Total Tumor Volume and Alpha Fetoprotein for selection of transplant candidates with hepatocellular carcinoma: a prospective validation

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Abbreviations: AASLD: American Association for the Study of Liver Diseases, AFP: alpha fetoprotein, HBV: hepatitis B virus, HCC: hepatocellular carcinoma, HCV: hepatitis C virus, IQR: interquartile range, MELD: Model for End-Stage Liver Disease, RFA: radio-frequency ablation, SRTR: Scientific Registry of Transplant Recipients, TACE: trans-arterial chemoembolisation, TARE: trans-arterial radioembolisation, TTV: total tumor volume, UCSF: University of California San Francisco.

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Abstract

The selection of liver transplant candidates with hepatocellular carcinoma (HCC) is currently validated based on Milan criteria. The use of extended criteria has remained a matter of debate, mainly because of the absence of prospective validation. The present prospective study recruited patients according to the previously proposed Total Tumor Volume (TTV ≤115 cm^3)/ alpha fetoprotein (AFP ≤400 ng/ml) score. Patients with AFP >400 ng/ml were excluded, and as such the Milan group was modified to include only patients with AFP <400 ng/ml; these patients were compared to patients beyond Milan, but within TTV/AFP. From January 2007 to March 2013, 233 patients with HCC were listed for liver transplantation. Of them, 195 patients were within Milan, and 38 beyond Milan but within TTV/AFP. The average follow-up from listing was 33,9 ±24,9 months. The risk of drop-out was higher for patients beyond Milan but within TTV/AFP (16/38, 42,1%), than for patients within Milan (49/195, 25,1%, p=0,033). In parallel, intent-to-treat survival from listing was lower in the patients beyond Milan (53,8% vs. 71,6% at four years, p<0,001). After a median waiting time of 8 months, 166 patients were transplanted, 134 patients within Milan criteria, and 32 beyond Milan but within TTV/AFP. They demonstrated acceptable and similar recurrence rates (4,5% vs. 9,4%, p=0,138) and post-transplant survivals (78,7% vs. 74,6% at four years, p=0,932).

Conclusion: Based on the present prospective study, HCC liver transplant candidate selection could be expanded to the TTV (≤115 cm^3)/ AFP (≤400 ng/ml) criteria in centers with at least 8-month waiting time. An increased risk of drop-out on the waiting list can be expected but with equivalent and satisfactory post-transplant survival.

Introduction

Liver transplantation is the most effective treatment for patients with early non-resectable hepatocellular carcinoma (HCC). However, some 5%-15% of patients experience a post-transplant recurrence, and candidate selection appears to be the most effective action for its prevention (1, 2). Milan criteria have been widely accepted for the selection of HCC patients for liver transplantation (3). With the use of Milan criteria, post-transplant survival rates of 75%-95% at two years and 70%-80% at five-years have been repeatedly observed, which represents the basis for use of Milan criteria as a gold standard when exploring new criteria (1, 3, 4). An international panel of experts also agreed that a modest expansion of the number of potential candidates could be considered on the basis of studies showing similar survivals for select patients outside Milan criteria (3).

In recent years, a wide range of expanded criteria have been proposed, including the externally validated University of California San Francisco (UCSF) criteria (one HCC ≤6·5 cm or ≤3 HCCs ≤4·5 cm and total tumor diameter ≤8 cm) and the registry-based up-to-seven score (size of largest HCC + number of HCCs ≤7) (1, 5-8). However, none of these criteria has reached wide acceptance thus far, mainly due to a lack of robust prospective validation.

The potential for candidate selection based on a composite of the total tumor volume (TTV ≤115 cm^3) and AFP ≤400 ng/ml without macrovascular invasion or extrahepatic disease has been recently explored. This score has been designed based on single-center, multi-center and registry based (SRTR) studies, and allowed a moderate expansion of the number of transplant candidates, without impacting on post-transplant outcome in these retrospective studies (6, 9, 10).

The main limitation of the TTV/AFP criteria to date has been the lack of prospective validation. The present study was designed as a multicentric web-based prospective assessment of patients listed utilizing the TTV/AFP criteria, to support validation of these criteria for selection of HCC candidates for liver transplant.

