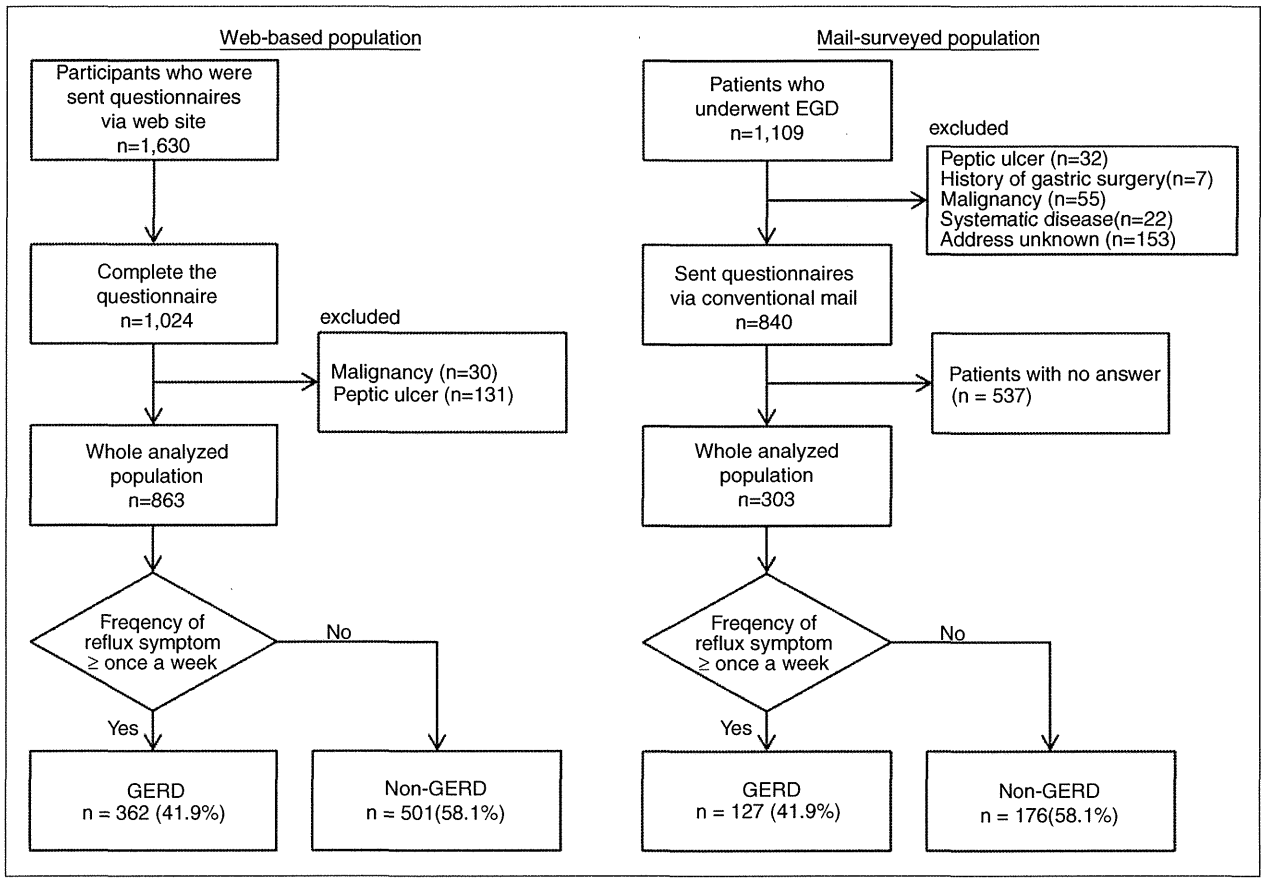

Figure 1. The web-based (a) and mail-surveyed (b) populations.
EGD, oesophagogastroduodenoscopy; GERD, gastro-oesophageal reflux disease.

participants were included in the GERD group. From the conventional mail survey, a total of 1109 patients were enrolled (Figure 1b). After excluding participants with peptic ulcers ($n=32$), histories of gastric surgery ($n=7$), malignant diseases ($n=55$), severe systematic diseases ($n=22$), and unknown addresses ($n=153$), 840 patients were sent questionnaires along with an informed consent form; of these, 303 patients gave complete responses. Among the 303, 127 participants were included in the GERD group. Demographic characteristics of these two populations were totally different except for BMI, as shown in Table 1.

Optimal cutoff value of GerdQ for the prediction of reflux oesophagitis

Among the 303 included mail respondents, 21 patients had LA grade A oesophagitis, 12 had LA grade B oesophagitis, one had LA grade C oesophagitis, one had LA grade D oesophagitis, and the remaining 268 patients did not have reflux oesophagitis. To validate the optimal cutoff value of GerdQ for predicting the presence of reflux oesophagitis, the sensitivity, specificity, positive predictive values (PPVs), negative predictive values (NPVs), and area under the ROC curves (AUCs) were calculated (Table 2). When the cutoff value was set at 8, the PPV and AUC were the highest. A significant association between GerdQ-positive and the presence of reflux oesophagitis was also shown ($p=0.02$). These results revealed that a cutoff value of 8 was also most predictive of reflux oesophagitis in Japanese population. In addition, GerdQ score ≥ 8 showed higher specificity, PPV, and AUC than CDQ score ≥ 6 (Table 2). These data indicated that GerdQ is more useful than CDQ for predicting the presence of reflux oesophagitis, although the sensitivity of GerdQ score ≥ 8 was low (34.3%).

Differences in characteristics between CDQ score $\geq$ 6 and GerdQ score $\geq$ 8

The GerdQ and CDQ scores were significantly correlated in both the web-based and mail-surveyed population (Figure 2). However, many participants showed

