Polyomavirus Nephropathy: Ten-Year Experience

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ABSTRACT

Background. Polyomavirus nephropathy (BKVN) is an important cause of chronic allograft dysfunction (CAD). Recipient determinants (male sex, white race, and older age), deceased donation, high-dose immunosuppression, diabetes, delayed graft function (DGF), cytomegalovirus infection, and acute rejection (AR) are risk factors. Reducing immunosuppression is the best strategy in BKVN. The objective of our study was to evaluate CAD progression after therapeutic strategies in BKVN and risk factors for graft loss (GL).

Methods. Retrospective analysis of 23 biopsies, from patients with CAD and histological evidence of BKVN, conducted over a period of 10 years. Glomerular filtration rate was <30 mL/min in 16 patients at the time of the BKVN diagnosis.

Results. BKVN was histologically diagnosed in 23 recipients (19 men, 4 women). All patients were white, with age of 51.2 ± 12.1 years (6 patients, age >60 years), and 22 had a deceased donor. Diabetes affected 4 patients, DGF occurred in 3, cytomegalovirus infection in 2, and AR in 15. All patients were medicated with calcineurin inhibitors (CNI) (95.7% tacrolimus) and corticoids, and 16 also received an antimetabolite. One year after antimetabolite reduction/discontinuation and/or CNI reduction/switching and/or antiviral agents, graft function was decreased in 11 patients, increased/stabilized in 10, and unknown in 2. GL occurred in 9 patients. Older age (hazard ratio, 1.76; 95% confidence interval, 0.94–3.28) and DGF (hazard ratio, 2.60; 95% confidence interval, 0.54–12.64) were the main risk factors for GL. The lower GFR at the time of the BKVN diagnosis was associated with an increased risk of initiation of dialysis.

Conclusions. GL occurred in 39.1% of patients with BKVN and DGF; older age and lower GFR at the time of diagnosis were important risk factors. Early diagnosis of BKVN is essential to prevent GL.

BK VIRUS (BKV) is a non-enveloped DNA virus of the *polyomaviridae* family, and 80% to 90% of the general population is seropositive for BKV [1]. Primary infection usually occurs in the first decade of life, probably by respiratory or oral transmission, and then becomes latent mainly in the urinary tract [2]. Under sustained and intensive immunosuppression, the reactivation of latent BKV can occur, leading to polyomavirus-associated nephropathy (BKVN) in 1% to 10% of kidney transplant patients [2], most frequently in the first 2 years after transplantation [3]. This progressively affects graft function and increases the risk of chronic allograft dysfunction (CAD) and graft loss (GL) [2], more often with BKVN late diagnosis. BKV incidence has increased since the mid-90s with the use of new powerful immunosuppressor treatments [4]. Because effective and safe antiviral therapies are lacking [2,3],

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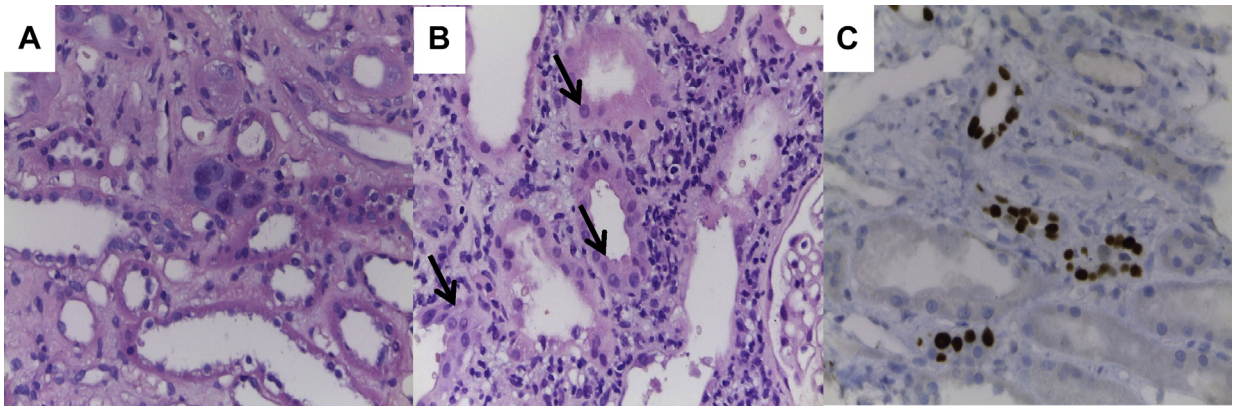