Patients and Methods

Study design

The study was based on a web-based multicentric database, which prospectively includes data on patients with hepatocellular carcinoma listed for liver transplantation. In the present analysis, patients from the University of Alberta, Edmonton, Canada (starting January 2007), the University of Geneva, Geneva, Switzerland (starting October 2009), and the University of Western Ontario, London, Canada (starting January 2012) were analyzed, as these three centers used the previously described TTV/AFP criteria for candidate selection (9, 10). The composite criteria allowed access to listing for transplantation to patients with TTV ≤115 cm^3 and AFP ≤400 ng/ml, in the absence of extra-hepatic disease and macrovascular HCC invasion on radiology (9, 10). All patients beyond the TTV/AFP cut-offs (whatever the level) were eligible for listing if they could be downstaged to within criteria according to mRECIST and stabilized within the criteria for a minimum of three months (11). Of note, only successfully downstaged patients were studied, as an accurate assessment of patients failing downstaging was difficult for geographical reasons (many who failed downstaging at distant centers were not referred for consideration of transplantation). A progression beyond TTV or AFP limits, while on the transplant waitlist, resulted in inactivation from the list. Inactivated patients considered suitable for locoregional therapy were offered further treatment. If responding and returning to within TTV/AFP criteria, and remaining stable for a minimum of 3 months, they could be reactivated on the waitlist. A complete radiological response to loco-regional HCC treatment was not a delisting criterion. The database was updated until September 2014, but only patients listed prior to March 2013 were analyzed to

allow sufficient follow-up. Four patients with non-cirrhotic underlying liver disease were excluded, because the selection of HCC liver transplant candidates without cirrhosis should be based on specific and different criteria, as size does not appear as a strong predictor of post-transplant outcome in this group of patients (12). The study was approved by the ethical review board of each institution.

Collected data

HCC data included listing, transplant and maximum levels for AFP and TTV. Only radiological assessments of HCC morphology were used throughout the study, as no pathological data is available for decision making at the time of listing and transplantation. TTV was calculated as the sum of the volume of each HCC ($(4/3)\pi r^3$) based on the maximum radius of each lesion (9, 10). An HCC was diagnosed by arterial phase contrast enhancement and early venous phase washout on augmented CT or MRI, or by biopsy in cases with equivocal imaging studies, as outlined in the AASLD guidelines (13).

The use of pre-transplant HCC-directed treatment was recorded and included the use of trans-arterial chemoembolization (TACE), radio-frequency ablation (RFA), surgical resection, alcohol ablation and trans-arterial radioembolization (TARE). Loco-regional treatments were used liberally in all eligible patients.

Outcome variables included the date and cause of waitlist drop-out, post-transplant recurrence and death. Waitlist HCC monitoring included AFP and contrast enhanced triphasic CT or MRI assessments every three months, and 6 monthly bone scans. Post-

transplant HCC monitoring included AFP, and CT or MRI imaging every six months for the first two years and yearly imaging thereafter.

Analysis

In order to assess the value of candidate selection based on the TTV/AFP score, patients within Milan criteria (and AFP <400 ng/ml) were used as the group of reference (3), and were compared to those beyond Milan, but within the TTV/AFP criteria. Patients within Milan included patients with a single tumor up to 5 cm in diameter or up to 3 tumors none larger than 3 cm (4). Of note, given the prospective study inclusion requirements included an AFP <400 ng/ml, the inclusion criteria excluded patients within Milan by morphological criteria but with AFP >400 ng/ml. As such, the "within Milan criteria" group in this prospective series was more highly selected than in standard practice, and so might be expected to have a better outcome. The two groups were compared regarding demographics, waitlist drop-out, intent-to-treat survival (from listing), post-transplant survival and post-transplant HCC recurrence. HCC characteristics of patients within vs beyond Milan were also represented graphically at listing and transplant as previously suggested (14).

In an effort to capture the impact of HCC changes on post-transplant survival, patients were assessed at their peak levels and at the time of transplantation according to Milan criteria (within vs. beyond), TTV/AFP criteria (within vs. beyond), and AFP (≤ 400 ng/ml vs. >400 ng/ml). Patients stable under the cut-off were compared to those downstaged from over the cut-off (peak) to below the cut-off at the time of transplant.

