ORIGINAL ARTICLE

Expanded living-donor liver transplantation criteria for patients with hepatocellular carcinoma based on the Japanese nationwide survey: the 5-5-500 rule - a retrospective study

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SUMMARY

Expansion of the liver transplantation indication criteria for patients with hepatocellular carcinoma (HCC) has long been debated. Here we propose new, expanded living-donor liver transplantation (LDLT) criteria for HCC patients based on a retrospective data analysis of the Japanese nationwide survey. A total of 965 HCC patients undergoing LDLT were included, 301 (31%) of whom were beyond the Milan criteria. Here, we applied the Greenwood formula to investigate new criteria enabling the maximal enrollment of candidates while securing a 5-year recurrence rate (95% upper confidence limit) below 10% by examining various combinations of tumor numbers and serum alpha-fetoprotein values, and maintaining the maximal nodule diameter at 5 cm. Finally, new expanded criteria for LDLT candidates with HCC, the 5-5-500 rule (nodule size ≤5 cm in diameter, nodule number ≤5, and alfa-fetoprotein value ≤500 ng/ml), were established as a new regulation with a 95% confidence interval of a 5-year recurrence rate of 7.3% (5.2–9.3) and a 19% increase in the number of eligible patients. In addition, the 5-5-500 rule could identify patients at high risk of recurrence, among those within and beyond the Milan criteria. In conclusion, the new criteria – the 5-5-500 rule – might provide rational expansion for LDLT candidates with HCC.

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Key words
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Introduction

Hepatocellular carcinoma (HCC) is the most frequent primary liver cancer with increasing incidence, and the third highest cause of cancer-related mortality [1,2]. The ideal treatment is liver transplantation, which both eliminates the HCC and cures the diseased liver, but early results of liver transplantation for HCC were disappointing because of the high recurrence rate and poor overall survival. In 1996, the results of a prospective cohort study led Mazzaferro *et al.* [3] to propose selection criteria for liver transplantation as a single HCC nodule up to 5 cm in diameter or up to three nodules no greater than 3 cm in size without vascular invasion or extrahepatic metastasis, well-known as the Milan criteria. Since then, liver transplantation has become widely accepted for HCC patients with favorable tumor morphology. Because of the strict limitations in patient selection, however, the Milan criteria were recently challenged by several studies aiming to expand the Milan criteria with comparable results over the last two decades [4–8].

The eligibility requirements for liver transplantation, which requires both the donor and recipient to meet specific criteria, are more complicated than those for other cancer treatments. Expanding the Milan criteria will recruit more candidates with HCC who cannot be treated by locoregional therapy and are currently excluded from waiting lists by conventional criteria because of the tumor burden. On the other hand, increasing the number of HCC patients on the waiting list for deceased donor liver transplantation (DDLT) will certainly decrease the opportunity of liver transplantation for those enlisted without a malignant diagnosis because of the limited donor pool [9]. Whether the indication criteria for HCC should be the same between living-donor liver transplantation (LDLT) and DDLT is a matter of debate [10]. The graft from a live donor in LDLT is a private donation, meaning it is only intended for a specific recipient, while the graft from a deceased donor is public. The differences in the number of listed patients and available grafts among regions, societies, and nations make it difficult to reach a worldwide consensus regarding the indications for liver transplantation for HCC, especially in the LDLT setting. Some centers strictly follow the Milan criteria, even for LDLT [11], while others have aggressively expanded the indications, even for those with macrovascular invasion [12].

In Japan, where LDLT is the mainstay for those requiring liver transplantation, the Milan criteria remain the government-approved criteria for insurance coverage for patients with HCC, aimed at restricting the undue expansion of the indications for LDLT for advanced HCC. On the other hand, some centers have proposed and utilized center-oriented expanded indications for those wishing to undergo LDLT in a private practice setting [13–15]. This private practice is allowed after obtaining the institutional ethics committee approval, but it is not covered by the national insurance system. During the last decade, there has been a strong demand and movement toward expanding the nationwide-approved criteria in Japan, as well as in other countries.

In expanding the indications for LDLT for patients with HCC, it is crucially important that the expanded criteria will achieve a socially and ethically accepted outcome, especially in terms of the HCC recurrence rate [16,17]. A 5-year recurrence rate of less than 10% and a 5-year survival rate over 70% seems both reasonable and socially acceptable in the setting of LDLT for HCC [18], which was achieved in the benchmark study by Mazzaferro and colleagues [3]. The aim of the present study is to propose Japanese national expanded criteria for LDLT for patients with HCC, achieving a 5-year recurrence rate of <10% based on the retrospective data analysis of a nationwide survey.

Patients and methods

Patients

Datasets of LDLT recipients for HCC between 1998 and 2009 were collected from each Japanese LDLT center in June 2011. This study population comprised 1122 patients who had undergone LDLT for HCC at 44

centers in Japan between 1998 and 2009. Inclusion criteria were as follows: (i) radiologic confirmation of the presence of at least one HCC nodule in the preoperative images, and (ii) histopathologic proof of HCC on the explanted liver. Exclusion criteria were as follows: (i) incidental HCC, (ii) the presence of macroscopic vascular invasion in preoperative images, (iii) tumors with complete necrosis, and (iv) retransplantation. In addition, we excluded all cases missing critical data such as the tumor number, the maximal tumor diameter, alfa-fetoprotein (AFP) value, des-gamma-carboxy prothrombin (DCP) value, and the chronological data for survival.

