Reproducibility of Occurrence of Paroxysmal Atrial Fibrillation in Patients Who Had Acetylcholine Testing

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Objectives: Paroxysmal atrial fibrillation (PAF) is observed in some certain patients with acetylcholine (ACh) testing. However, it is uncertain for the reproducibility of ACh-induced PAF. We investigated the reproducibility of occurrence of ACh-induced PAF. **Methods:** We retrospectively investigated the reproducibility of occurrence of PAF in 70 patients who had ACh testing two times apart from at last one month. Incremental intracoronary injection of ACh into the right coronary artery was 20, 50, and 80 µg, while incremental dose of ACh into the left coronary artery was 20, 50, 100, and 200 µg. Positive provoked spasm was defined as > 90% transient narrowing with ischemic ECG changes or chest symptoms. **Results:** Among 70 patients, concordance was observed in 59 patients including 50 patients with both sinus rhythm and 9 patients with both positive PAF. The remaining 11 patients had discordance between the first and the second ACh tests. There were no clinical and angiographical differences except organic stenosis between the first and second ACh testing. All 70 patients had neither past PAF nor future PAF. The rate of coincidence was 84%, while discordance was 16%. ACh-inducible PAF was reproduced in 45% of patients who had two times of ACh testing, while the remaining 55% of patients revealed the discordance of ACh-inducible PAF. **Conclusions:** Less than half patients disclosed the reproducibility of ACh-inducible PAF.

KEY WORDS: acetylcholine, acetylcholine-induced paroxysmal atrial fibrillation, paroxysmal atrial fibrillation, vagal stimulation

Introduction

In 1986, intracoronary acetylcholine (ACh) testing was first reported¹⁾. Since then, intracoronary ACh test has become popular as a spasm provocation test as well as ergonovine (ER) test in the world^{2–6)}. Intracoronary injection of ACh has a short half life of this agent and so intracoronary ACh testing considered to be a relatively safe method. However, we experienced some complications such as ventricular fibrillation or tachycardia necessary for electric cardioversion, severe hypotension or left main trunk equivalent spasm during the ACh tests. Furthermore, we also experienced transient paroxysmal atrial fibrillation (PAF) in a sixth of patients who underwent ACh testing based on the Japanese Circulation Society guidelines^{7, 8)}. ACh-inducible PAF is one of a mechanism of a vagally-mediated PAF⁷⁾. There are no reports

concerning the reproducibility of ACh-inducible PAF in the same patients. In this article, we retrospectively investigated the reproducibility of ACh-inducible PAF in the same patients.

Methods

Study patients

From January 1991 to February 2019, we performed total 1,864 ACh spasm provocation tests. During these periods, we tried to perform the selective spasm provocation tests to examine the incidence of provoked spasm in patients who had undergone coronary angiography whenever possible. Among 1,864 patients with ACh testing, we enrolled 70 patients who had at least two times ACh tests apart from more than one month. As shown in Table 1, female was observed in 10 patients (14%) and mean age was 64 years old. Ischemic heart disease (IHD) was observed in all 70 patients including 48 patients with angina pectoris, 9 patients with myocardial infarction, and 13 patients with post percutaneous coronary intervention. Organic stenosis (>75%) was recognized in 38 patients (54%), while 60 patients (86%) had a history of smoking. Hypertension was observed in 38 patients (54%), whereas 42 patients (60%) had dyslipidemia. Diabetes mellitus was found in 23 patients (33%). Subjects were excluded and the provocation test was not performed if patients had left

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Table 1 All patient clinical characteristics