Results were provided as mean ± standard deviation or median ± inter-quartile range (IQR) according to variable normality, which was assessed graphically. Groups were compared with the use of Student-t test, or Mann-Whitney test, and Chi-square test, or Fisher test according to normality. Survivals were displayed according to Kaplan Meier, and groups were compared with log-rank test. Standard alpha level of 0·05 indicated statistical significance. Analyses were conducted using SPSS 18·0 (SPSS, Chicago, IL).

Results

Listing characteristics

During the study period, 233 patients with HCC were listed for liver transplantation (Table 1). They included 43 females and 190 males, with a mean age of 57,1 ±6,4 years. Cirrhosis was most often related to hepatitis C virus (HCV), hepatitis B virus (HBV) and alcohol. The mean calculated model for end-stage liver disease (MELD) score was 9,9 ±4,9.

At the time of listing, 195 patients were within Milan criteria and 38 beyond Milan, but within TTV/AFP. Most patients had a limited number of small HCCs; the median AFP was 11 ±25 ng/ml (Table 1, Figure 1A).

Risk of drop-out and intent-to-treat outcome

Follow-up from listing was 33,9 ±24,9 months (median: 27,4 months, IQR: 29,8). At the time of writing, 166 (71%) patients had been transplanted, 65 (27%) had dropped from the list, one (1%) had been removed from the list (treated by TACE, without sign of recurrence after several years), and one (1%) was still active on the list.

Drop-out was related to HCC progression (n=34), non-HCC related death (n=10) and deterioration of medical conditions considered beyond transplantable state (n=21). The overall risk of drop-out was significantly higher for patients beyond Milan but within TTV/AFP (16/38, 42,1%) than for patients within Milan (49/195, 25,1%, p=0.033). HCC progression was the most frequent cause of drop-out in both groups, and was nearly twice as frequent in patients beyond Milan but within TTV/AFP vs patients within Milan (9/38, 21% vs 25/195, 12.8%, p=0.18, log-rank: p=0.082). Other causes of drop-out included

non-HCC related deaths (4/38, 10.5% vs. 6/195, 6.3%, p=0.038), and deterioration of medical conditions considered beyond transplantable state (2/38, 5.3% vs 6/195, 6.3%, p=0.5). Six and 12 months after listing, the rates of drop-out were 8,4 ±7,4 and 56,2 ±10,9% beyond Milan, but within TTV/AFP, and 10 ±2,3 and 18,9 ±3,4% within Milan (Figure 2A, log-rank, p<0,001). As expected from the drop-out rates, patients beyond Milan, but within TTV/AFP had significantly lower intent-to-treat survivals from listing than those within Milan (four-year survivals 53,8 ±10,1% vs. 71,6 ±3,9%, p<0,001, Figure 2B).

Transplant characteristics and outcome

Mean waiting time was 11,9 ±11,7 months (median: 8, IQR: 11), and was similar between patients within Milan vs. beyond Milan, but within TTV/AFP (12.4 ±12.3 vs 10.5 ±8.7 months, p=0.40). Most patients underwent loco-regional treatment during this time, including TACE (n=135 patients), RFA (n=91), alcohol ablation (n=25) and surgical resection (n=15). The use of these treatments was not distributed differently between patients listed within Milan (169/195, 86,7%) and those beyond Milan, but within TTV/AFP (36/38, 94,7%, p=0,162). Of the 15 patients with surgical resection, 11 had recurrent HCC present on pre-transplant imaging. Overall, both HCC size and number remained stable from listing until transplantation (in part because 33 patients with irreversible progression of TTV or AFP were dropped out), and most patients presented HCCs with only a limited expansion from Milan criteria (Table 1, Figure 1B). Of the transplanted patients, 56 patients demonstrated a complete pre-transplant radiological response, including 50/134 (37.3%) patients within Milan, and 6/32 (18.8%)

patients beyond Milan, but within TTV/AFP (0.046). Of all 56 patients with complete radiological response, 24 (43%) demonstrated no residual viable HCC on the explant.