Fig 1. BK virus nephropathy in kidney allograft biopsies. (**A**) Tubular epithelial cells with large, irregular nuclei, nucleoli, basophilic chromatin, and vesicular changes; hematoxylin and eosin (HE) stain, magnification ×400. (**B**) Polyomavirus intranuclear inclusions (*arrows*) in the tubular epithelial cells; HE stain, magnification ×400. (**C**) Characteristic nuclear staining reaction (antibody directed against the BKV SV40 T antigen) demonstrated by immunohistochemistry; magnification ×400.

identification of risk factors and screening for BKV replication is essential.

Some of the risk factors for BKVN are donor determinants (HLA mismatches and deceased donation), recipient determinants (older age, male sex, white race), and modulating factors after transplantation (acute rejection, delayed graft function [DGF], steroid exposure, lymphocyte-depleting antibody medication, high dose of immunosuppressive medication, and tacrolimus–mycophenolic acid compared with cyclosporine-mycophenolic acid or mTOR inhibitor combinations) [2].

Viremia by polymerase chain reaction (PCR) detection is the recommended screening test for BKVN. A level superior to 10^4 copies/mL is associated with a 93% specificity [2] and can establish "presumptive BKVN" [2,5]. However, only histological findings of BKVN (cytopathic tubular epithelial cell changes, intra-nuclear viral inclusion bodies in tubular epithelial cells, and immunohistochemistry detection of monoclonal antibodies directed against BKV SV40 T antigen) in the allograft biopsy allows its definitive diagnosis [2,5]. Viremia can guide a stepwise reduction of immunosuppression, which appears to be the best therapeutic strategy to delay the progression of CAD. Most studies consider that a period of more than 12 weeks of reduced immunosuppression is required to significantly reduce viral load levels [5]. The effectiveness of additional strategies such as switching immunosuppressive medication (eg, calcineurin inhibitors (CNI) to mTOR inhibitor), with no randomized, controlled trial recommending one over the other, or use of antiviral therapy (quinolone or leflunamide), are still not proven [2,3].

The objective of the study was to evaluate CAD progression after therapeutic strategies in BKVN and risk factors for GL.

METHODS

We retrospectively reviewed 23 kidney allograft biopsies from patients with CAD, between January 2005 and December 2015. All patients had histological evidence of BKVN, with intra-nuclear viral inclusions in the tubular epithelial cells, stained with hematoxylin-

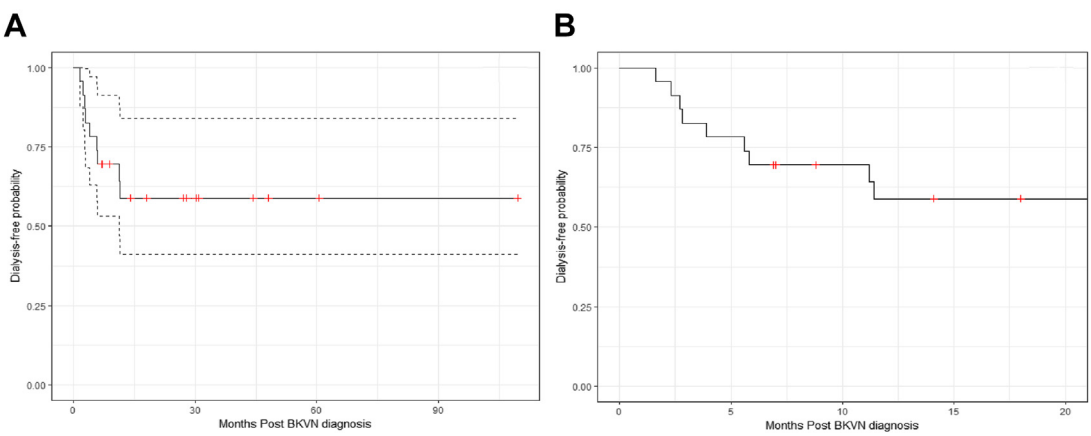

Fig 2. Kaplan-Meier estimate of time to dialysis after BKVN diagnosis (including 95% CIs), from the time of BKVN diagnosis until the end of the study (**A**) and for the first 20 months after the diagnosis of BKVN (**B**).

