1. Sperm output over time. Plot of sperm output (in millions per ejaculate) against time from baseline before and after bone marrow transplantation. The non-linear line of best fit to this data is a smoothing curve using locally weighted, non-parametric linear regression (LOESS curve) suitable for the non-monotonic data.

Table 1. Baseline associations with final sperm output.

Variables	N	Azoospermia *	Oligozoospermia *	Normozoospermia *	*P*
N		34	19	9	
Age, years	62	30±1	26±2	31±2	0.074
Height, cm	55	177±1	178±1	171±3	0.2
Weight, kg	56	75.4±2.4	81.3±3.6	74.8±5.9	0.4
Diagnosis, N (%) Aplastic anemia Other	61	 0 (0.0) 34 (100.0)	 0 (0.0) 18 (100.0)	 4 (44.4) 5 (55.6)	<0.001
Cryptorchidism, N (%)	61	1 (2.9)	0 (0.0)	0 (0.0)	>0.9
Testicular pathology, N (%)	59	3 (9.1)	0 (0.0)	0 (0.0)	0.7
Prior fertility, N (%)	61	6 (17.6)	3 (15.8)	2 (25.0)	0.8
Smoking, N (%) None Past Current	57	 27 (81.8) 4 (12.1) 2 (6.1)	 14 (87.5) 0 (0.0) 2 (12.5)	 7 (87.5) 1 (12.5) 0 (0.0)	0.6
Alcohol, N (%) None Moderate/social	57	 9 (27.3) 24 (72.7)	 4 (25.0) 12 (75.0)	 3 (37.5) 5 (62.5)	0.8
Fever, N (%)	58	11 (34.4)	9 (50.0)	2 (25.0)	0.4
Weight loss, N (%)	57	6 (19.4)	5 (27.8)	2 (25.0)	0.7
Prior chemotherapy, N (%)	47	26 (100.0)	12 (92.3)	4 (50.0)	<0.001
Prior radiotherapy, N (%)	55	6 (20.0)	2 (12.5)	0 (0.0)	0.4
BMT type, N (%) Autologous Allogeneic	60	 8 (25.0) 24 (75.0)	 5 (26.3) 14 (73.7)	 0 (0.0) 9 (100.0)	0.3
Conditioning regimen, N (%) Chemotherapy Chemotherapy and TBI	56	 12 (38.7) 19 (61.3)	 13 (81.3) 3 (18.8)	 9 (100.0) 0 (0.0)	<0.001
TBI, N (%)	57	19 (61.3)	4 (23.5)	0 (0.0)	<0.001
Drugs, N (%) Alkylating Non-alkylating	55	 31/31 (100.0) 14/30 (46.7)	 16/16 (100.0) 7/16 (43.8)	 9/9 (100.0) 5/9 (55.6)	0.9 >0.9 0.9
Multiple BMT, N (%)	55	7 (23.3)	1 (6.3)	1 (11.1)	0.4
Total testicular volume, mL	56	41±2	40±2	40±5	>0.9
Luteinizing hormone, IU/L	61	6.5±0.9	5.7±0.8	6.0±1.1	0.8
Follicle stimulating hormone, IU/L	61	5.4±0.6	4.9±0.7	4.6±1.0	0.8
Testosterone, nmol/L	60	13.3±1.2	12.6±1.1	13.9±2.4	0.8
Sex hormone binding globulin (nmol/L)	61	39.1±3.3	33.4±6.0	28.4±5.0	0.2
N of semen samples at initial cryostorage, N (%)	62	3±1	2±0	2±0	0.6
Semen volume, mL	62	2.9±0.3	2.9±0.4	3.3±0.5	0.8
Sperm output, x10^6 per ejaculate (IQR)	62	151 (49-270)	183 (78-460)	306 (119-589)	0.13
Sperm density, x10^6/mL (IQR)	62	70.4 (22.7-121.7)	74.2 (32.8-106.6)	95.5 (65.9-123.2)	0.2
Sperm motility, %	60	46±3	54±3	57±1	0.023
Sperm forward progression, 0-5 (IQR)	56	2.00 (0.00-4.00)	2.00 (1.00-3.00)	2.00 (2.00-2.00)	
Atypical spermatozoa, %	58	90±1	90±1	89±2	>0.9

*Normozoospermia is defined as >20 million sperm per ejaculate, oligozoospermia as 0.1-20 million sperm per ejaculate and azoospermia as <0.1 million sperm per ejaculate[14]. Continuous data is presented as mean ± standard error of the mean (SEM) (if normal distribution) or median (interquartile range [IQR]) otherwise. Categorical data is presented as number (proportion) and ordinal data (forward progression) as mode (range). BMT: bone marrow transplant; TBI: total body irradiation.