This study was conducted in collaboration with the Japanese Liver Transplantation Society. The study protocol was approved as project number 016-0131 by the Research Ethics Committee/Institutional Review Board of the Graduate School of Medicine and Faculty of Medicine, University of Hokkaido. All efforts were made to protect patient privacy and anonymity during the preparation of this manuscript.

Preoperative tumor characteristics

Preoperative data, including patient, donor, and graft characteristics, were collected. Regarding preoperative tumor staging, the extent of the HCC was evaluated with contrast-enhanced computed tomography within 1 month before LDLT. Data regarding tumor size, tumor number, and the presence of macrovascular invasion were determined and collected by a specialized radiologist at each center. As for tumor markers, des-gamma-carboxy prothrombin (DCP, also known as protein induced by vitamin K absence or antagonist II [PIVKA-II]), and AFP, measured within 1 month prior to LDLT, were collected.

Histopathologic data

Histopathologic examination was performed on the explanted liver. The presence of HCC nodules was regarded as the definite diagnosis. Data on tumor size, tumor number, tumor differentiation, and macrovascular/microvascular invasion were collected from the pathologic reports. Each tumor was characterized as being well, moderately, or poorly differentiated according to modified Edmondson criteria. In cases with heterogeneous grades of differentiation, the most progressive grade was applied. The tumor with complete necrosis was not considered as HCC, while HCC tumor with partial necrosis was counted and sized as the whole nodule.

Operative procedure, postoperative management, and follow-up

The operative procedure, including selection of the graft, depended on each center following standard LDLT procedures. Immunosuppression was the conventional double or triple regimen including calcineurin inhibitors (cyclosporin A or tacrolimus) and steroids with or without mycophenolate mofetil. Mammalian target of rapamycin inhibitors has never been used in the present cohort, as it is not approved for liver transplant recipients in Japan. As for surveillance for HCC recurrence, serum levels of AFP and DCP were measured at every visit (at least once per 3 months), and contrast-enhanced computed tomography (or magnetic resonance imaging) was performed at least once a year in all participating centers. Additional contrast-enhanced computed tomography and other radiologic modalities, mandatory for a definite diagnosis of recurrence, were performed on-demand when the recurrence was suspected. The diagnosis of the HCC recurrence was based on the radiologic confirmation of the tumor, mainly in computed tomography images.

Statistical analysis

Endpoints

The primary endpoint was the 5-year recurrence rate, because predicting the risk of recurrence should be the main goal in establishing the indication criteria for liver transplantation for patients with HCC. The secondary endpoint was 5-year overall survival. Recurrence-free survival and patient survival were computed by the Kaplan–Meier method and compared by the log-rank test.

Designing new indication criteria for HCC

The aim of this study was to establish new, practical extended criteria enabling the maximal enrollment of candidates, while securing a 5-year recurrence rate (95% upper confidence limit) below 10%, and not to search for factors associated with recurrence or establish a prediction model for recurrence. The maximal diameter of the tumors was set at 5 cm, because all extended criteria proposed by Japanese major liver transplant centers have set 5 cm as the upper limit of the tumor size as an indication for LDLT [19]. Regarding tumor markers, both AFP and DCP proved to be significant predictors for HCC recurrence after LDLT in our previous nationwide survey [20]. Therefore, the upper limit of the

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Table 1. Patient demographics.

	Median [range]/number
Age	
(years)	56 [12–73]
Gender	
Male	704
Female	261
Etiology	
No virus	86
HBV	282
HCV	582
Both	15
Child-Pugh-Turcotte classification	
A	109
B	328
C	473
Unknown	55
MELD score	13 [–0.68 to 45]
Pretreatment	
Yes	668
No	297
Graft type	
Left lateral	4
Left	289
Right posterior	18
Right	645
Whole	5
Dual	1
Unknown	3
Tumor number (radiology)	
1	360
2–3	387
4–5	101
6–10	79
≥11	38
Tumor size (radiology)	
(cm)	2.4 [0.4–23]
Milan (radiology)	
Within	664
Beyond	301
AFP	27 [undetectable-280 074]
DCP	41 [undetectable-43 600]
Tumor number (pathology)	
No viable tumor	25
1	262
2–3	323
4–5	133
6–10	123
≥11	98
Unknown	1
Tumor size (pathology)	
(cm)	2.4 [0.2–22.5]
Differentiation (pathology, *1)	
Well	188
Moderate	602
Poor	135
Combined/mixed	2
Unknown	13
Portal venous invasion (pathology*1)	
vp0	743
vp1	166
vp2	21
vp3	8
Unknown	2
Hepatic venous invasion (pathology*1)	
vv0	879
vv1	55
vv2	4
Unknown	2
Milan (pathology*1)	
Within	500
Beyond	438
Unknown	2

*1 N = 940, total necrosis: 25. AFP, alfa-fetoprotein; DCP, des-gamma-carboxy prothrombin; MELD, model for end-stage liver disease.

tumor number and serum AFP/DCP value satisfying a 5-year recurrence rate (95% upper confidence limit) below 10% with the maximal enrollment of candidates was computed and investigated using the Greenwood formula as follows; the upper (and the lower limit) of confidence interval was computed as "actual recurrence rate + 1.96*standard error" (and "actual recurrence rate − 1.96*standard error"). In this analysis, patients with a categorized value (i.e., <5 ng/ml) were excluded for statistical reasons.

Continuous variables and categorical variables are expressed as median (with range) and number (%) respectively. A P value of <0.05 was considered to indicate statistical significance. All calculations were performed with SPSS statistical software (ver 22.0 for Windows, IBM, Chicago, IL, USA).