Of the 166 transplanted patients, 134 were within Milan and 32 beyond Milan, but within TTV/AFP at the time of transplantation. As expected, patients beyond Milan had larger and more numerous HCCs, and a trend towards higher AFP (Table 2). Of the 134 patients transplanted within Milan, 31 (23%) demonstrated HCCs beyond Milan on explants, most often because of a number of HCCs >3.

Post-transplant follow-up was 30 ±22,1 months (median: 23,2, IQR: 32). Nine patients had recurrences, 8,5 to 45 months after transplantation, for a rate of recurrence of 5,4%. The most frequent sites of recurrence were the liver (n=4), lung (n=3), bone (n=1), spleen (n=1), peritoneum (n=1) and abdominal wall (n=1). Six patients with recurrence were within Milan (6/134, 4,5%) and three were beyond Milan but within TTV/AFP (3/32, 9,4%, p=0,272).

Post-transplant overall survivals were similar between patients within Milan (and with AFP <400 ng/ml), and patients beyond Milan, but within TTV/AFP (two-year survivals: 86,1 ±3,5% vs 83,4 ±3,8%; four-year survivals: 78,7 ±4,9 vs 74,6 ±10,3%, p=0,932, Figure 2C). Post-transplant disease-free survivals were also similar between patients within Milan, and patients beyond Milan, but within TTV/AFP (two-year survivals: 83,4 ±3,8% vs 87,9 ±6,6%; four-year survivals: 77,9 ±4,7 vs 68,0 ±11,3%, p=0,930).

Impact of TTV and AFP downstaging

In order to assess the impact of downstaging, post-transplant survival was assessed according to the maximum observed tumor volumes and AFP. Patients beyond Milan at

any time, but downstaged to within Milan at the time of transplant (n=27) had similar post-transplant disease-free survivals as those continuously within Milan (n=101, two-year survivals: 87,6 ±6,7% vs 81,7 ±4,5%; four-year survivals: 76,6 ±11,8 vs 78,1 ±5%, p=0,753, Figure 3A). Two recurrences appeared in patients downstaged to Milan (2/27, 7,4%), and four in patients continuously within Milan (4/101, 4%, p=0,452).

None of the 12 patients downstaged from beyond TTV/AFP and stabilized within TTV/AFP demonstrated a recurrence after a mean follow-up of 28,2 ±18,4 months (7 patients were originally with AFP> 400 ng/ml and 5 were originally with TTV> 115 cm^3). They had similar post-transplant disease-free survivals as those continuously within TTV/AFP (n=154, two-year survivals: 100% vs 83,1 ±3,5%; four-year survivals: 100% vs 74,5 ±4,6%, p=0,140, Figure 3B).

Seven of these patients had high peak AFP values between 412 and 5845 ng/ml and were successfully downstaged and stabilised at ≤400 ng/ml until the time of transplant. They were all alive and free of HCC recurrence after a mean follow-up of 29,5 ±18,7 months, and with similar post-transplant disease-free survivals as patients with AFPs continuously ≤400 ng/ml (two-year survivals: 100% vs 83,7 ±3,6%; four-year survivals: 100 vs 74,7 ±4,8%, p=0,252, Figure 3C).

Discussion

The present prospective study suggests that HCC liver transplant candidate selection could be expanded to the TTV/AFP criteria in centers with at least 8-month median waiting times.

These results confirm previous studies demonstrating similar post-transplant outcomes with the use of the TTV/AFP score as with Milan criteria, despite the inclusion of approximately 20% more patients (6, 9, 10). With a TTV cut-off of 115 cm3, any HCC size and number combination can be used, including patients with one HCC ≤6 cm, two HCCs ≤4,8 cm or three HCCs ≤4,2 cm. While some 30% more patients overall could be included based on HCC size and number compared to Milan, all patients with high AFPs (>400 ng/ml) were excluded, an important factor as these patients have been shown to have poor outcomes even when within Milan (15). This likely explains the very low tumor recurrence rate of 4,5% in the "Milan" group in the present study. Our group of patients beyond Milan, but within TTV/AFP would compare even more favorably to a standard Milan group unselective for AFP.