Table 1. Individual Characteristics (1)

Id	G	Age (Years)	Donor	DGF	Immunosuppression at Diagnosis	Therapeutic Strategy
1	M	56	D	No	FK + PDN	Quinolone + antimetabolite reduction/discontinuation + conversion FK to EVE
2	M	55	D	No	MY + FK + PDN	Quinolone + antimetabolite reduction/discontinuation + conversion FK to EVE
3	M	63	D	No	MY + FK + PDN	Quinolone + CNI reduction + antimetabolite reduction/discontinuation
4	F	42	D	No	FK + PDN	Quinolone + antimetabolite reduction/discontinuation + conversion FK to EVE
5	M	53	D	No	MY + FK + PDN	antimetabolite reduction/discontinuation + conversion FK to EVE
6	M	31	D	No	MY + FK + PDN	CNI reduction
7	M	25	D	Yes	MMF + FK + PDN	CNI reduction + antimetabolite reduction/discontinuation
8	F	38	D	No	MMF + FK + PDN	antimetabolite reduction/discontinuation + CNI reduction
9	M	65	D	No	FK + PDN	Quinolone + CNI reduction + antimetabolite reduction/discontinuation
10	M	34	D	No	FK + PDN	conversion FK to EVE
11	M	53	D	No	FK + PDN	Quinolone + CNI reduction + antimetabolite reduction/discontinuation
12	F	48	D	No	MY + FK + PDN	Quinolone + Leflunamide + antimetabolite reduction/discontinuation + conversion FK to EVE
13	M	52	D	No	MMF + FK + PDN	Quinolone + antimetabolite reduction/discontinuation + conversion FK to CyP
14	F	61	D	No	MY + FK + PDN	Quinolone + antimetabolite reduction/discontinuation + conversion FK to CyP
15	M	54	L	No	MY + FK + PDN	Quinolone + Leflunamide + CNI reduction + antimetabolite reduction/discontinuation
16	M	47	D	No	MMF + CyP + PDN	Leflunamide + antimetabolite reduction/discontinuation + conversion CyP to EVE
17	M	46	D	Yes	MMF + FK + PDN	Leflunamide + antimetabolite reduction/discontinuation + conversion FK to CyP
18	M	48	D	No	MY + FK + PDN	Leflunamide + CNI reduction + antimetabolite reduction/discontinuation
19	M	44	D	No	MMF + FK + PDN	Quinolone + conversion FK to CyP + antimetabolite reduction/discontinuation
20	M	66	D	No	MMF + FK + PDN	antimetabolite reduction/discontinuation
21	M	74	D	Yes	FK + PDN	Quinolone + CNI reduction + antimetabolite reduction/discontinuation
22	M	56	D	No	AZA + FK + PDN	CNI reduction
23	M	66	D	No	FK + PDN	CNI reduction + antimetabolite reduction/discontinuation

Abbreviations: AZA, azathioprine; CNI, calcineurin inhibitor; CyP, cyclosporine; D, deceased donor; DGF, delayed graft function; EVE, everolimus; F, female; FK, tacrolimus; G, gender; L, living donor; M, male; MFA, mycophenolic acid; MMF, mycophenolate mofetil; PDN, prednisone.

eosin, and positive polyomavirus SV40 large T-antigen expression, by immunohistochemistry (Fig 1). Data regarding transplant recipient and donor demographics, plasma BKV (viremia) values, glomerular filtration rate (GFR) and immunosuppressive medication at the time of the biopsy, BKVN therapeutic strategy and posterior viremia, and graft function evolution were collected. At the time of the biopsy, viremia was $>10^4$ copies/mL in 13 patients, $\leq 10^4$ copies/mL in 5 patients, and not tested in 5 patients; plasmatic creatinine was 3.3 ± 1.2 mg/dL and GFR (MDRD) was <30 mL/min in 16 patients. BKV DNA was detected through the use of a quantitative PCR assay and BKV viremia was defined positive when there were $>10^4$ copies/mL and negative when there were $\leq 10^4$ copies/mL. Date of diagnosis of BKVN was defined as the date of the first biopsy with positive immunohistochemistry for BKV.