Results

Patient characteristics

A total of 965 patients were included in the study, and the patient demographics are shown in Table 1. Twelve patients with a categorized AFP value were excluded from the dataset used to create the new criteria. Among the 965 patients, 664 patients were within the Milan criteria, while the remaining 301 (31%) were beyond the Milan criteria according to the preoperative imaging findings. The distributions of the diameter of the largest

nodule, the number of tumors, the serum AFP, and the serum DCP values are presented in Fig. 1(a–d). Other findings, including the pathology of explant livers and donor characteristics, are shown in Table 1. The median follow-up period after LDLT was 54 (1–154) months.

5-year recurrence rates along with the number of tumors and the serum AFP level

As described in the Methods section, the maximal diameter of the tumor was set to 5 cm. Then, 5-year recurrence rates were calculated with various combinations of tumor numbers and serum AFP levels. The 95% confidence interval of 5-year recurrence rates with the number of patients satisfying the various combinations are shown in Figures S1–S8 in order of increasing tumor number. The number of patients within the upper limit of the tumor number and AFP value is presented with the number of patients meeting both the new criteria and the Milan criteria, and the number of those meeting only the new criteria, but beyond the Milan criteria. In addition, the number of patients meeting the Milan criteria, but beyond the new criteria is shown in the figures. When the tumor number was <4, the 95% upper confidence limit of recurrence rate was <10% regardless of the serum AFP value, but when the upper limit of the tumor number was set to 8 or more, it was above 10% regardless of the serum AFP value. The 5-year recurrence rate (95% CI) and the number of patients meeting each set of criteria with the variable AFP cut-offs when the tumor number upper limit was set to 5 are shown in Fig. 2, demonstrating that the AFP cut-off should be 500 ng/ml to satisfy the target.

The optimal cut-off value for the tumor number and the serum AFP (DCP) value satisfying a 5-year recurrence rate (95% upper confidence limit) below 10% with the maximal enrollment of candidates

Figure 3 summarizes the number of patients within the expanded criteria along with the number of tumors and the AFP values among the eligible patients ($n = 953$). The combinations of tumor numbers and AFP cut-off values producing a 95% upper confidence limit of recurrence rate below 10% are indicated in gray color. The maximal number included in the new criteria was 725 when the upper limit of the tumor number and the AFP value was set at 5 and 500 respectively. Consequently, the new criteria, i.e., tumor size ≤5 cm in diameter, tumor number ≤5, and AFP value ≤500 ng/ml, namely the 5-5-500 rule, proved to be the optimal cutoff value as assessed by the Greenwood formula. When these criteria were used to indicate LDLT for the entire cohort ($n = 965$), 71 additional patients were included over the conventional criteria (11% increase) while achieving a 95% upper confidence limit of a recurrence rate below 10%. When the upper limit of AFP was only used for those beyond the Milan criteria, meaning LDLT was indicated for those "within the 5-5-500 rule OR the Milan criteria", a total of 792 patients were included, increasing the number of eligible patients by 19%.

The same analytic procedure was done for DCP value among the eligible patients ($n = 953$), which demonstrated that the DCP cut-off value of 1000 mAU/ml and the tumor number of four will satisfy the target (data not shown). However, the maximal patient number included in the criteria based on the DCP value (5-4-1000) was 708 (Figure S9), which was smaller than that obtained by the 5-5-500 rule based on the AFP value.

Cumulative 5-year recurrence rate, disease-free survival, and patient survival according to the indication criteria

The number of patients indicated for LDLT, the 5-year recurrence rate, the 5-year recurrence-free survival rate, and the 5-year patient survival rate according to the Milan criteria, the 5-5-500 rule, and the Milan criteria and 5-5-500 rule combined is summarized in Table 2. The Kaplan–Meier curves for recurrence-free survival and overall patient survival are shown in Fig. 4a and b, respectively, stratified by the indication criteria. The outcome did not differ among the different selection criteria, while the outcome was poorest when LDLT was indicated for those "within the 5-5-500 rule OR the Milan criteria". In addition, the Kaplan–Meier curves for recurrence-free survival and overall patient survival according to the combination of the Milan criteria and the 5-5-500 rule are shown in Fig. 5a and b respectively. Both recurrence-free survival ($P < 0.001$) and patient survival ($P < 0.001$) among those within the Milan criteria were significantly different between patients within the 5-5-500 rule and those beyond it. This was also the case among those beyond the Milan criteria ($P < 0.001$ and $P < 0.001$ respectively). These results clearly demonstrate that an AFP cut-off value of 500 ng/ml can identify patients at high-risk of recurrence among those within the Milan criteria, and that the 5-5-500 rule can be a reasonable expanded indication for those beyond the Milan criteria.