Table 1. Characteristics of analysed patients

	Web-based population				Mail-survey population				Web vs. mail
	Whole ($n=863$)	Non-GERD ($n=501$)	GERD ($n=362$)	p-value	Whole ($n=303$)	Non-GERD ($n=176$)	GERD ($n=127$)	p-value	p-value
Age (years)	41.1 ± 9.3	40.4 ± 9.4	41.9 ± 9.1	0.02[a]	50.3 ± 15.4	51.2 ± 14.4	49.1 ± 16.3	0.02[a]	<0.001[a]
Gender									
Men	391 (45.3)	218 (43.5)	173 (47.8)	0.24[b]	168 (55.4)	100 (56.8)	68 (53.5)	0.64[b]	0.003[b]
Women	472 (54.7)	283 (56.5)	189 (52.2)		135 (44.6)	76 (43.2)	59 (46.5)		
Smoking habits									
Non-smokers	409 (47.4)	246 (49.1)	163 (45.0)	0.58[c]	180 (59.4)	112(64.0)	68(53.5)	0.13[c]	<0.001[c]
Ex-smokers	253 (29.3)	146 (29.1)	107 (29.6)		88 (29.1)	47(26.9)	41(32.3)		
1-15/day	104 (12.1)	57 (11.4)	47 (13.0)		11 (3.6)	7(4.0)	4(3.1)		
> 15/day	97 (11.2)	52 (10.4)	45 (12.4)		23 (7.6)	9(4.6)	14(11.0)		
Alcohol habits									
Abstainers	161 (18.7)	114 (22.8)	47 (13.0)	0.004[c]	61 (20.1)	29(16.6)	32(25.2)	0.22[c]	<0.001[c]
Social drinkers	329 (38.1)	182 (36.3)	147 (40.6)		89 (29.4)	48(27.4)	41(32.3)		
Stop drinking	43 (5.0)	28 (5.6)	15 (4.1)		16 (5.3)	9(5.1)	7(5.5)		
1-2 days/week	58 (6.7)	36 (7.2)	22 (6.1)		46 (15.2)	31(17.7)	15(11.8)		
3-4 days/week	76 (8.8)	40 (8.0)	36 (9.9)		38 (12.5)	26(14.9)	12(9.4)		
5-7 days/week	196 (22.7)	101 (20.2)	95 (26.2)		52 (17.2)	32(18.3)	20(15.7)		
BMI (kg/m^2)	23.2 ± 4.3	23.0 ± 4.3	23.4 ± 4.3	0.26[a]	22.9 ± 7.8	22.9 ± 7.3	22.9 ± 8.3	0.99[a]	0.27[a]
Diabetes mellitus	33 (3.8)	13 (2.6)	20 (5.5)	0.03[b]	27 (8.9)	16(9.1)	11(8.7)	1.00[b]	0.001[b]
Hypertension	101 (11.7)	53 (10.6)	48 (13.3)	0.23[b]	55 (18.2)	32(18.1)	23(18.1)	1.00[b]	0.01[b]
Dyslipidaemia	75 (8.7)	32 (6.4)	43 (11.9)	0.005[b]	52 (17.2)	32(18.1)	20(15.7)	0.65[b]	<0.001[b]
Fatty liver	63 (7.3)	34 (6.8)	29 (8.0)	0.50[b]	37 (12.2)	16(9.1)	21(16.5)	0.07[b]	0.01[b]
Medications for upper gastrointestinal tract									
PPI	42 (4.9)	20 (4.0)	22 (6.1)	0.16[b]	73 (24.1)	35 (20.2)	38 (30.6)	0.04[b]	<0.001[b]
H_2RA	44 (5.1)	17 (3.4)	27 (7.5)	0.007[b]	34 (11.2)	16 (9.2)	18 (14.5)	0.20[b]	<0.001[b]
Prokinetics	24 (2.8)	10 (2.0)	14 (3.9)	0.10[b]	30 (9.9)	9 (5.2)	21 (17.1)	0.001[b]	<0.001[b]
Others	35 (4.1)	16 (3.2)	19 (5.2)	0.08[b]	35 (11.6)	12 (7.0)	23 (18.5)	0.003[b]	<0.001[b]
OTC medications	175 (20.3)	55 (11.0)	120 (33.1)	<0.001[b]	30 (9.9)	5 (2.8)	25 (19.7)	<0.001[b]	<0.001[b]

Values are mean ± standard deviation or n (%). [a]Student's t-test; [b]Fisher's Exact test; [c]Pearson's χ^2 test.
BMI, body mass index; H_2RA, histamine H_2-receptor antagonist; PPI, proton pump inhibitor; OTC, over-the-counter.

Table 2. Sensitivity and specificity of CDQ and GerdQ for the diagnosis of reflux oesophagitis among the mail-survey population (n = 303)

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	p-value
CDQ ≥ 6	51.4	61.9	15.0	90.7	0.567	0.14
GerdQ ≥ 6	88.6	14.6	11.9	90.7	0.516	0.80
GerdQ ≥ 7	42.9	70.5	16.0	90.4	0.567	0.12
GerdQ ≥ 8	34.3	82.5	20.3	90.6	0.584	0.02
GerdQ ≥ 9	17.1	89.6	17.6	89.2	0.533	0.25

p-values calculated using Fisher's Exact test.
CDQ, Carlsson-Dent questionnaire; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve.

CDQ score ≥ 6 and GerdQ score < 8, or CDQ score < 6 and GerdQ score ≥ 8, even in the GERD groups. This suggests that there are differences in the patient characteristics between populations of patients with CDQ score ≥ 6 and GerdQ score ≥ 8 populations. Since CDQ cutoff score is sometimes set at 4 in Japan, kappa coefficients between CDQ score ≥ 4 and GerdQ score ≥ 8 were also calculated. Using CDQ cutoff score of 6,