The statistical analysis was event-driven, being the event of interest initiation of dialysis. Overall probabilities of not initiating dialysis were obtained and plotted by use of the Kaplan-Meier method and compared by means of the log-rank test when applicable. Univariate analysis was performed to identify potential risk factors, using the Cox proportional hazards model to obtain hazard ratios (HR). The Fisher exact test was also calculated, when possible, to assess the individual predictive value of each factor regardless of the time factor. All statistical analysis were conducted using R Statistical Software version 3.2.5.

RESULTS

BKVN was histologically diagnosed in 23 kidney recipients during the 10-year period analyzed, 17.8 ± 33.3 months after transplant. Figure 2 shows that the risk of GL is higher during the first 12 months after the diagnosis of BKVN. The individual characteristics of these patients are shown in Tables 1 and 2. All patients were white, mean age was 51.2 ± 12.1 years (6 patients were >60 years of age), and the majority (19 patients) were men. Almost all patients had a deceased donor (22 patients), and basiliximab was the most commonly used induction therapy agent (18 patients). At the time of the biopsy, all patients were medicated with CNI (22 patients with tacrolimus [FK], 1 patient with cyclosporine [CyP]) and corticoids, in combination with an antimetabolite in 16 patients (mycophenolic acid in 8 patients, mycophenolate mofetil in 7 patients, and azathioprine in 1 patient). Diabetes affected 4 patients: 1 patient had type 2 diabetes mellitus previous to the transplant and 3 patients were diagnosed with new-onset diabetes after transplantation. DGF occurred in 3 patients. There was cytomegalovirus infection in 1 patient at the time of the biopsy and in 1 patient before the biopsy. Acute rejection (AR) was diagnosed in 3 patients at the time of BKVN diagnosis (one with borderline rejection and one with T-cell–mediated rejection type IB, both medicated with methylprednisolone pulse therapy [MTP]; one with acute antibody-mediated rejection, medicated with immunoglobulin therapy, plasmapheresis, and rituximab) and in 12 patients before the biopsy (6 patients medicated with MTP, 3 patients medicated with MTP + thymoglobulin, and 3 non-medicated patients).

Table 2. Individual Characteristics (2)

Id	Diabetes Mellitus	CMV Infection	Acute Rejection	GFR Group at Diagnosis (mL/min)	Viremia $>10^4$ Copies/mL at Diagnosis	Viremia After Therapy	Decreased Graft Function (1 Year After Therapy)	GL
1	Yes	No	No	15–29	Yes	Decreased	Increased	No
2	No	No	No	15–29	Yes	Decreased	U	No
3	Yes	No	Yes	15–29	Yes	U	U	No
4	No	No	Yes	15–29	Yes	Negative	Increased	No
5	No	D	Yes	30–44	No	Decreased	Stabilized	No
6	No	No	No	45–59	U	Negative	Yes	No
7	No	No	Yes	30–44	U	U	Increased	No
8	No	No	Yes	30–44	No	Negative	Stabilized	No
9	No	No	Yes	<15	No	Decreased	Yes	Yes
10	No	No	Yes	30–44	Yes	Decreased	Stabilized	No
11	No	No	No	15–29	Yes	Negative	Increased	No
12	No	No	No	15–29	Yes	Increased	Stabilized	No
13	No	No	Yes	15–29	No	Negative	Increased	No
14	No	PrD	No	<15	Yes	Decreased	Yes	Yes
15	No	No	Yes	30–44	Yes	Decreased	Yes	No
16	No	No	No	30–44	Yes	Decreased	Yes	Yes
17	No	No	No	15–29	U	U	Yes	Yes
18	No	No	Yes	<15	Yes	U	Yes	Yes
19	No	No	Yes	15–29	No	U	Yes	Yes
20	Yes	No	Yes	<15	U	Decreased	Increased	No
21	No	No	Yes	<15	Yes	Decreased	Yes	Yes
22	No	No	Yes	15–29	Yes	U	Yes	Yes
23	Yes	No	Yes	15–29	U	U	Yes	Yes

Abbreviations: CMV, cytomegalovirus; D, at diagnosis of BKVN; DGF, delayed graft function; GFR, glomerular filtration rate; GL, graft loss; PrD, previous to diagnosis of BKVN; U, unknown.