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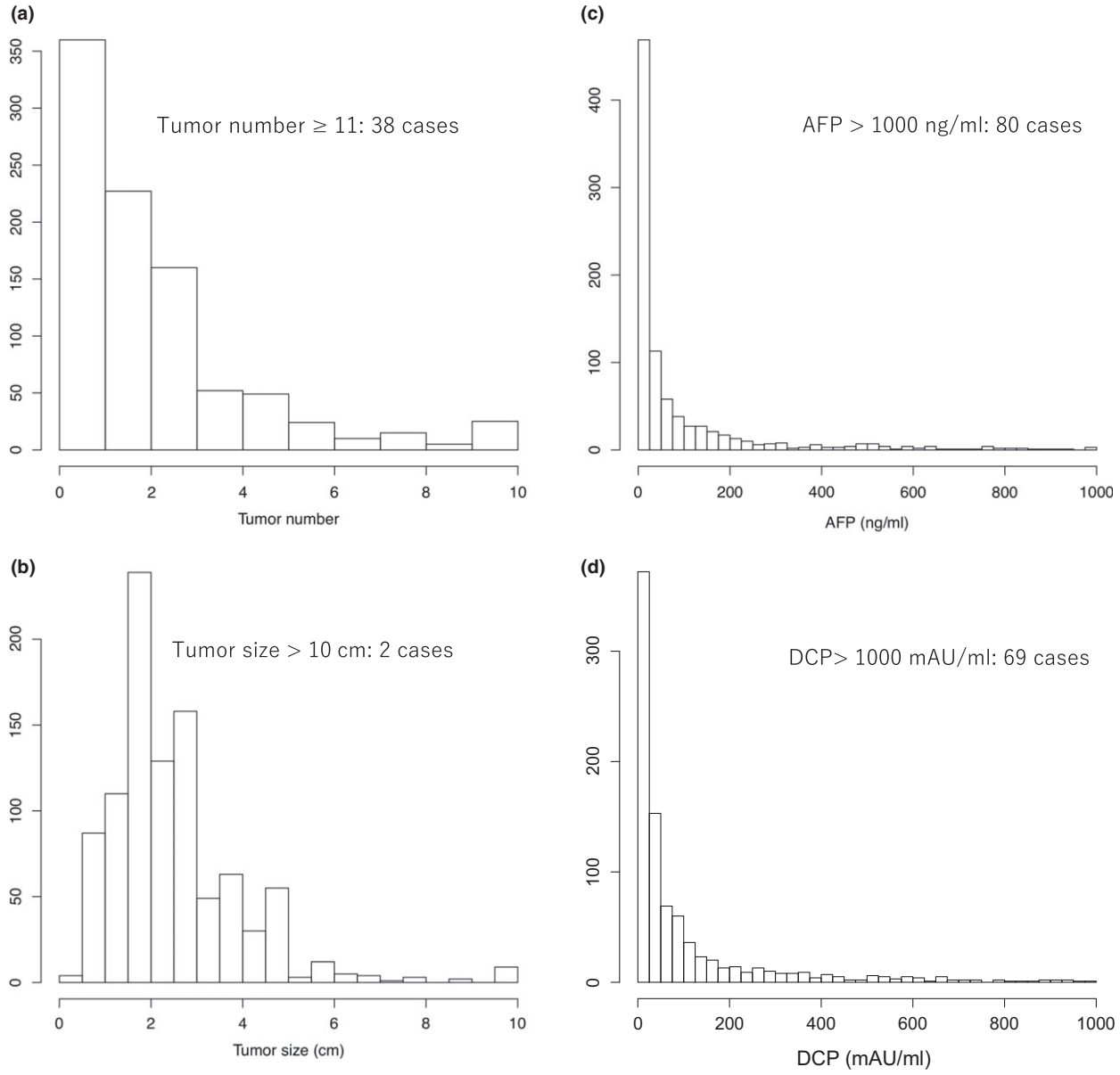

Figure 1 The distribution of the tumor size (a), the tumor number (b), and the serum AFP (c) and DCP (d) values.

Discussion

This study aimed to establish new, practical extended selection criteria for patients with HCC in the LDLT setting that could achieve a socially and ethically acceptable outcome, and not to advocate a statistically significant prediction model for HCC recurrence. For practical application, new extended criteria should be as simple as the Milan criteria, as objective as possible, and easily verifiable retrospectively and prospectively. Based on the Japanese nationwide survey, we developed new expanded criteria for LDLT candidates with HCC – the 5-5-500 rule, i.e., tumor size ≤5 cm in diameter, tumor number ≤5, and AFP value ≤500 ng/ml, as a new regulation. The new criteria satisfied our target of a 5-year recurrence rate <10% and a 5-year survival rate over 70%.

Liver transplantation for patients with HCC has become highly successful because of the landmark study by Mazzaferro and colleagues in 1996 [3], but recently the Milan criteria have become seen as too restrictive, preventing access to liver transplantation by many patients with HCC (who could benefit from transplantation compared with other therapies) [8]. Many studies have proposed extended criteria for HCC patients with a comparable long-term outcome when compared with the Milan criteria; nevertheless, the Milan criteria