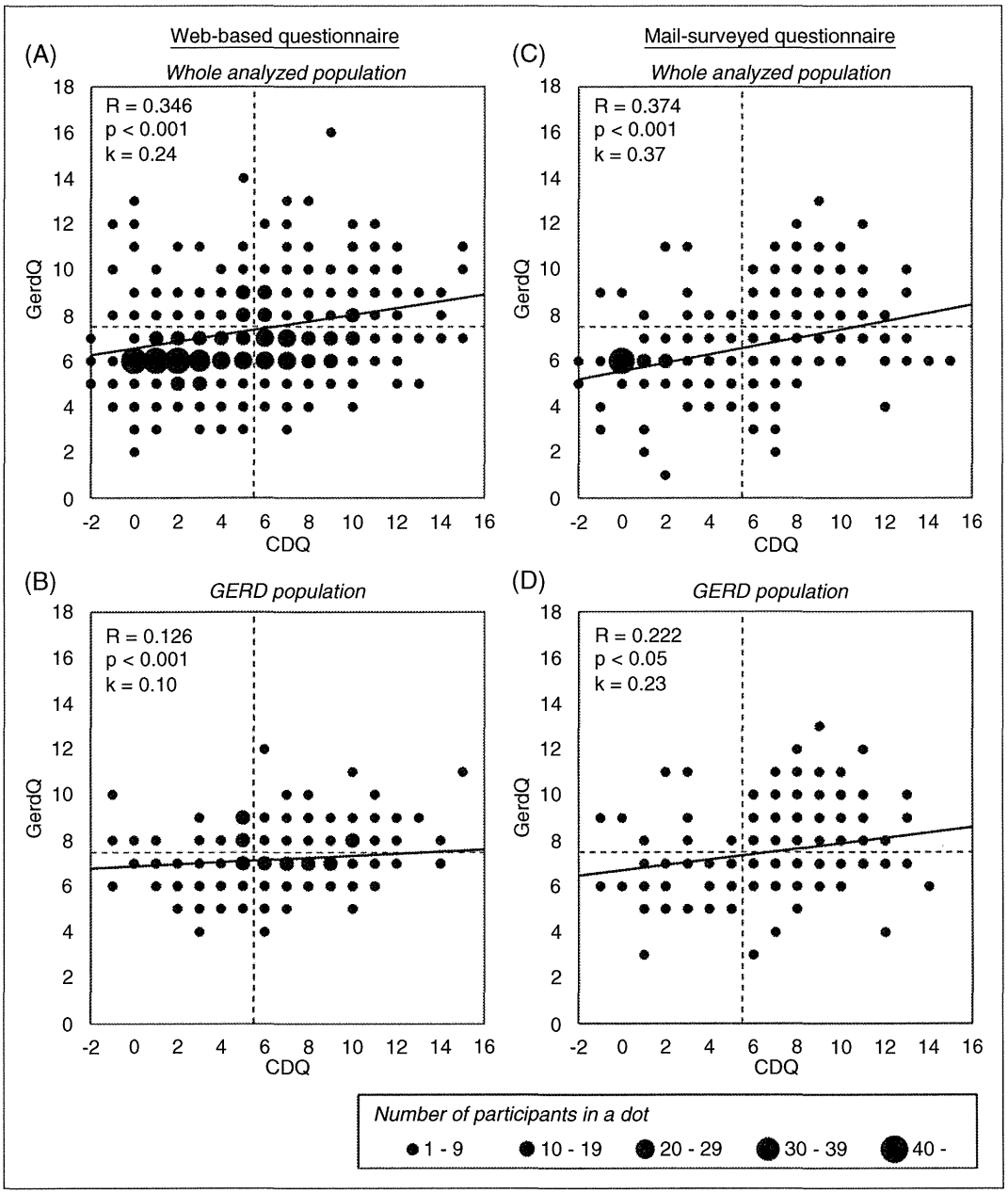

Figure 2. Correlation and concordance between CDQ and GerdQ scores in the total analysed population and in the GERD groups. CDQ, Carlsson-Dent questionnaire; GERD, gastro-oesophageal reflux disease.

kappa coefficients were 0.24 in web-based population and 0.27 in mail-surveyed population, whereas, using CDQ cutoff score of 4, kappa coefficients were 0.22 in web-based population and 0.25 in mail-surveyed population. All of the kappa coefficients were < 0.4, which indicates poor concordance.

Therefore, the average score of each question of GerdQ was compared between participants with CDQ score ≥ 6 and CDQ score < 6 (Table 3). The scores of question 1, 2, 5, and 6 in GerdQ were significantly higher in CDQ score ≥ 6 than CDQ score < 6. On the other hand, the score of question 3 in GerdQ was significantly lower in CDQ score ≥ 6 than CDQ score < 6. The score of question 4 in GerdQ was not different between CDQ score ≥ 6 and CDQ score < 6. These showed that question 3 and 4 in GerdQ caused poor concordance about the diagnosis of GERD using GerdQ and CDQ.

Subsequently, the associations of CDQ score ≥ 6 and GerdQ score ≥ 8 with demographic information

Table 3. Scores for each question of GerdQ in CDQ-positive and -negative participants

GerdQ	Web-based population			Mail-survey population		
	CDQ < 6 ($n = 135$)	CDQ ≥ 6 ($n = 227$)	p-value	CDQ < 6 ($n = 40$)	CDQ ≥ 6 ($n = 87$)	p-value
1. How often did you have a burning feeling behind your breastbone (heartburn)?	0.38 ± 0.71	**0.96 ± 0.89**	<0.001	0.14 ± 0.50	**0.84 ± 0.93**	<0.001
2. How often did you have stomach contents (liquid or food) moving upwards to your throat or mouth (regurgitation)?	0.36 ± 0.63	**0.68 ± 0.80**	<0.001	0.26 ± 0.64	**0.83 ± 0.92**	<0.001
3. How often did you have a pain in the centre of the upper stomach?	**2.65 ± 0.70**	2.42 ± 0.83	<0.001	**2.66 ± 0.74**	2.29 ± 0.91	<0.001
4. How often did you have nausea?	2.57 ± 0.74	2.53 ± 0.70	0.37	2.77 ± 0.66	2.74 ± 0.69	0.72
5. How often did you have difficulty getting a good night's sleep because of your heartburn and / or regurgitation?	0.18 ± 0.51	**0.32 ± 0.63**	<0.001	0.06 ± 0.28	**0.29 ± 0.63**	<0.001
6. How often did you take additional medication for your heartburn and / or regurgitation, other than what the physician told you to take?	0.23 ± 0.65	**0.49 ± 0.84**	<0.001	0.08 ± 0.39	**0.33 ± 0.77**	<0.001

Values are mean ± standard deviation. p-values calculated using Student's t-test.
CDQ, Carlsson-Dent questionnaire.

were analysed among GERD groups using logistic regression models. The results of univariate analysis involving GERD patients responding to the web-based survey showed that, men and the presence of metabolic syndrome were associated with CDQ score ≥ 6, whereas older age, the presence of metabolic syndrome, and the use of both prescription and OTC medications were associated with GerdQ score ≥ 8 (Table 4). Among GERD patients in the mail-survey population, no association was observed between CDQ score ≥ 6 and demographic factors, whereas men and the presence of metabolic syndrome were associated with GerdQ score ≥ 8. The use of both prescription and OTC medications was marginally associated with a GerdQ score ≥ 8 ($p = 0.06$).

Finally, multivariate logistic regression analysis was performed with adjustments for age, gender, the presence of metabolic syndrome, and the use of both prescription and OTC medications (Table 5). According to the multivariate analysis results, among GERD patients in the web-surveyed population, men were associated with CDQ score ≥ 6, whereas older age and the use of both prescription and OTC medications were independently associated with GerdQ score ≥ 8. Contrarily, among GERD patients in the mail-surveyed population, men and the use of both prescription and OTC medications were independently associated with GerdQ score ≥ 8. These results suggest that the use of both prescription and OTC medications would be associated with GerdQ score ≥ 8, but not CDQ score ≥ 6, in any population.

Discussion

When the GerdQ was developed as an exploratory part of the DIAMOND study, a cutoff of 8 showed the highest specificity (71.4) and sensitivity (64.6) for GERD. The present study also showed that a GerdQ cutoff of 8 gave the best balance with regard to sensitivity and specificity for reflux oesophagitis in Japanese populations. The reason for the low positive predictive value (20.3) would be that the prevalence of reflux oesophagitis is low in GERD patients. Previous data showed that the prevalence of non-erosive reflux disease (NERD) in medical check-up studies was about 70–80% in Asian GERD population.[14] Patients with GerdQ score ≥ 8 but without reflux oesophagitis were thought to be NERD patients.