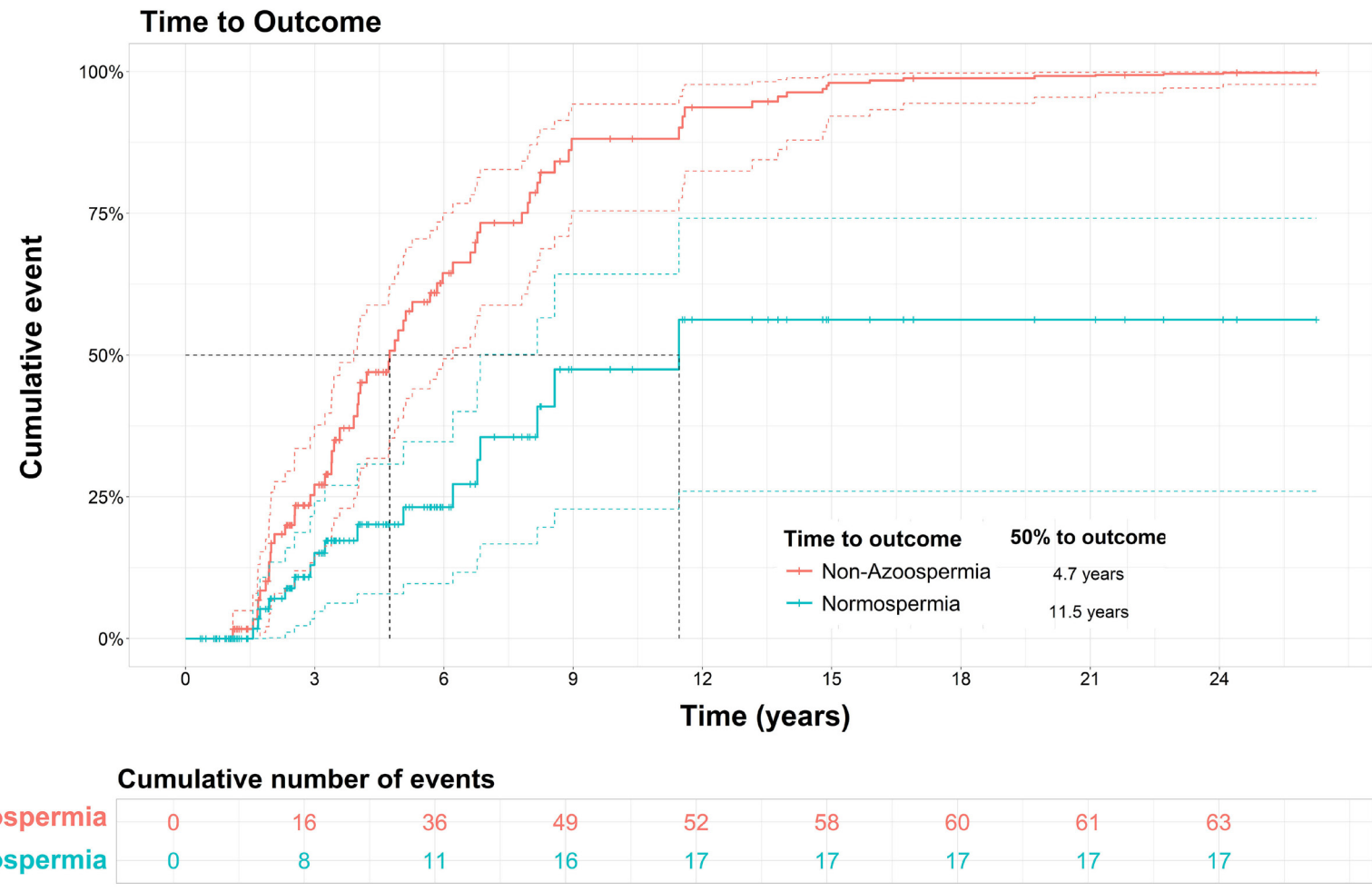

Figure 2. Survival analysis of sperm output over time. Cumulative survival plot of sperm output over time since bone marrow transplantation with separate curves for normozoospermia (≥20 million sperm per ejaculate) or non-azoospermia (any sperm appearing in the ejaculate) with their estimated median times to achieving those thresholds.

in this study who underwent TBI conditioning (40%), compared to 66% or greater among other cohorts.

The severe detrimental effect of TBI on spermatogenesis is widely reported.[6,7] In the present study, age at the time of BMT was not associated with sperm recovery[4,15] consistent with another study of comparably aged patients.[10] By contrast, age was a predictive factor in previous studies which included much younger pediatric patients.[4] The present study did neither have data on chronic graft-*versus*-host disease,[4,15] nor elevated ferritin levels[7] which are reported as influential factors in recovery of spermatogenesis after BMT. Moreover, the predictive analysis was limited by the lack of granular treatment data including detailed conditioning regimen protocols and chemotherapy/radiotherapy dosages. The greater propensity for recovery of sperm production after BMT for aplastic anemia has been previously identified.[6] This better prognosis likely reflects the general absence of prior treatment with chemotherapy, as well as less intense conditioning regimens. Mathiesen *et al*.[7] reported that the cyclophosphamide-equivalent dose of the conditioning regimen predicted azoospermia at follow-up among those not treated with TBI, so it is likely that prior chemotherapy would add to this cumulative gonadotoxic burden. However, pre-BMT treatment dosage data were unavailable, and the extent of confounding between aplastic anemia diagnosis, prior chemotherapy and TBI-based conditioning (or lack thereof) was not assessed in this study.

Strengths of this study include a relatively large number of men with reproductive function studied prospectively before as well as longitudinally after BMT using time-to-event analysis to accommodate the irregular follow-up. On the other hand, limitations include its retrospectivity over decades leading to incomplete data with loss-to-follow-up introducing survivorship bias. Furthermore, the sperm cryostorage program as a source for recruitment may have introduced participation bias in omitting men who did not undertake such storage. Finally, the study sample size may have limited the ability to detect smaller effects.

In summary, this study has produced a contemporary picture of the reproductive effects of BMT on adult male patients, with novel features regarding the use of time-to-event analysis to define the time course of such impacts. It may thus serve to further inform clinicians and encourage patients to have sperm cryostorage for fertility preservation insurance prior to, as well as fertility counseling following, BMT.

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Disclosures

No conflicts of interest to disclose.

Contributions

JD was the primary investigator and his role included study design, data collection and analysis, and manuscript preparation. SS and AI assisted with database maintenance/data entry, as well as manuscript review. VJ and AJC also assisted with study design and manuscript review. TZ performed the statistical analysis and table/figure generation. DJH was the supervising author, with his role including study design, overseeing of the statistical analysis, and manuscript preparation and review.

Data-sharing statement

Original study data can be provided upon reasonable request.

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