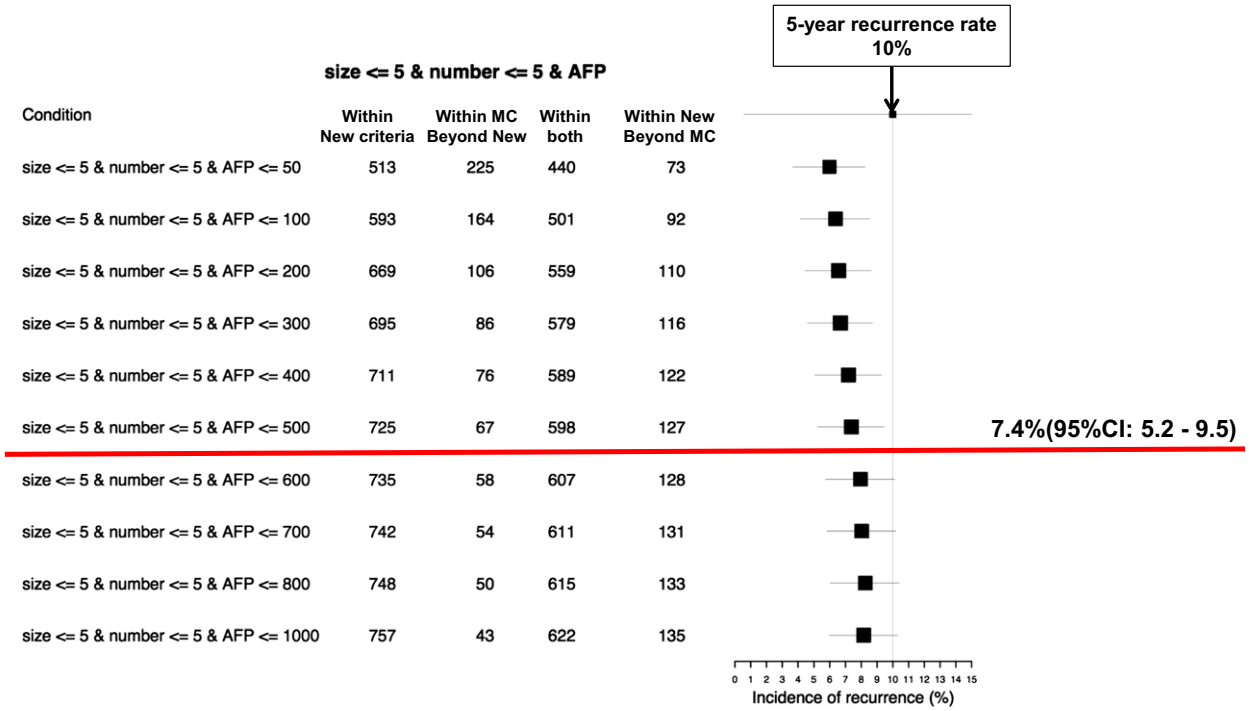

Figure 2 The 5-year recurrence rate (95% confidence interval) and the number of patients meeting each set of criteria with the variable AFP cut-offs when the tumor number upper limit was set to 5.

remain the current benchmark for selecting HCC patients for liver transplantation, and the basis for comparison with other suggested criteria, as recommended by western experts at the international conference held in 2010 [16]. Key aspects of expanded selection criteria are as follows: (i) they should identify those patients who, despite exceeding the Milan criteria, will still do well without an increase in HCC recurrence after liver transplantation; (ii) they should ideally identify those within the Milan criteria who will have a high-risk of recurrence; (iii) the expansion of candidates with HCC should be balanced with other candidates without HCC in a DDLT setting. Because the aim of this study was to establish new extended criteria for LDLT recipients, the third point was not considered.

The Milan criteria are based on the tumor size and number, and therefore the initial attempts to expand the Milan criteria focused on increasing the upper limit of the tumor size and number. It is now clear, however, that selection based only on tumor size and number is not optimal and that the parameters reflecting the biologic behavior of the tumor are mandatory. Previous studies reported an extended maximal diameter of the tumor [4,21], the total tumor volume [6], the sum of the maximal tumor diameters [5,21], and various definitions for the upper limit of the tumor number [7,13–15,22], as new expanded criteria. In the present study, we set the upper limit of the maximal diameter of the tumor as 5 cm for the following reasons: (i) this size limit is consistent with the conventional Milan criteria; (ii) all expanded criteria utilized in Japan have used 5 cm as the maximal tumor diameter [19]: (iii) HCC tumors over 5 cm in diameter are associated with the presence of vascular invasion [23,24], which is also a significant predictor of recurrence. As described by the "Metroticket paradigm", we should always be aware of the fact that the farther we wander from the conventional criteria; the higher the price we will pay by higher recurrence [5]. Therefore, in the present study, the most suitable number of tumors for the upper limit with a fixed maximal tumor diameter (5 cm) was investigated with a variable limitation of the upper limit of the AFP value to suppress the recurrence rate within the target.

Pretransplant tumor markers such as serum AFP and DCP levels are increasingly studied to exclude patients who are at-risk for post-transplant recurrence [25,26]. While both AFP and DCP have proven to be significant predictors in our previous nationwide survey [20], AFP value of 500 ng/ml was finally selected as part of the new criteria based on the maximal inclusion number. DCP has been reported as the most significant predicting factor for venous invasion of HCC [27] and intra- and extra-hepatic spread of the disease [28,29], and was reported to be useful prognostic factor for those beyond