According to the univariate logistic regression analysis, the presence of metabolic syndrome was associated with GerdQ score ≥ 8 in both the web- and mail-surveyed populations. Because metabolic syndrome is well known to be associated with the development and progression of GERD,[15] the association between GerdQ score ≥ 8 and metabolic syndrome also suggests that GerdQ is useful for diagnosing GERD in Japanese individuals. On the other hand, CDQ score ≥ 6 was also associated with the presence of metabolic syndrome in the web-surveyed population,

Table 4. Association between the scores of CDQ and GerdQ and demographic factors among GERD patients (univariate analysis)

	Web-based population						Mail-survey population					
	CDQ < 6 (*n* = 135)	CDQ ≥ 6 (*n* = 227)	OR (95% CI)	GerdQ< 8 (*n* = 183)	GerdQ≥ 8 (*n* = 179)	OR (95% CI)	CDQ < 6 (*n* = 40)	CDQ ≥ 6 (*n* = 87)	OR (95% CI)	GerdQ < 8 (*n* = 71)	GerdQ ≥ 8 (*n* = 87)	OR (95% CI)
Age (years)	40.8 ± 9.2	42.6 ± 9.0	1.02 (1.00–1.05)	40.2 ± 9.1	43.7 ± 8.8	**1.05 (1.02–1.07)**	50.3 ± 6.0	48.5 ± 9.0	0.97 (0.93–1.02)	49.1 ± 7.9	49.0 ± 8.6	1.00 (0.96–1.04)
Gender (men)	52 (38.5)	121 (53.3)	**1.82 (1.18–2.81)**	88 (48.1)	85 (47.5)	0.98 (0.65–1.48)	22 (55.0)	46 (52.9)	0.92 (0.43–1.95)	29 (40.8)	39 (69.6)	**3.32 (1.58–6.97)**
Heavy smoking (> 15/day)	17 (12.6)	28 (12.3)	0.98 (0.51–1.86)	20 (44.4)	25 (55.6)	1.32 (0.71–2.48)	2 (5.0)	12 (13.8)	3.04 (0.65–14.3)	7 (9.9)	7 (12.5)	1.31 (0.43–3.97)
Heavy alcohol consumption (5–7 days/week)	28 (20.7)	67 (29.5)	1.60 (0.97–2.65)	46 (25.1)	49 (27.4)	1.12 (0.70–1.79)	6 (15.0)	14 (16.1)	1.09 (0.38–3.07)	11 (15.5)	9 (16.1)	1.04 (0.40–2.73)
Metabolic syndrome	49 (36.3)	109 (48.0)	**1.62 (1.05–2.51)**	68 (37.2)	90 (50.3)	**1.71 (1.13–2.60)**	20 (50.0)	35 (40.2)	0.67 (0.32–1.43)	24 (33.8)	31 (55.4)	**2.43 (1.18–4.99)**
Using both prescription and OTC medications	8 (5.9)	26 (11.5)	2.05 (0.90–4.68)	6 (3.3)	28 (15.6)	**5.47 (2.21–13.6)**	4 (10.0)	7 (8.0)	0.79 (0.22–2.86)	3 (4.2)	8 (14.3)	3.78 (0.95–15.0)

Values are mean ± SD or *n* (%), unless otherwise stated. Bold indicates significant differences.
CDQ, Carlsson-Dent questionnaire; OTC, over-the-counter.

but not in mail-surveyed population. CDQ cannot be used to distinguish between GERD and functional heartburn,[16] and functional heartburn is not associated with metabolic syndrome.[17] CDQ score ≥ 6 was not associated with metabolic syndrome in the mail-surveyed population because more patients with functional heartburn were included in the population. In addition, epigastric pain (question 3 in GerdQ) was more frequent in participants with CDQ score ≥ 6 than those with CDQ score < 6, suggesting that participants with CDQ score ≥ 6 were likely to have functional dyspepsia. These results suggest that GerdQ would be more useful for distinguishing between GERD and functional upper gastrointestinal disorders (heartburn and dyspepsia) than CDQ. However, GerdQ has limitations to distinguish between GERD and functional heartburn. Although positive PPI response and SAP were taken into account to diagnose GERD in the development of GerdQ, some researchers have reported that positive PPI response cause overestimation of functional heartburn patients.[18,19] SAP might be overinterpreted in patients with refractory GERD especially when low reflux rates are observed.[20] In addition, non-acid reflux, which is reported to be involved in the development of reflux symptoms, was not taken into account in GerdQ.[21] Therefore, to validate how useful for distinguishing between GERD and functional heartburn, GerdQ should be evaluated using combined impedance pH monitoring.

According to the multivariate logistic regression analysis, the additional use of OTC medications was well associated with GerdQ score ≥ 8 in both populations. The additional use of OTC medications may indicate that prescription medicines are inadequate for treating reflux symptoms. This result suggests that the GerdQ would be useful for evaluating treatment response and for detecting unmet medical needs.

Limitations of the present study include the absence of pH monitoring for the mail-surveyed population. The pH monitoring data might have revealed the prevalence of NERD and functional heartburn in this population, and the optimal cutoff value of GerdQ and the differences between GerdQ and CDQ might have been more clearly evaluated. The low response rate for the questionnaires in the mail-surveyed population might have caused selection bias.

In conclusion, the present study is the first study to evaluate the usefulness of GerdQ in Japanese population. The GerdQ cutoff score of 8 was found to also be appropriate for the Japanese population. The GerdQ was better able to detect unmet therapeutic needs. Symptom-based management of GERD using GerdQ would be beneficial in Japan, as was revealed in Western countries.