	All patients	Both negative PAF	Both positive PAF	Concordance	Discordance
Number	70	50	9	59	11
Sex (female)	10 (14)	6 (12)	1 (11)	7 (12)	3 (27)
Age, y, mean $\pm$ SD	64 $\pm$ 11	64 $\pm$ 11	60 $\pm$ 13	64 $\pm$ 11	64 $\pm$ 9
Organic stenosis	38 (54)	28 (56)	7 (78)	35 (59)	3 (27)
Follow-up duration (month)	54 $\pm$ 34	52 $\pm$ 31	44 $\pm$ 37	51 $\pm$ 32	71 $\pm$ 43
Angina pectoris	48 (69)	38 (76)	5 (56)	43 (73)	5 (45)
Myocardial infarction	9 (13)	5 (10)	3 (33)	8 (14)	1 (9)
Post percutaneous coronary intervention	13 (19)	7 (14)	1 (11)	8 (14)	5 (45)*
LVEF by UCG (%) mean $\pm$ SD	65 $\pm$ 9	66 $\pm$ 9	63 $\pm$ 10	65 $\pm$ 9	62 $\pm$ 11
Coronary risk factors					
Hypertension	38 (54)	28 (56)	4 (44)	32 (54)	6 (55)
Dyslipidemia	42 (60)	27 (54)	6 (67)	33 (56)	9 (82)
Diabetes mellitus	23 (33)	15 (30)	2 (22)	17 (29)	6 (55)
History of smoking	60 (86)	45 (90)	7 (78)	52 (88)	8 (73)
Medications before ACh testing					
Calcium channel blocker	50 (71)	36 (72)	6 (67)	42 (71)	8 (73)
Dihydropyridine r	40 (57)	27 (54)	6 (67)	33 (56)	7 (64)
Benzodiazepine	22 (31)	18 (36)	1 (11)	19 (32)	3 (27)
Both calcium channel blocker	12 (17)	9 (18)	1 (11)	10 (17)	2 (18)
Nitrates or nicorandil	40 (57)	29 (58)	6 (67)	35 (59)	5 (45)
Beta blocker	6 (9)	3 (6)	2 (22)	5 (8)	1 (9)
ACEI or ARB	12 (17)	7 (14)	4 (44)	11 (19)	1 (9)
Statin	19 (27)	12 (24)	3 (33)	15 (25)	4 (36)
Antiplatelet	42 (60)	27 (54)	6 (67)	33 (56)	9 (82)

ACh: acetylcholine, LVEF: left ventricular ejection fraction, UCG: ultrasound cardiography, ARB: angiotensin receptor blocker, ACEI: angiotensin converting enzyme inhibitor, PAF: paroxysmal atrial fibrillation, *: $p < 0.05$ vs. concordance

main narrowing ($>50\%$), triple-vessel disease, two-vessel disease with total occlusion, heart failure (New York Heart Association functional class III or IV), renal failure (creatinine > 2.0 mg/dl), if spontaneous spasm was observed or if isosorbide dinitrate was initially used to relieve spasm in the coronary artery tested.

The definition of PAF

We defined positive PAF as at least 30 seconds' duration of PAF, and also defined spontaneous relief of ACh-induced PAF as < 15 minutes' duration. When ACh-induced PAF continues for > 15 minutes, 50 mg of disopyramide or cibenzoline 70 mg solved in warmed 0.9% saline solution was administered intravenously for 5 minutes.

The definition of positive spasm and spasm configuration

Generally, we defined positive spasm as $\geq 90\%$ transient stenosis and usual chest symptom or ischemic ECG changes. The degree of ST-segment depression was measured 80 msec after the J point. We considered a result to be positive when at least 1 of the following ischemic ECG changes was demonstrated during and/or after the ACh test: (1) ST-segment elevation of ≥ 0.1 mV in at least 2 contiguous leads; (2) ST-segment depression of 0.1 mV in at least 2 contiguous leads. We also considered negative U wave as positive ischemic ECG change. Focal spasm was defined as a discrete transient vessel narrowing $\geq 90\%$ localized in a major coronary artery, whereas diffuse spasm was diag-

nosed when transient vessel narrowing $\geq 90\%$, compared with baseline coronary angiography, was observed in ≥ 2 adjacent coronary segments of epicardial coronary arteries.

Spasm provocation test

All drugs except for nitroglycerine were discontinued for ≥ 24 hours before the study, and nitroglycerine was also discontinued ≥ 4 hours before the study. Cardiac catheterization was performed from 9:00 am to 4:00 pm in the fasting state. After control coronary arteriograms of the left coronary artery (LCA) in the right anterior oblique with caudal projection and of the right coronary artery (RCA) in the left anterior oblique with cranial projection were obtained by injection of 8–10 ml of contrast medium, a temporary pacemaker was inserted into the right ventricle of each ACh testing patient and the pacing rate was set at 40–45 beats/min.