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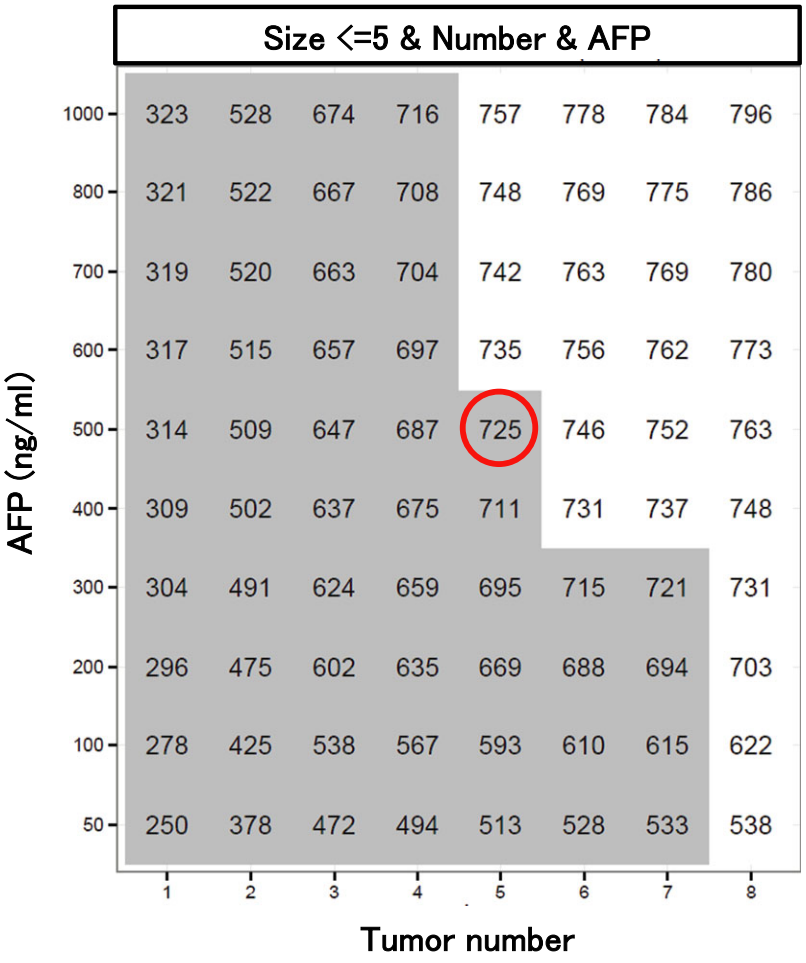

Figure 3 The number of patients within the expanded criteria along with the number of tumors and the AFP values. The combinations of tumor numbers and AFP cut-off values producing a 95% upper confidence limit of recurrence rate below 10% are indicated in gray color.

the Milan criteria in LDLT [14,15,26]. On the contrary, DCP is criticized for its not being a routine laboratory value in the west [30], and is often affected by the vitamin K status and warfarin administration in clinical settings. Various AFP cut-offs, 20 ng/ml [31], 400 ng/ml [6,32,33], and 1000 ng/ml [34,35], have been proposed as a predictor for HCC recurrence. In the present study, an AFP value of 500 ng/ml was finally selected as the cut-off to limit the 5-year recurrence rate within 10% and to maximize the included patients under the extended size and number criteria – 5 nodules no greater than 5 cm in size.

The importance of biomarkers, other than the morphologic characteristics of tumors, has recently increased. Not only tumor makers such as AFP and DCP, but the neutrophil-to-lymphocyte ratio [36,37], fluorine-18-fluorodeoxyglucose positron emission tomography [38–40], and tumor progression during the waiting period [37] are reportedly able to predict recurrence, and the model to predict tumor recurrence calculated by the combination of these markers seem promising in predicting HCC recurrence after liver transplantation. Despite the promise, however, these biomarkers (besides tumor size and number) have not yet reached the conventional practical decision-making criteria, nor has there been a consensus regarding which biomarker should be incorporated into the conventional selection criteria based on the tumor morphologic features.

In the present study, both the new extended criteria, the 5-5-500 rule, and the Milan criteria achieved a 95% CI of a 5-year recurrence rate <10%, while selection allowing either the 5-5-500 rule or Milan criteria resulted in the slight deviance from the target (the 95% upper confidence limit of 11.2%). Exclusion of patients from liver transplantation by the new criteria, who are otherwise indicated by the Milan criteria, seems not socially accepted and not rational at present, considering the worldwide prevalence and acceptance of the Milan criteria. Therefore, in enforcing the new extended criteria, patients within the Milan criteria but beyond the new criteria, namely those meeting the Milan

Table 2. The number of patients indicated for LDLT, the 5-year recurrence rate, the 5-year recurrence-free survival rate, and the 5-year patient survival rate according to the Milan criteria, the 5-5-500 rule, and the Milan criteria and 5-5-500 rule combined.

Selection criteria	*N*	5-year recurrence rate [95% confidence interval)	5-year recurrence-free survival rate [95% confidence interval)	5-year overall survival rate [95% confidence interval)
Milan criteria				
Within	664	7.5% [5.3–9.7]	73.1% [69.7–76.7]	75.3% [71.9–78.8]
Beyond	301	34.7% [28.4–40.4]	50.6% [45.1–56.8]	58.7% [53.1–64.8]
5-5-500 rule				
Within	735	7.3% [5.2–9.3]	73.2% [70.0–76.6]	75.8% [72.6–79.1]
Beyond	230	43.8% [36.3–50.5]	43.4% [37.4–50.5]	52.1% [45.8–59.2]
Milan or 5-5-500				
Within	792	9.1% [6.8–11.2]	71.8% [68.6–75.1]	74.8% [71.7–78.0]
Beyond	173	47.8% [38.8–55.4]	40.0% [33.1–48.2]	48.6% [41.4–57.0]

criteria with a serum AFP value above 500 ng/ml, should still be indicated for liver transplantation.