Table 5. Association between the scores of CDQ and GerdQ and demographic factors among GERD patients (multivariate analysis)

	Web-based population		Mail-survey population	
OR (95% CI)	CDQ	GerdQ	CDQ	GerdQ
Age	1.01 (0.99–1.04)	**1.04 (1.02–1.07)**	0.98 (0.93–1.03)	0.96 (0.92–1.02)
Gender (men)	**1.66 (1.05–2.61)**	0.78 (0.50–1.23)	1.12 (0.49–2.54)	**3.66 (1.57–8.53)**
Metabolic syndrome	1.29 (0.80–2.07)	1.38 (0.86–2.20)	0.73 (0.32–1.68)	1.88 (0.82–4.28)
Using both prescription and OTC medications	1.96 (0.85–4.52)	**5.11 (2.04–12.8)**	0.87 (0.23–3.26)	**4.61 (1.06–20.2)**

Values are odds ratio (95% CI). Bold indicates significant differences.
CDQ, Carlsson-Dent questionnaire; OTC, over-the-counter.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Fujiwara Y and Arakawa T. Epidemiology and clinical characteristics of GERD in the Japanese population. *J Gastroenterol* 2009; 44: 518–534.
2. Kahrilas PJ, Shaheen NJ, Vaezi MF, et al. American Gastroenterological Association Medical Position Statement on the management of gastroesophageal reflux disease. *Gastroenterology* 2008; 135: 1383–1391.
3. Jones R, Junghard O, Dent J, et al. Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. *Aliment Pharmacol Ther* 2009; 30: 1030–1038.
4. Jonasson C, Wernersson B, Hoff DA, et al. Validation of the GerdQ questionnaire for the diagnosis of gastro-oesophageal reflux disease. *Aliment Pharmacol Ther*2013.
5. Jonasson C, Moum B, Bang C, et al. Randomised clinical trial: a comparison between a GerdQ-based algorithm and an endoscopy-based approach for the diagnosis and initial treatment of GERD. *Aliment Pharmacol Ther* 2012; 35: 1290–1300.
6. Wong BC and Kinoshita Y. Systematic review on epidemiology of gastroesophageal reflux disease in Asia. *Clin Gastroenterol Hepatol* 2006; 4: 398–407.
7. Kinoshita Y, Kawanami C, Kishi K, et al. *Helicobacter pylori* independent chronological change in gastric acid secretion in the Japanese. *Gut* 1997; 41: 452–458.
8. Kinoshita Y, Adachi K, Hongo M, et al. Systematic review of the epidemiology of gastroesophageal reflux disease in Japan. *J Gastroenterol* 2011; 46: 1092–1103.
9. Carlsson R, Dent J, Bolling-Sternevald E, et al. The usefulness of a structured questionnaire in the assessment of symptomatic gastroesophageal reflux disease. *Scand J Gastroenterol* 1998; 33: 1023–1029.
10. Mishima I, Adachi K, Arima N, et al. Prevalence of endoscopically negative and positive gastroesophageal reflux disease in the Japanese. *Scand J Gastroenterol* 2005; 40: 1005–1009.
11. Iwasaki E, Suzuki H, Sugino Y, et al. Decreased levels of adiponectin in obese patients with gastroesophageal reflux evaluated by videoesophagography: possible relationship between gastroesophageal reflux and metabolic syndrome. *J Gastroenterol Hepatol* 2008; 23(Suppl 2): S216–S221.
12. Vakil N, van Zanten SV, Kahrilas P, et al. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; 101: 1900–1920.
13. Alberti KG, Zimmet P and Shaw J. The metabolic syndrome – a new worldwide definition. *Lancet* 2005; 366: 1059–1062.
14. Jung HK. Epidemiology of gastroesophageal reflux disease in Asia: a systematic review. *J Neurogastroenterol Motil* 2011; 17: 14–27.
15. Lee YC, Yen AM, Tai JJ, et al. The effect of metabolic risk factors on the natural course of gastro-oesophageal reflux disease. *Gut* 2009; 58: 174–181.
16. Netinatsunton N, Attasaranya S, Ovartlarnporn B, et al. The value of Carlsson-dent questionnaire in diagnosis of gastroesophageal reflux disease in area with low prevalence of gastroesophageal reflux disease. *J Neurogastroenterol Motil* 2011; 17: 164–168.
17. Matsuzaki J, Suzuki H, Iwasaki E, et al. Serum lipid levels are positively associated with non-erosive reflux disease, but not with functional heartburn. *Neurogastroenterol Motil* 2010; 22: 965–970, e251.
18. Zerbib F, Belhocine K, Simon M, et al. Clinical, but not oesophageal pH-impedance, profiles predict response to proton pump inhibitors in gastro-oesophageal reflux disease. *Gut* 2012; 61: 501–506.

19. Savarino E, Marabotto E, Zentilin P, et al. The added value of impedance-pH monitoring to Rome III criteria in distinguishing functional heartburn from non-erosive reflux disease. *Dig Liver Dis* 2011; 43: 542–547.
20. Slaughter JC, Goutte M, Rymer JA, et al. Caution about overinterpretation of symptom indexes in reflux monitoring for refractory gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2011; 9: 868–874.
21. Savarino E, Tutuian R, Zentilin P, et al. Characteristics of reflux episodes and symptom association in patients with erosive esophagitis and nonerosive reflux disease: study using combined impedance-pH off therapy. *Am J Gastroenterol* 2010; 105: 1053–1061.

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CASE REPORT

Progressive liver failure induced by everolimus for renal cell carcinoma in a 58-year-old male hepatitis B virus carrier

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Abstract A 58-year-old man was diagnosed as a hepatitis B virus (HBV) carrier approximately 30 years ago. He was diagnosed with renal cell carcinoma when he was 57 years old. Radical nephrectomy was performed, and everolimus was administered to treat his lung metastasis. After beginning the everolimus, intermittent fever, general fatigue, and jaundice developed. He was admitted under a diagnosis of flare (acute exacerbation) of chronic B hepatitis due to HBV reactivation. Despite intensive care, he died of hepatic failure and fungus infection. The autopsy findings were compatible with hepatic failure due to HBV reactivation by everolimus. Antiviral prophylaxis must be taken into consideration before beginning immunosuppressive therapy such as everolimus in HBV carriers.

Keywords Everolimus · Immunosuppressive therapy · Hepatitis B virus · Liver failure · Nucleoside analogue

Introduction

Many molecular target agents have been developed for anticancer therapy. Some of them also have immunosuppressive effects. Everolimus is one molecular target drug that inhibits the mammalian target rapamycin (mTOR). It is widely used to treat renal cell carcinoma. In terms of its molecular mechanism, it stabilizes tumor progression, leading to prolonged progression-free survival [1]. The molecule mTOR exists in the middle of the signal cascade following nuclear factor-kappa B (NF-κB). Inhibition of mTOR is also able to block interleukin (IL)-2 signaling, which induces T cell growth and suppresses Th1-cell function [2]. These effects control cellular immunity, and everolimus has both anticancer and immunosuppressive effects.

Reactivation of hepatitis B virus (HBV), which is defined as the recurrence or abrupt rise in HBV replication, occurs both in patients in the inactive carrier state and in those with resolved hepatitis [3]. One million or more HBV carriers are present in Japan. It is thought that many patients do not undergo medical examinations or regular treatment. The Japan de novo Hepatitis B Research Group reported that the prevalence of and mortality associated with fulminant hepatitis were significantly higher among patients with HBV reactivation than among those with acute HBV infection [4]. Furthermore, recent reports showed that patients with malignant lymphoma treated with rituximab, an anti-CD20 agent, had a high risk of HBV reactivation [5, 6]. Reactivation can lead to clinically apparent acute hepatitis, which can be severe and result in acute liver failure and death [3]. Teng et al. [7] reported that inhibition of the mTOR signal could induce HBV replication.