Although not statistically significant, older age (HR, 1.76; 95% confidence interval [CI], 0.94–3.28) and DGF (HR, 2.60; 95% CI, 0.54–12.64) Cox regression estimates were high (Table 3), which may denote them as important risk factors for GL.

The lower GFR at the time of the BKVN diagnosis was associated with an increased risk of initiation of dialysis. Table 3 shows that patients with GFR 15 to 29 mL/min were approximately one-third less likely of starting dialysis than patients with GFR <15 mL/min. Kaplan-Meier plots (Fig 3) also support these findings.

The immunosuppressive medication was reduced in all patients, and at least one of the following therapeutic

Table 3. Risk Factors for Initiation of Dialysis

Factor	n (N)	HR (95% CI)	Cox Log-Likelihood Test	Fisher Exact Test
Age (range, 10 years)	NA	1.76 (0.94–3.28)	0.062	NA
Male sex	8 (19)	NE	0.396	1
Deceased donor type	9 (22)	NE	0.294	.640
Immunosuppression therapy				
FK + PDN	3 (7)	1.32 (0.33–5.30)	0.690	1
MY + FK + PDN	2 (8)	0.47 (0.09–2.29)	0.323	.399
MMF + FK + PDN	2 (6)	0.678 (0.14–3.30)	0.618	1
MMF + CyP + PDN	1 (1)	3.14 (0.38–26.09)	0.290	.391
AZA + FK + PDN	1 (1)	10.49 (0.95–115.73)	0.055	.391
Diabetes mellitus	1 (4)	0.64 (0.08–5.19)	0.660	1
DGF	2 (3)	2.60 (0.54–12.64)	0.280	.538
CMV infection	1 (2)	1.07 (0.13–8.57)	0.952	1
Acute rejection	6 (15)	1.06 (0.26–4.27)	0.932	1
Viremia $>10^4$ copies/mL at the diagnosis	5 (13)	1.12 (0.21–5.89)	0.894	1
GFR group at BKVN diagnosis				
<15 mL/min	4 (5)	RG	RG	RG
15–29 mL/min	4 (11)	0.35 (0.09–1.41)	0.143	.281
30–44 mL/min	1 (6)	0.12 (0.01–1.09)	0.060	.134
45–59 mL/min	0 (1)	NE	1	1

Abbreviations: AZA, azathioprine; CyP, cyclosporine; FK, tacrolimus; MFA, mycophenolic acid; MMF, mycophenolate mofetil; n, subjects who had undergone dialysis; N, subjects at risk; NA, not applicable; NE, non-estimable; PDN, prednisone; RG, reference group.