There are two ongoing debates regarding the difference between LDLT and DDLT when discussing liver transplantation for HCC patients: (i) should the indication criteria be the same? And (ii) Is there a difference in the post-transplant biologic behavior of HCC recurrence? [10,41–43] In contrast with the graft as a public gift in DDLT, living grafts are given to patients through a close donor-recipient relationship on a case-by-case basis considering patient expectation, recipient survival outcome, and donor will and safety. In this respect, expansion of the indication criteria seems easier and more acceptable in the LDLT setting, but it still depends on the socially accepted norms in terms of the recipient outcome, and the documented donor morbidity and mortality must be considered. As mentioned above, in Japan, LDLT is allowed by the national insurance system only for those within the Milan criteria, although some centers perform LDLT as private practice with center-oriented expanded criteria achieving 5-year patient survival over 80% and a 5-year recurrence rate of <10% [19,44]. Consequently, not a few patients have given up the chance for LDLT because of financial reasons, despite the potential of a live donor, and demands to expand the insurance coverage are strong. Therefore, we are trying to establish new extended criteria that enable the inclusion of a considerable number of additional patients with socially acceptable outcomes, which will be approved only in LDLT setting. With the severe shortage of deceased donors, the Milan criteria should remain the gold standard in the DDLT setting in Japan. This latter issue, too, remains controversial, as some have raised the alarm regarding an increase in tumor recurrence in the LDLT setting [45]. Fast-tracking, i.e., indicating LDLT for those would otherwise be delisted because of tumor progression during the waiting period, could be one reason for the increased recurrence rate in LDLT [46]. The possibility of a patient being rushed for liver transplantation with an available live donor, however, will not happen in Japan, because the majority of patients with HCC re followed up for a long time by hepatologists and the time from the last treatment to liver transplantation should be at least 3 months according to the national regulation.

The present study has several limitations. First, this is a retrospective study based on a certain degree of old cases and lacking a validation cohort, although the size of the population was quite large. A further nationwide study validating the present results with prospective patient recruitment or based on recent cases is mandatory. The developments and advances in imaging modalities (especially multi-detector computed tomography), anti-viral treatments (especially direct-acting anti-virals for hepatitis C), and immunosuppression regimens might have considerably changed the practice in the management of liver transplantation within the last two decades, which could be a bias in this type of study. There was no difference in the recurrence-free and patient survival between old and recent era (1998–1999 vs. 2000–2009, data was not presented), however, no evaluation was done regarding the center-volume effect in the present study, which could also be a bias. Another limitation of this study was the absence of the consideration for the effect of the pre-transplant treatment for HCC and the duration between the last treatment and LDLT. In Japan, most patients with chronic liver diseases are closely followed up, and once they develop HCC, they will undergo locoregional

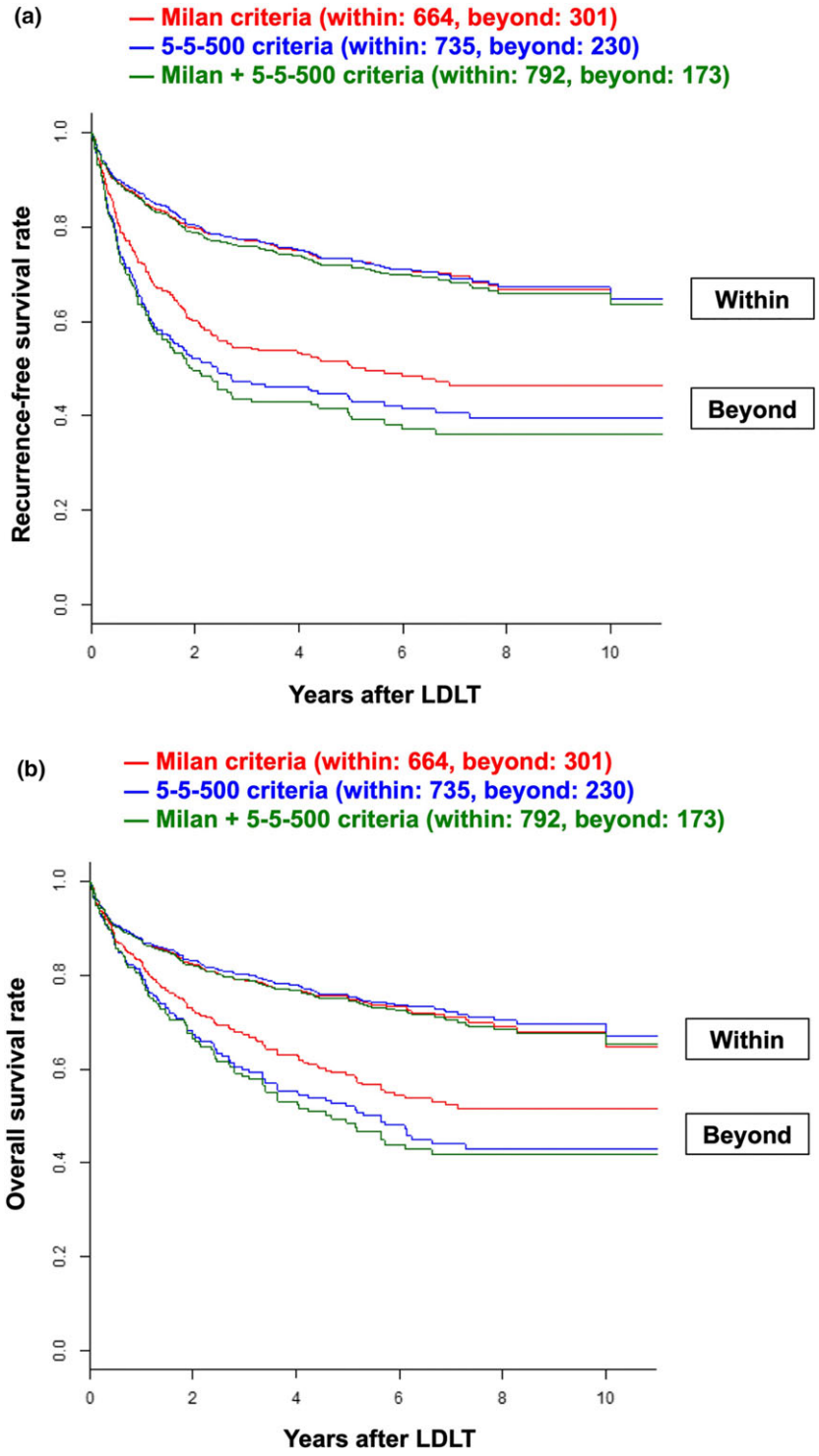

Figure 4 The Kaplan–Meier curves for recurrence-free survival (a) and overall patient survival (b), stratified by the indication criteria.