Taken together, mTOR inhibitors, including everolimus, may induce immune suppression in HBV carriers and lead to HBV reactivation. This type of reactivation induced by immunosuppressive therapy in HBV carriers is an important issue that demands prompt action.

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Case report

A 58-year-old Japanese man was diagnosed as hepatitis B surface (HBs) antigen (Ag)–positive by medical examination approximately 30 years ago, but he was not undergoing medical consultation.

He was diagnosed with right renal cell carcinoma at a previous hospital, and radical right nephrectomy was performed when he was 57 years old. Three months after nephrectomy, lung metastases were identified. Interferon (IFN) alpha was started, but it could not control the disease progression. IFN alpha was stopped 2 months later, and sorafenib was started. Eight months later, because of peritoneal dissemination, sorafenib was switched to sunitinib, which was continued for 3 months until general fatigue developed. During the therapies the patient never had liver injury and his serum HBV DNA was not checked.

Approximately 18 months after the diagnosis of renal cell carcinoma, the patient began everolimus therapy. General fatigue and jaundice followed by intermittent fever appeared 5 months after starting everolimus. Laboratory findings showed liver injury, and the patient was admitted to his previous hospital with a diagnosis of acute exacerbation of chronic B hepatitis. Everolimus was stopped and entecavir (1 mg/day, oral administration) was started, but the liver injury and jaundice progressively worsened. Eight days later, he was transferred to our hospital for further medical treatment.

At the time of transfer, his serum aspartate aminotransferase (AST) level was 1920 IU/L, and his alanine aminotransferase (ALT) level was 878 IU/L. He had a marked coagulation disorder with a prothrombin time-international normalized ratio (PT-INR) of 1.83. The serum bilirubin level was elevated with direct bilirubin (D-Bil) predominance; the total bilirubin (T-Bil) was 11.0 mg/dL, and the D-Bil was 7.8 mg/dL. The serum HBV DNA level was 7.2 log copies/mL as measured by real-time polymerase chain reaction, and the HBV genotype was C. Other results of HBV-related serology tests were HBs-Ag-positive, HBe-Ag-negative, HBe-Ab-positive, and HBc-IgM-negative (chemiluminescent enzyme immunoassay). Contrast-enhanced computed tomography (CT) revealed cavity formation in the upper lobe of his right lung, and small nodular lesions were scattered in both lung fields. Ascites and slight liver atrophy were also seen (Fig. 1a).

We continued oral entecavir (0.5 mg/day) and started steroid pulse therapy (methylprednisolone, 1000 mg/day for 3 days with gradual tapering). His serum AST and ALT were gradually decreasing (AST, 101 IU/L; ALT, 112 IU/L) after 2 weeks of therapy, but his serum HBV DNA level was still high (6.7 log copies/mL), and his serum T-Bil was elevated at >20 mg/dL. His PT was still prolonged (PT- % 39). We added IFN beta therapy (3×10^6 IU/day as daily intravenous injections) to decrease the viral load and improve his liver function.

Three weeks after transfer, his plasma (1 → 3)-beta-D-glucan level suddenly increased to 61.2 pg/mL, and his chest X-ray showed a reticular shadow in his left lung. *Aspergillus* infection was suspected because he became positive for serum *Aspergillus* antigen at the same time. We then began amphotericin B at 150 mg/day as a daily intravenous infusion. In addition, he showed a decreased level of consciousness and flapping tremor at the time. That was comparable to hepatic encephalopathy level II(or III).

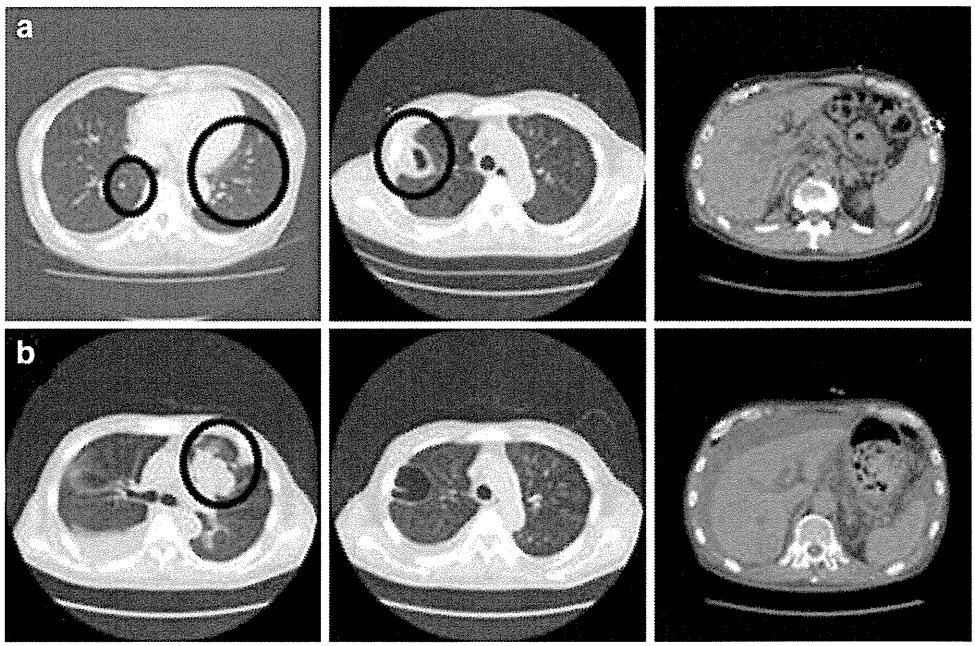

Fig. 1 **a** Contrast-enhanced computed tomography (CT) performed on the eighth hospital day showed small nodular lesions scattered in both lung fields (*left*), cavity formation in the upper lobe of the right lung (*middle*), and ascites and slight liver atrophy (*right*). **b** CT performed on the 38th hospital day showed a nodular lesion, lung metastases, and massive pleural effusion in the right thoracic cavity (*left* and *middle*). Liver atrophy was aggravated compared with the above image (*right*) (**b**)

Four weeks after transfer, CT revealed that the nodular lesions and lung metastases had extended and that massive pleural effusion had appeared in his right thoracic cavity (Fig. 1b). Furthermore, his renal function was worsening; his serum creatinine level had increased from 1.03 to 3.5 mg/dL. To improve his renal function and support his liver function, we began hemodialysis and plasma exchange. Despite intensive therapy, he died of hepatic failure and fungus infection on the 45th hospital day. Figure 2 shows his clinical course.