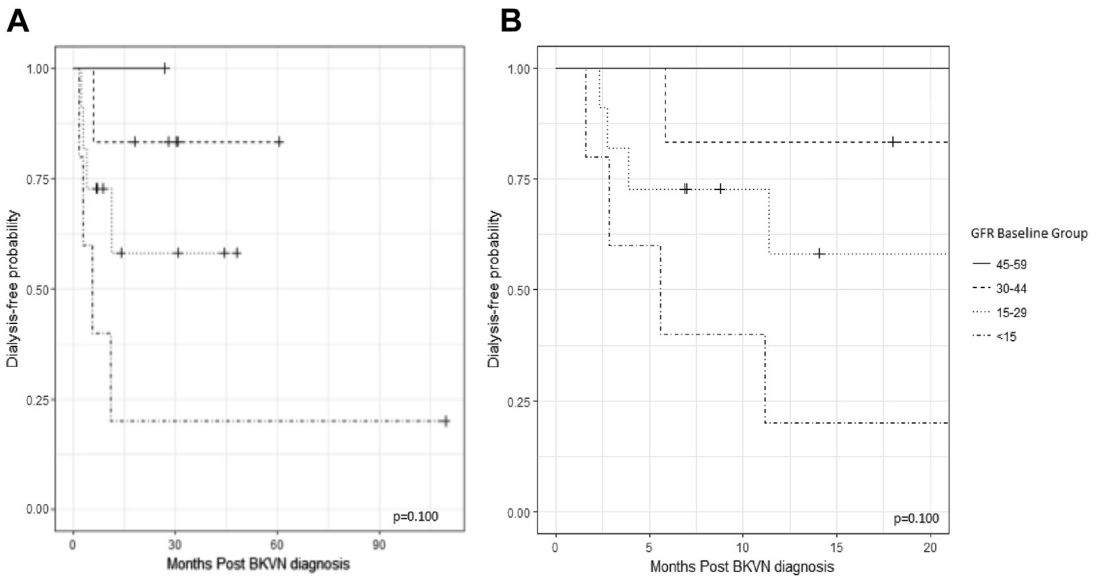

Fig 3. Kaplan-Meier estimate of time to dialysis after BKVN diagnosis by GFR baseline group: from the time of BKVN diagnosis until the end of the study (**A**) and for the first 20 months after the diagnosis of BKVN (**B**).

strategies was used: antimetabolite reduction/discontinuation in 20 patients, CNI substitution in 11 patients (FK to everolimus in 6 patients, FK to CyP in 4 patients, and CyP to everolimus in 1 patient), and CNI reduction in 11 patients. Antiviral therapy was used in 15 patients (quinolones during 6 months in 10 patients, oral leflunamide in 3 patients, or both in 2 patients). One year after the initiation of these therapeutic strategies, viremia was increased in 1 patient, reduced in 10 patients, negative ($\leq 10^4$ copies/mL) in 5 patients, and was not evaluated in 7 patients; allograft function was increased (total recovery in 1 patient, partial recovery in 5 patients) or stabilized in 10 patients (43.5%), decreased in 11 patients, and unknown in 2 patients. GL occurred in 9 patients (39.1%), 8 of them with GFR <30 mL/min (mean GFR of 17.6 mL/min) at the time of biopsy. Of these patients, 4 were >60 years of age, 8 were men, 1 was diabetic, 1 had CMV infection, 6 had previous AR (mostly medicated with corticoid + thymoglobulin), and 5 had viremia $>10^4$ copies/mL at diagnosis (Tables 1 and 2). The mean post-diagnosis follow-up of the patients was 21.1 ± 21.6 months.

DISCUSSION

In our study, we reviewed renal allograft biopsies that were performed in 23 patients in the setting of CAD and established the diagnosis of BKVN. These patients were white and mainly of male sex, had a deceased donor, and had AR, well-known risk factors for BKVN. On the other hand, this study revealed that the main risk factors associated with GL were older age and DGF. Although high values of HR have also been seen for MMF + CyP + PDN and AZA + FK + PDN medication at the time of diagnosis of BKVN (Table 3), this finding should be interpreted carefully because only one patient in each group was available to estimate the HR, which may yield inaccurate estimates.

Therapeutic strategies often promote resolution of infection and prevent further decline in the kidney allograft function, but the damage from BKVN may not be reversed, leading to chronic allograft dysfunction [1]. In fact, the results demonstrated that even after strategies had been implemented, a significant percentage of patients had progressive allograft dysfunction. The potential for BKVN to cause high rates of GL is well-documented [6]. We found that 39.1% of patients (9 patients) had GL after the diagnosis of BKVN, a higher percentage than the 15% GL previously described in Wadei et al [6], in which 55 kidney transplant recipients, also with biopsy-proven BKVN, were analyzed during 20 ± 11 months of follow-up. Even when patients had increased graft function after the diagnosis of BKVN, more patients had partial recovery than total recovery of the graft function.

This study had some limitations, such as a low number of cases of BKVN histologically diagnosed, because surveillance ("protocol") biopsies were not performed and some patients had contraindications to kidney allograft biopsy. At the time of the biopsy, 16 patients had GFR <30 mL/min, and this late diagnosis of BKVN could also be a limitation to the study because these patients were already at a high risk for GL.

Chung et al [7] considered BK viremia to be a useful predictor for BKVN and suggested that its regular monitoring was effective in preventing this pathology. The use of this screening test could promote the early diagnosis of BKVN and early initiation of the therapeutic strategies. The BK viremia screening test was not performed in only 5 patients in this study because this test was not available in the first years of the study.

CONCLUSIONS

We found that DGF and older age of the kidney transplant recipients at the time of BKVN diagnosis were important risk factors for GL. Patients with lower GFR at the time of BKVN diagnosis were also at high risk for GL. Therefore, early diagnosis of BKVN is essential to prevent GL.

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