Provocation of coronary artery spasm was performed with an intracoronary injection of ACh, as previously reported^{9, 10}. ACh chloride was injected in incremental doses of 20, 50 and 80 μ g into the RCA and of 20, 50, 100 and 200 μ g into the LCA over 20 seconds with at least a 3-minute interval between each injection. Coronary arteriography was performed when ST-segment changes and/or, chest pain occurred or 1–2 minutes after the completion of each injection. When an induced coronary spasm did not resolve spontaneously within 3 minutes after the comple-

Table 2 Clinical and angiographical findings in patients with both PAF positive

No	Age	Sex	Diagnosis	First ACh test			Second ACh test		
				ACh dose	Spasm	PAF	ACh dose	Spasm	PAF
1	56	M	Rest AP	RCA (50) LCA (10/20)	1(d) 7(f)	Disopyramide 50 mg	RCA (50) LCA (50/100)	No spasm 8 (t)	Natural Natural
2	47	M	Effort AP	RCA (20/50) LCA (50)	2(d) 7(f)	Disopyramide 50 mg	RCA (50/80) LCA (50/100)	No spasm No spasm	50 Natural 80 disopyramide 50 mg
3	62	F	MI	RCA (80) LCA (50/100)	No spasm 8(d)	Disopyramide 50 mg	RCA (50/80) LCA (50/100)	No spasm No spasm	Disopyramide 50 mg
4	64	M	MI	RCA (20/50/ 80) LCA (50/100)	1(d) 6(d) 11(d)	Disopyramide 50 mg	RCA (20/ 50/80) LCA (50)	3 (t) 7 (t)	Natural
5	60	M	Rest AP	RCA (25/ 50) LCA (25/50)	No spasm 7(d)	Cibenzoline 70 mg	RCA (50) LCA (25/50/100)	No spasm No spasm	Cibenzoline 70 mg
6	39	M	MI	RCA (50) LCA (50/100)	No spasm 7(t)	Natural	RCA (50) LCA (25/50)	4(d) 7(d)	Natural
7	72	M	Effort AP	RCA (25/ 50) LCA (25/50/100)	4(d) 8(d) 13(t)	Natural	RCA (75) LCA (25/50/100/200)	4(d) 8(d) 11(d)	Cibenzoline 70 mg
8	63	M	Post P	RCA (50/80) LCA (20/50/100)	No spasm 8(d)	Cibenzoline 70 mg	RCA (50) LCA (25/ 50/100)	No spasm No spasm	Natural Natural
9	85	M	Post P	RCA (25/ 50/75) LCA (25/50/100/200)	No spasm	Cibenzoline 70 mg	RCA (80) LCA (100)	No spasm No spasm	Cibenzoline 70 mg

ACh: acetylcholine, AP: angina pectoris, MI: myocardial infarction, Post P: post percutaneous coronary intervention, RCA: right coronary artery, LCA: left coronary artery, (d): diffuse spasm, (f): focal spasm, (t): total spasm, PAF: paroxysmal atrial fibrillation, bold number: PAF induced dose of ACh

tion of ACh injections or when hemodynamic instability occurred as the result of coronary spasm, 2.5 to 5.0 mg of nitrate was injected into the involved vessel. A standard 12-lead electrocardiogram was recorded every 30 seconds. We used the ECG findings when ACh, saline and contrast medium were not injected into the responsible vessels for at least 60 seconds. After the spasm provocation tests were completed, an intracoronary injection of 5.0 mg isosorbide dinitrate was administered, and coronary arteriography was then performed in multiple projections.

During the study, arterial blood pressure and ECG were continuously monitored on an oscilloscope by Nihon-Kohden polygraphy (Tokyo, Japan). Patients with catheter-induced spasms were excluded from this study. Significant organic stenosis was defined as > 75 percent luminal narrowing according to the American College of Cardiology/American Heart Association classification¹¹. We defined hypoplastic RCA as a very small RCA when the RCA supplies only to the right ventricle and ends before reaching the crux of the heart and prior to splitting off the posterior descending artery and posterolateral artery. We did not perform ACh testing in the hypoplastic RCA¹².

The study protocol complied with the Declaration of Helsinki. Written informed consent was obtained from all patients before performing the pharmacological spasm provocation tests and the protocol of this study was in agreement with the guidelines of the ethical committee at our institution.