At autopsy, the liver weight was 1,040 g. The macroscopic view of the liver showed mild liver atrophy and

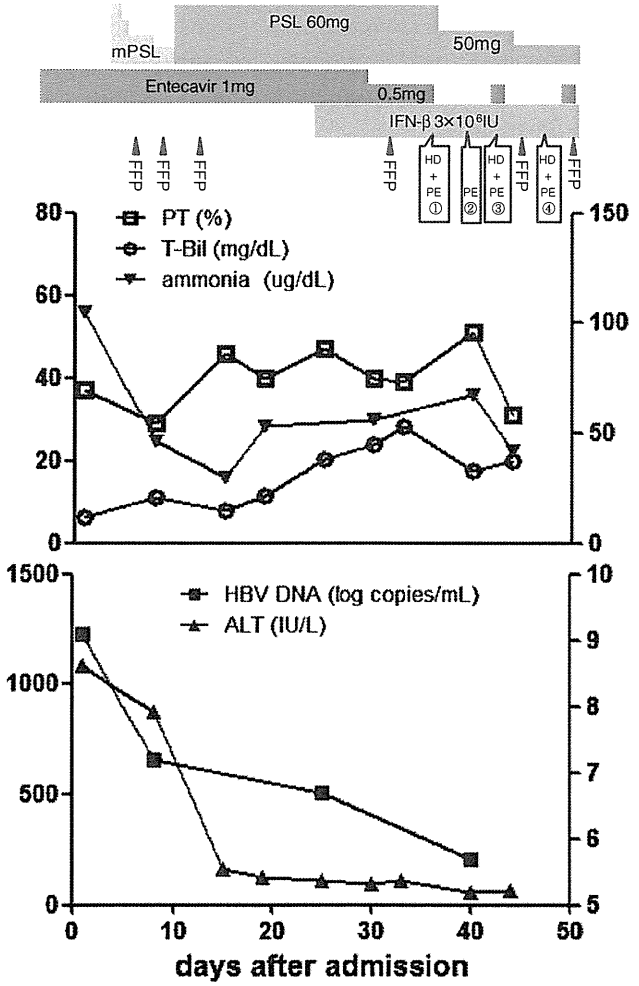

Fig. 2 Clinical course of the present case. *Upper panel* shows the treatment course, and *lower panel* shows the course of the laboratory findings. Left longitudinal axis of the *upper line graph* shows PT (%) and T-Bil, and right axis shows ammonia. Left longitudinal axis of the *lower line graph* shows ALT, and right axis shows HBV DNA. *mPSL* methylprednisolone, *PSL* prednisolone, *HD* hemodialysis, *PE* plasma exchange, *FFP* fresh frozen plasma, *T-Bil* total bilirubin, *ALT* alanine aminotransferase, *PT* prothrombin time

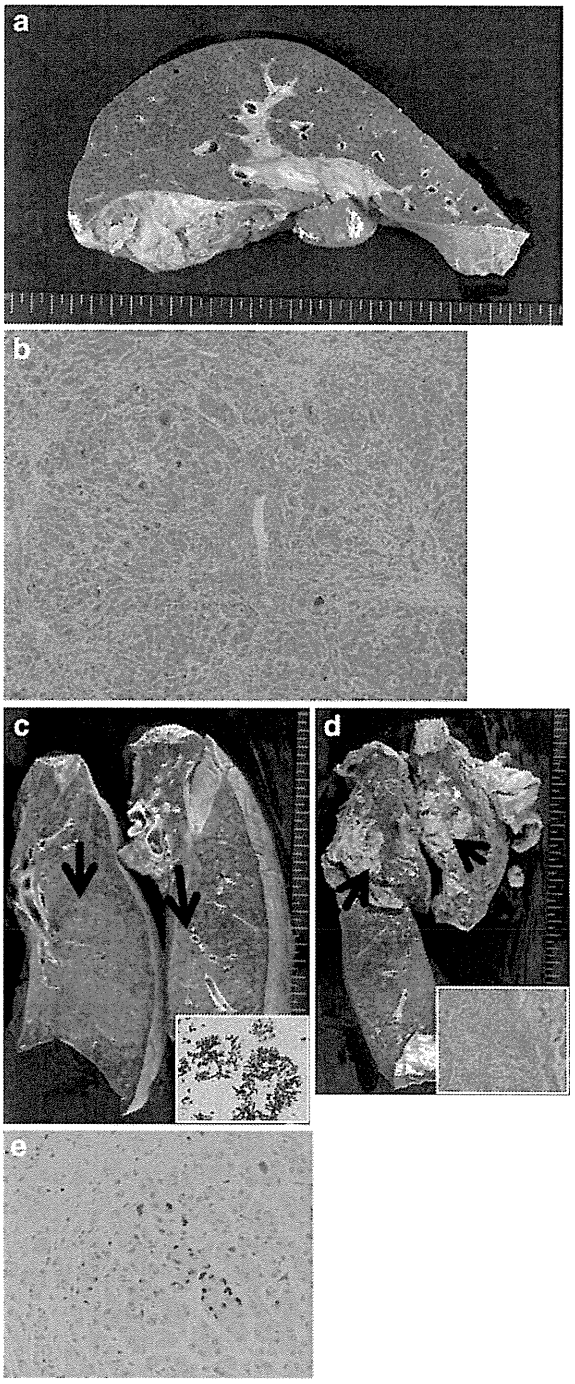

Fig. 3 Autopsy findings. **a** Macroscopic view of the liver shows mild atrophy and marked cholestasis. **b** H&E staining of the liver shows massive liver necrosis and moderate lymphocyte infiltration, and cholestasis (magnification 10×). **c** Congested lung and marked overgrowth of *Aspergillus* mycelia in the bilateral lungs. *Arrows* show overgrowth of *Aspergillus* mycelia. Lower right box shows Grocott's methenamine silver staining of *Aspergillus* mycelia in these lesions. **d** Metastatic lesions in the bilateral lungs. *Arrows* show these lesions. *Lower right box* shows H&E staining of metastatic renal carcinoma in these lesions. **e** HBs-Ag immunostaining in the liver

cholestasis (Fig. 3a). The pathological findings showed massive liver necrosis and moderate lymphocyte infiltration and cholestasis (Fig. 3b). There was alveolar hemorrhage, lung congestion, and marked overgrowth of *Aspergillus* mycelia in both lungs. There were large metastatic lesions in the left hilar region, left thoracic wall, and right lung. The pathological findings revealed that the renal cell carcinoma had also spread to left adrenal gland, cardiac muscle of the left ventricle, and bilateral hilar lymph nodes (Fig. 3c, d). Positive HBs-Ag immunostaining for HBs-Ag was seen (spread) in the liver (Fig. 3e).