treatment promptly, if the liver function allows. Liver transplantation is considered for those with cirrhosis of Child-Pugh B and worse. In addition, in LDLT setting, once the live donor is fixed, LDLT will be performed without delay, meaning no waiting time. The interval between LDLT and the last treatment for HCC should be more than 3 months, which is defined by the government regulation. As a whole the present results may be difficult to be interpreted and operated in the same way in the West where the DDLT is the mainstay and the etiology of the disease are different, however, may be of relevance in the general management of HCC worldwide.

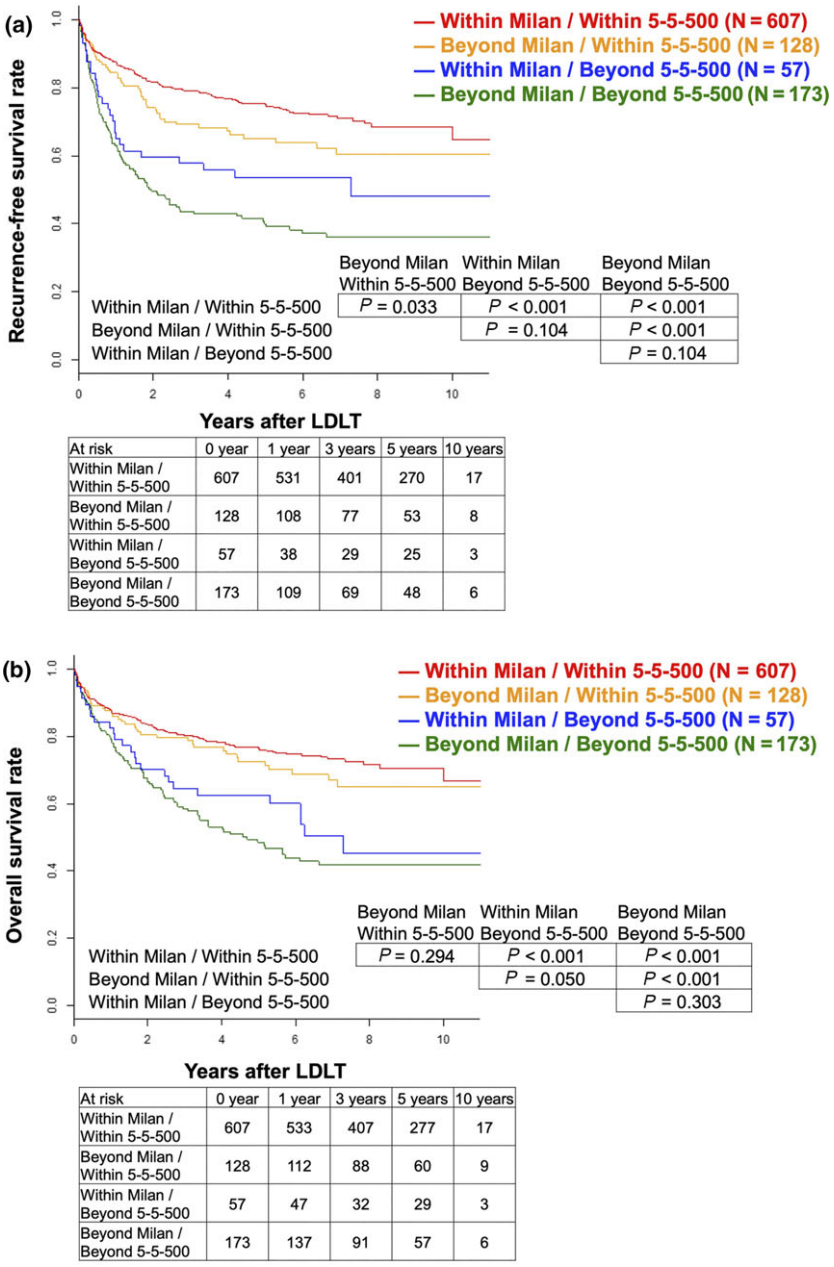

Figure 5 The Kaplan–Meier curves for recurrence-free survival (a) and overall patient survival (b) according to the combination of the Milan criteria and the 5-5-500 rule.

In conclusion, we propound the 5-5-500 rule: 5 HCC nodules no greater than 5 cm in size with an AFP value below 500 ng/ml, as a new expanded indication for HCC patients in the LDLT setting. The new criteria could secure the 95% upper confidence limit of a recurrence rate below 10%, and coupled with the Milan criteria, could increase the number of eligible LDLT candidates by 19%.

Authorship

TS, NA, SK, SU, NK, HO, HE, HF and ST: planed the study design and collected data. MF, AK and SM: performed the statistical analysis. Finally, TS and NA: wrote the manuscript. KH: performed critical review. All authors have confirmed and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1-S8. The 95% confidence interval of 5-year recurrence rates with the number of patients satisfying the various combinations in order of increasing tumor number.

Figure S9. The number of patients within the expanded criteria along with the number of tumors and the DCP values. The combinations of tumor numbers and DCP cut-off values producing a 95% upper confidence limit of recurrence rate below 10% are indicated in gray color.

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APPENDIX

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