Of note, more patients were listed within Milan criteria (n=195) compared to the number of patients beyond Milan, but within TTV/AFP (n=38). Although we cannot exclude that some patients beyond Milan may have not been referred, or were missed internally, we consider this unlikely from the policy of discussing all stages of HCC in our multidisciplinary meetings. The balance between both groups is similar to the one of a previous population-based study (6), and probably corresponds to the fact that in units

serving a population where screening for HCC is well implemented, more patients are caught within Milan and with an AFP < 400, than beyond Milan, but within TTV/AFP. The originality and added value of the TTV/AFP score also lays in the absence of a strict cut-off by number of HCC, and a greater weighting for tumors of larger size. This leads to a more stringent selection of patients based on radiology, as HCC size (radius) is cubed in the calculation of volume, and larger lesions, unequivocally characterised by radiology, have more weight in the score (10). In other words, small, less than 1-cm lesions have minimal weight in including/excluding an individual from transplantation. Informal discussions with members of leading units reveal that ignoring a fourth or even a fifth lesion that is <1 cm is a relatively common practice in centers currently following "Milan" criteria.

In practice, the current use of TTV/AFP criteria corresponds to the modest expansion of the "up to 7 without vascular invasion" advocated by the Metroticket study (1), by integrating similar size-volume limits, and by adding AFP as a surrogate marker for vascular invasion. The idea to combine morphological and biological factors has recently gained attraction with several centers reporting retrospective results of liver transplantation for patients with HCC (16-19). AFP >1000 ng/ml has been incorporated with morphological criteria by the UCSF liver transplant program (18).

The expansion of morphological criteria due to the use of the TTV/AFP score led to higher rates of drop-out from the list and lower intent-to-treat survival rates. However, post-transplant outcomes were similar to what is currently accepted for non-HCC patients, suggesting non-HCC patients would not be penalized unfairly by the expansion in patients with HCC eligible for a graft. Because of the potential importance of the

waiting time to exclude patients with an aggressive tumor phenotype, the use of the TTV/AFP score cannot currently be recommended to centers with shorter waiting times than ours (close to 8 months). While waiting for specific data, a similar delay should also be advocated in case of living-donor liver transplantation, possibly in combination with aggressive loco-regional therapy: it is likely that the waiting time, before or after listing, allows exclusion of the more biologically unfavorable HCCs, especially within the group beyond Milan, and that early transplantation could worsen post-transplant outcomes (20). The present study included an aggressive wait-list loco-regional HCC management (33.7% of the transplanted patients demonstrated a complete radiological response), which might have contributed to the low incidence of post-transplant HCC recurrence. This policy also provided data on downstaging, as patients initially beyond the TTV/AFP criteria could be listed, provided they had been downstaged to within the limits and stabilized for a minimum of three months. Successfully downstaged patients with high peak AFPs (>400 ng/ml) had similar post-transplant HCC-free outcomes as those with stable low (≤400 ng/ml) AFPs. This observation, if confirmed, would validate previous registry-based data, suggesting that patients with transplant AFPs ≤400 ng/ml do well after transplantation, whatever the highest AFP level (15). Similarly, patients downstaged based on Milan criteria also demonstrated similar outcomes as those continuously within Milan. These data supports previous retrospective observations, and further reinforce the value of downstaging and the “ablate and wait” strategy (11, 21, 22).

Altogether, the present prospective study shows that an expansion of HCC liver transplant candidate selection criteria to the TTV/AFP criteria achieves post-transplant

tumor-free survival equivalent to Milan criteria while allowing a 20% increase in the number of eligible patients. This observation was made in centers with median wait times of 8 months, and was accompanied by an increased rate of waitlist drop-out in the expanded group.

We consider that the waitlist dropout as part of the selection process to achieve good long term results is a fair price to pay for equity in access to liver transplantation between HCC candidates and non-tumor candidates alike. The current report includes the first prospective series of patients transplanted after selection based on such combined morphological, biomarker and tumor-evolution criteria and argues for such a policy change.

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Figure legends

Figure 1: Radiologically-assessed number of HCC and size of largest HCC for patients at listing (A, with or without drop-out for HCC progression) and at transplant (B, with or without post-transplant HCC recurrence). Grey lines show the size / number limits of Milan criteria. Some dots represent more than one patient.