Statistical analysis

Data analysis was carried out with SPSS (version 22.0, IBM Japan, Ltd., Tokyo, Japan). All data were presented as the mean±1 SD. Clinical characteristics among three groups and angiographical findings and medications between the first and second tests were analyzed by the Fisher's exact test with correction or the Mann-Whitney test. $P < 0.05$ was considered significant.

Results

During the follow-up periods (64±11 months), we found no previous PAF and no future PAF in all 70 patients. According to the response of ACh testing, we classified 70 patients into the three groups: 50 patients with both sinus rhythm, 9 patients with both positive PAF, and 11 patients with either of one PAF positive. We could not perform ACh tests in 5 RCAs (hypoplastic RCA: 2 vessels, obstructive stenosis > 90%: 2 vessels, after the administration of nitroglycerine into the LCA: 1 vessel) and one LCA (after the administration of nitroglycerine into the RCA) of the first ACh testing, whereas we also could not perform these tests in 2 RCAs (hypoplastic RCA) and one LCA (after the administration of nitroglycerine into the RCA) of the second ACh tests. We could perform both times and both coronary ACh testing in 63 patients (90%) of the study subjects.

Concordance of PAF or sinus rhythm

As shown in Table 1, 59 patients had the same response be-

Table 3 Clinical and angiographical findings in patients with discordance PAF positive

No	Age	Sex	Diagnosis	First ACh test			Second ACh test		
				ACh dose	Spasm	PAF	ACh dose	Spasm	PAF
1	54	M	Post P	RCA (50/80)	No spasm	Disopyramide 50 mg	RCA (50/80)	No spasm	(-)
				LCA (50/100)	No spasm	Natural	LCA (50/100)	No spasm	(-)
2	71	M	R & E AP	RCA (50)	4(d)		RCA (50)	No spasm	(-)
				LCA (100)	8(d)	Natural	LCA (50/100)	8(d)	(-)
3	65	F	Post P	RCA (20/50/80)	No spasm	Natural	RCA (80)	No spasm	(-)
				LCA (20/50/100)	No spasm		LCA (50/100/20)	No spasm	(-)
4	72	M	MI	RCA (20/50)	2(d)		RCA (20/50/80)	No spasm	(-)
				LCA (100)	8(d) 11(f)	Natural	LCA (50/100/200)	8(t) 13(d)	(-)
5	69	F	Rest AP	RCA (20/50)	2(t)	Natural	RCA (80)	No spasm	(-)
				LCA (20/50/100/200)	No spasm		LCA (20/100/200)	No spasm	(-)
6	73	M	Post P	RCA (50/80)	4(d)	Disopyramide 50 mg	RCA (25/50)	No spasm	(-)
				LCA (25/50/100)	7(d)		LCA (50/100)	Spastic	(-)
7	45	M	Rest AP	RCA (20/50/ 80)	3(f)	Natural	RCA (80)	4(d)	(-)
				LCA (20/50/100)			LCA (20/50/100/200)	7(d)	(-)
8	66	F	Post P	RCA (50/80)	No spasm	(-)	RCA (50/ 80)	No spasm	Disopyramide 50 mg
				LCA (50/100)	No spasm	(-)	LCA (50/100)	No spasm	
9	69	M	Post P	RCA (25/50)	No spasm	(-)	RCA (75)	No spasm	Natural
				LCA (25/50/100)	7(d)	(-)	LCA (50/100)	7(d)	
10	54	M	MI	RCA (20)	2(d)	(-)	RCA (20/50)	4(d)	Natural
				LCA (20/50/100)	7(t) 13(f)	(-)	LCA (20/50/100)	7(d)	
11	65	M	R & E AP	RCA (20/50)	1(d)	(-)	RCA (50)	4(d)	
				LCA (50/100)	6(d) 11(d)	(-)	LCA (100)	6(f) 11(f)	Disopyramide 50 mg Etilefrine 2 mg

ACh: acetylcholine, AP: angina pectoris, MI: myocardial infarction, Post P: post percutaneous coronary intervention, RCA: right coronary artery, LCA: left coronary artery, (d): diffuse spasm, (f): focal spasm, (t): total spasm, PAF: paroxysmal atrial fibrillation, bold number: PAF induced ACh dose, under PAF test

tween the two ACh testing. There was no clinical difference between both sinus rhythm and both positive PAF. Table 2 showed the clinical and angiographical findings in patients with both occurrence of PAF. PAF was observed in all 9 patients in the first RCA ACh testing, whereas, PAF was observed in all 9 RCA ACh testing and two LCA ACh tests in the second ACh test. Positive spasm in 59 patients with concordance groups was observed in 49 patients (83%) in the first ACh testing, while positive spasm in the second ACh test was recognized in 41 patients (69%). In 9 patients with both PAF, positive spasm of first ACh testing was observed in 8 patients (89%), whereas positive spasm in the second ACh test was found in 4 patients (44%).