Discussion

In the present case, everolimus was started for treatment of metastatic renal cell carcinoma, and HBV reactivation accompanied by severe liver dysfunction subsequently developed. The risk of fulminant hepatitis is significantly higher in HBV reactivation than in acute HBV infection, as described in Introduction [4]. Lamivudine, a nucleoside analogue, may reduce the risk for HBV reactivation of HBs-Ag-positive patients treated with chemotherapy [8, 9]. Lubel et al. [10] stated that prevention of HBV reactivation must be considered during immunosuppressive therapy or chemotherapy. EASL Clinical Practice Guidelines recommend that HBs-Ag-positive candidates for chemotherapy and immunosuppressive therapy should undergo pre-emptive nucleoside analogue administration during therapy and for 12 months after cessation of therapy [11]. Moreover, Li et al. [12] reported that entecavir is more effective than lamivudine in preventing hepatitis B reactivation in patients with lymphoma under chemotherapy. EASL Clinical Practice Guidelines also recommend that patients with a high HBV DNA level and/or repeated cycles of immunosuppression should be protected with a nucleoside analogue with high viral potency and a high barrier resistance; i.e., entecavir or tenofovir. A Japanese study group also recommended pre-emptive nucleoside analogue administration for HBs-Ag-positive patients before receiving immunosuppressive therapy or chemotherapy [13, 14]. The present patient was administered entecavir and IFN after HBV reactivation and severe liver dysfunction developed.

Although anti-HBV therapy and intensive liver and renal support (i.e., plasma exchange and hemodialysis) were performed, the patient developed liver failure and died. Autopsy findings showed massive liver necrosis equivalent to fulminant hepatitis. As mentioned above, everolimus may have a strong potential for immune suppression, and in the present case, drug-induced HBV reactivation with liver failure occurred. It is suggested that antiviral therapy may not be effective once HBV reactivation with liver dysfunction develops. The serum HBV DNA level did not decrease after starting entecavir in the present case. Remarkable necrosis and an inflammatory reaction secondary to *Aspergillus* infection were observed in his lungs; he finally developed respiratory failure in addition to liver failure, leading to his death. No previous history of liver injury before starting everolimus and positive immunostaining of HBs Ag with massive liver necrosis may support the incidence of HBV replication induced by everolimus treatment.

Because Drug Information warns that everolimus can cause hepatitis virus reactivation, there are no previous articles of case reports on everolimus-related HBV reactivation. Taken into consideration of the above-mentioned findings, this patient should have received anti-HBV prophylaxis with a nucleoside analogue such as entecavir or lamivudine before starting everolimus because he was HBs-Ag-positive.

In the clinical trial of everolimus, one HBV carrier died of everolimus-induced HBV reactivation. Detailed information about that case cannot be acquired because it was part of a clinical trial.

This case report is the first to include detailed clinical information. We also obtained the pathological autopsy findings of HBV reactivation by everolimus. This case raises an alert over the importance of prophylactic administration of a nucleoside analogue to HBs-Ag-positive patients. Baseline HBV serology must be tested for all patients who may receive everolimus, and a nucleoside analogue should be started for HBs-Ag-positive patients before treatment to decrease the risk of HBV reactivation.

We need to treat this case as a lesson, and hepatologists must enlightened other doctors about a standard procedure of decreasing that risk. It's preferable that doctors who consider the possibility of starting immunosuppressive therapy to their patients must check their HBV serology whether their HBs-Ag is positive or negative.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Chan HY, Grossman AB, Bukowski RM. Everolimus in the treatment of renal cell carcinoma and neuroendocrine tumors. Adv Ther. 2010;27:495–511.
2. Matsuda C, Ito T, Song J, Mizushima T, Tamagawa H, Kai Y, et al. Therapeutic effect of a new immunosuppressive agent, everolimus, on interleukin-10 gene-deficient mice with colitis. Clin Exp Immunol. 2007;128:348–59.

3. Hoofnagle JH. Reactivation of hepatitis B. Hepatology. 2009;49: S156–65.
4. Umemura T, Tanaka E, Kiyosawa K, Kumada H, Japan de novo Hepatitis B Research Group. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. Clin Infect Dis. 2008;47: e52–6.
5. Hui CK, Cheung WW, Zhang HY, Au WY, Yueng YH, Leung AY, et al. Kinetics and risk of de novo hepatitis B infection in HBs Ag-negative patients undergoing cytotoxic chemotherapy. Gastroenterogy. 2006;131:59–68.
6. Yeo W, Chan TC, Leung NW, Lam WY, Mo FK, Chu MT, et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. J Clin Oncol. 2009;27:605–11.
7. Teng CF, Wu HC, Tsai HW, Shiah HS, Huang W, Su IJ. Novel feedback inhibition of surface antigen synthesis by mammalian target of rapamycin (mTOR) signal and its implication for hepatitis B virus tumorigenesis and therapy. Hepatology. 2011;54: 1199–207.
8. Manzano-Alonso ML, Castellano-Trtajada G. Reactivation of hepatitis B virus infection after cytotoxic chemotherapy or immunosuppressive therapy. World J Gastroenterol. 2011;17:1531–7.
9. Loomba R, Rowley A, Wesley R, Liang TJ, Hoofnagle JH, Pucino F, et al. Systematic review: the effect of preventive lamivudine on hepatitis B reactivation during chemotherapy. Ann Intern Med. 2008;148:519–28.
10. Lubel JS, Testro AG, Angus PW. Hepatitis B virus reactivation following immunosuppressive therapy: guidelines for prevention and management. Intern Med J. 2007;37:705–12.
11. European Association for the study of the liver. EASL Clinical practice guidelines: management of chronic hepatitis B virus infection. J Hepatol. 2012; [Epub ahead of print].
12. Li HR, Huang JJ, Guo HQ, Zhang X, Xie Y, Zhu HL, et al. Comparison of entecavir and lamivudine in preventing hepatitis B reactivation in lymphoma patients during chemotherapy. J Viral Hepat. 2011;18:877–83.
13. Tsubouchi H, Kumada H, Kiyosawa K. Prevention ofi mmunosuppressive therapy or chemotherapy-induced reactivation of hepatitis B virus infection-Joint report of the Intractable Liver Diseases Study Group of Japan and the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis. Acta Hepatol Jpn. 2009; 50:38–42 (in Japanese).
14. Oketani M, Ido A, Uto H, Tsubouchi H. Prevention of hepatitis B virus reactivation in patients receiving immunosuppressive therapy or chemotherapy. Hepatol Res. 2012;42:627–36.