Figure 2: Drop-out rate (A, $p<0.001$), intent-to-treat (B, $p<0.001$) and overall (C, $p=0.378$) survivals for patients within Milan and beyond Milan, but within TTV/AFP

Figure 3: Impact of Milan (A), TTV/AFP (B) and AFP (C, cut-off 400 ng/ml) downstaging on post-transplant disease-free survival.

Table 1: Patient and tumor characteristics at listing and transplant

		Listing	Transplant	*p*
Patients (number)		233	166	
Mean age (years ±SD)		57.1 ±6.4	57.6±6.5	0.41
Gender (ratio)		female:43/ male:190	female:25/ male:141	0.37
Cause of liver disease (%)				
	HCV (±alcohol, ±HBV)	142 (60)	102 (61)	0.912
	HBV	28 (12)	20 (12)	0.99
	Alcohol	35 (15)	24 (14)	0.878
	NASH	12 (5)	8 (5)	0.883
	Hemochromatosis	5 (2)	4 (2)	0.86
	Other	14 (6)	10 (6)	0.993
Mean MELD score (±SD)		9.9 ±4.9	11.5±5.9	0.004
Median largest HCC diameter (cm ±IQR)		1.7 ±2.8	1.3 ±2.6	0.533
Median Total Tumor Volume (cm^3 ±IQR)		3.5 ±12.8	1.4 ±10.9	0.787
Median an number of HCC (±IQR)		1 ±2	1 ±2	0.437
Median serum alpha fetoprotein level (ng/ml ±IQR)		11 ±25	9 ±22	0.944

HCV: hepatitis C virus infection, HBV: hepatitis B virus infection
MELD: Model for End-Stage Liver Disease
NASH: non-alcoholic steato-hepatitis, HCC: hepatocellular carcinoma

Table 2: Radiological pre-transplant characteristics within Milan and outside Milan,but within TTV/AFP

	Within Milan	Beyond Milan, but within TTV/AFP	*p*
Patients (number)	134	32	
Median largest HCC diameter (cm ±IQR)	1 ±1.9	3.1 ±2	<0.001
Median Total Tumor Volume (cm^3 ±IQR)	0.5 ±4.2	21 ±43	<0.001
Median number of HCC (±IQR)	1 ±1	3 ±3	<0.001
Median serum alpha fetoprotein level (ng/ml ±IQR)	9 ±13	27 ±39	0.071