Comparisons of concordance and discordance PAF

Discordance occurrence of PAF between the two ACh tests was found in 11 patients, as shown in Table 3. In the first ACh testing, 7 patients had PAF including 4 RCA ACh tests, 2 LCA ACh tests and one both ACh test, while 4 patients had PAF including 3 RCA ACh tests and one LCA ACh test in the second ACh testing. Positive spasm was found in 7 patients (64%) in the first ACh testing, whereas positive spasm in the second ACh test

was revealed in 6 patients (55%). However, there were no clinical differences between the concordance group and discordance group as shown in Table 1.

Comparisons between first and second ACh testing

As shown in Table 4, the frequency of organic stenosis in second ACh testing was significantly lower than that in first ACh tests. However, there was no differences concerning the provoked spasm and medications between the two ACh testing.

Complications of ACh testing

Left amin trunk equivalent spasm was observed in just one patient. However, no irreversible complications were observed in this study.

Discussion

In this article, we found no correlation of reproducibility of PAF between first ACh testing and second ACh tests. Discordance of ACh-inducible PAF was observed in 14% of patients who had two times ACh tests apart from more than one month. Furthermore, during the follow-up periods (64±11 months), we found no previous PAF and no future PAF in these 70 patients.

Table 4 Angiographical findings and medications before acetylcholine tests

	First ACh testing 70	Second ACh testing 70	p value
Number			
Organic stenosis	38 (54)	16 (23)	< 0.001
Right coronary artery	13 (19)	6 (9)	0.1387
Left circumflex coronary artery	19 (27)	3 (4)	< 0.001
Left anterior descending coronary artery	48 (69)	8 (11)	< 0.001
1 vessel disease	33 (47)	15 (21)	< 0.01
2 vessel diseases	5 (7)	1 (1)	0.2106
Provoked positive spasm	54 (77)	46 (66)	0.1344
Right coronary artery	36 (51)	32 (46)	0.4987
Left circumflex coronary artery	17 (24)	13 (19)	0.4100
Left anterior descending coronary artery	45 (64)	37 (53)	0.1698
1 vessel spasm	23 (33)	19 (27)	0.4606
2 vessel spasm	19 (27)	19 (27)	1.0000
3 vessel spasm	12 (17)	8 (11)	0.3339
Medications before ACh testing			
Calcium channel blocker	50 (71)	57 (81)	0.1633
Dihydropyridine r	40 (57)	43 (61)	0.6058
Benzodiazepine	22 (31)	21 (30)	0.8546
Both calcium channel blocker	12 (17)	7 (10)	0.2172
Nitrates or nicorandil	40 (57)	44 (63)	0.4901
Beta blocker	6 (9)	3 (4)	0.4907
ACEI or ARB	10 (14)	12 (17)	0.6423
Statin	19 (27)	20 (29)	0.8504
Antiplatelet	42 (60)	44 (63)	0.7284

ACh: acetylcholine, ARB: angiotensin receptor blocker, ACEI: angiotensin converting enzyme inhibitor

This may suggest that ACh-inducible PAF is a reaction of individual response to just ACh at that time. The clinical implications of ACh-inducible PAF may have limitations of estimating previous and future occurrence of PAF.