TTV: total tumor volume, AFP: alpha foeto-protein, HCC: hepatocellular carcinoma

Figure 1

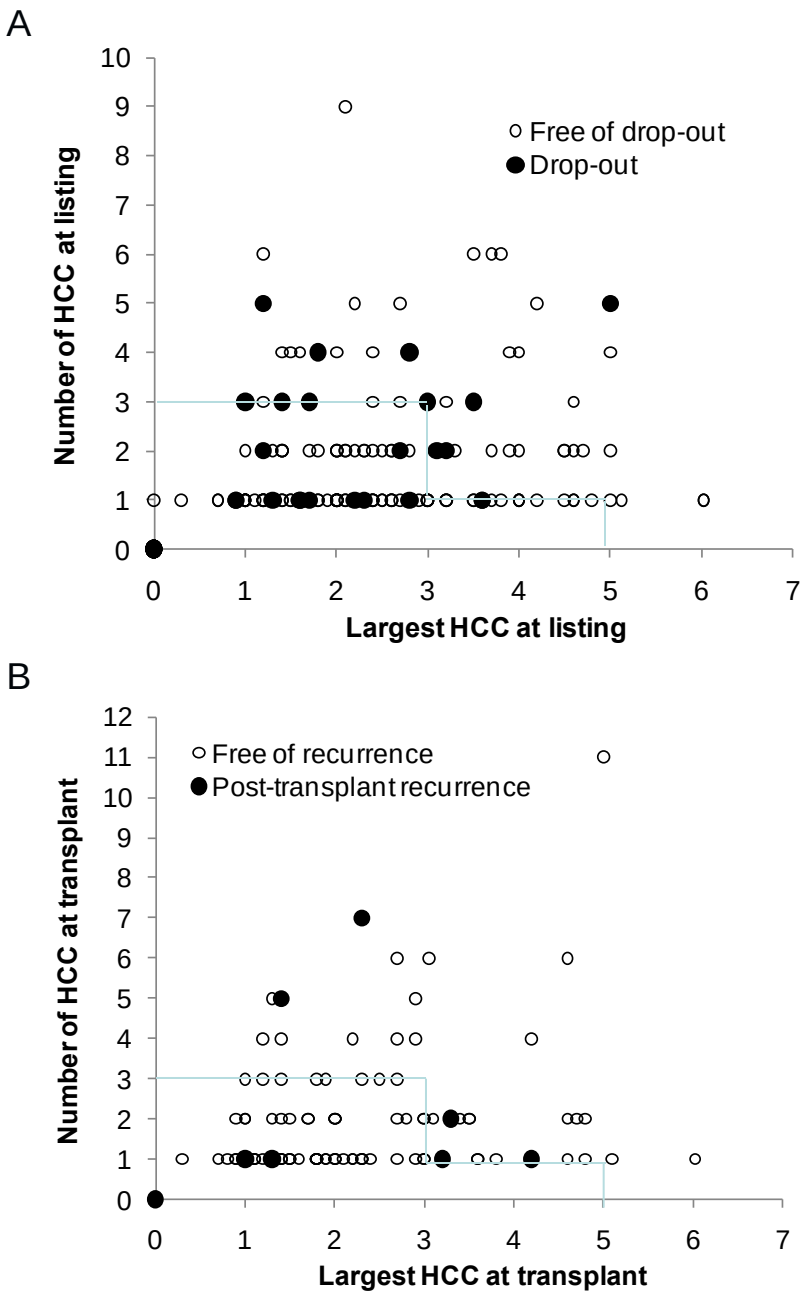
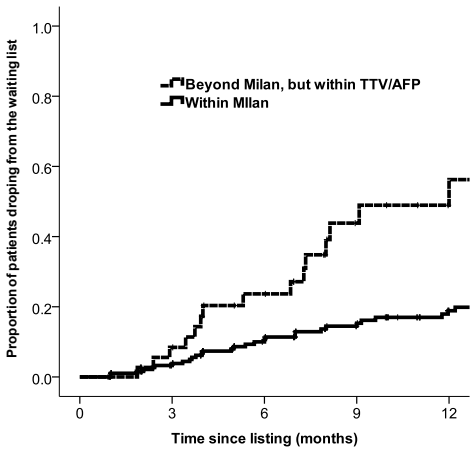
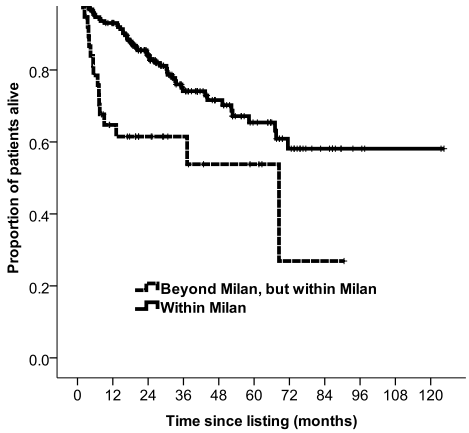


Figure 2

A



B



C

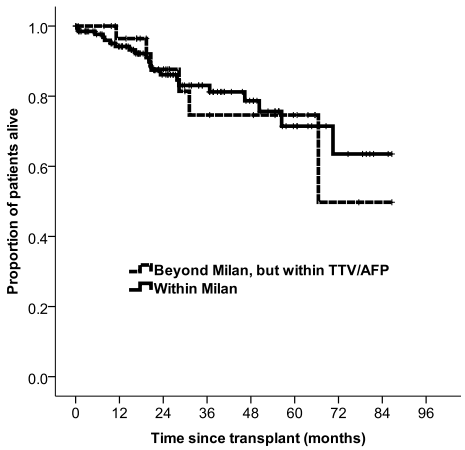


Figure 3