ACh-inducible PAF in Western and Japanese populations

In the past report of ours, we had no correlation between the daily PAF and ACh-inducible PAF⁷⁾. Among 717 patients without persistent atrial fibrillation, we experienced transient PAF during ACh testing in 116 patients (16.2%). Daily PAF in patients with ACh-induced PAF was not different from that in those with ACh-induced no PAF (2.6% (3/116) vs. 1.0% (6/601), ns). However, Saito et al. reported that a history of PAF and body mass index were independent predictors for occurrence of PAF during intracoronary ACh provocation test by multivariate logistic regression analysis¹³⁾. PAF during ACh testing was observed in just 8% (31 patients) among 377 patients without persistent atrial fibrillation who underwent intracoronary ACh provocation tests. The occurrence rate of PAF during ACh testing was lower than the data of previous ours. Maximum ACh dose of 80 µg into the RCA and 200 µg into the LCA of our study might be concerned. They employed maximum ACh dose of 50 µg into the RCA and 100 µg into the LCA. In contrast, in a Western report, one patient had fast PAF that resolved spontaneously after discontinuing the ACh injection¹⁴⁾. The observation of short phases

of PAF during ACh testing was not uncommon, but was not registered as a complication in their study. Incremental doses of 2, 20, 100, and 200 µg of ACh were manually infused over a period of 3 minutes into the LCA without a pacemaker. If no proved spasm in the LCA was revealed, 80 µg of ACh was injected into the RCA. However, intracoronary RCA ACh testing was performed in just 34.4% (291/847) of their study. Accurate occurrence of PAF in their study was obscure. In another Caucasian report, PAF is a rare complication with ACh infusion over 1 minute into the LCA (1.1%). While 6.9% of patients developed atrioventricular block and 3.3% developed significant bradycardia, these conditions readily reversed once the administration of ACh was stopped¹⁵⁾. Western cardiologists administered intracoronary ACh over 1–3 minutes without a pacemaker, whereas we injected intracoronary ACh within 20 seconds under pacemaker. Intracoronary RCA ACh testing without a pacemaker is difficult to perform possibly due to bradycardia and block. According to our previous report, PAF was observed in 109 patients (94%) in the RCA ACh testing, whereas 9 patients (8%) had PAF in the LCA ACh tests⁷⁾. Two patients (2%) had PAF on both coronary arteries. In this study, PAF was recognized in 26 RCAs, while 6 LCAs had PAF. PAF was observed in 3 patients on both coronary arteries (one patient with discordance PAF of first ACh test & two patients with concordance PAF of second ACh tests).

Clinical implications of ACh-inducible PAF

The majority of ACh-inducible PAF was observed in the RCA ACh testing. However, the occurrence of ACh-inducible PAF in Western reports was unclear. Furthermore, we could not find the reproducibility of ACh-inducible PAF in the previous reports. This is the first report regarding the reproducibility of ACh-inducible PAF. In this study, we could perform both times and both coronary ACh testing in 90% of study populations. The administration time of ACh may contribute to the occurrence of PAF and vagally mediated PAF is a mechanism of ACh-inducible PAF. In this study, a previous history of PAF or future PAF in patients with ACh-induced PAF was not found. We suggest that ACh-induced PAF may react the individual response of ACh in the cardiac catheterization laboratory at that time. Mechanism favoring the occurrence of PAF are complex and the occurrence of PAF greatly depends on variation of the autonomic tone, with a primary increase in adrenergic tone followed by an abrupt shift toward vagal predominance^{16, 17}. It may be difficult to estimate the occurrence of clinical daily PAF or persistent atrial fibrillation in the future by intracoronary ACh-inducible PAF alone. Further research is necessary to clarify the reproducibility of the occurrence of ACh-inducible PAF in Western and Japanese subjects.

Limitations

Several limitations were recognized in this study. First is a single center, retrospective, small study sample and not prospective study. Second, we could not perform Holter monitoring in all patients during follow-up periods at a constant examination. We may miss a transient silent PAF. Third, follow-up duration was short to verify the correlation between ACh-inducible PAF and future PAF or persistent atrial fibrillation. It may be better more-longer duration of follow-up periods than 10 years. Fourth, we could not perform the complete ACh testing on both times. However, we could perform both times and both coronary ACh testing in 90% of study populations. Further study is necessary for clarifying the correlation between ACh-inducible PAF and future occurrence of PAF or persistent atrial fibrillation.

Conclusions

There was no correlation regarding the reproducibility between first and second ACh-inducible PAF. This study suggests that it may be difficult to estimate the past and future occurrence of PAF according to the ACh-inducible PAF alone.

Conflicts of interest

The authors declare that they have no conflict of interest.

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None.

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