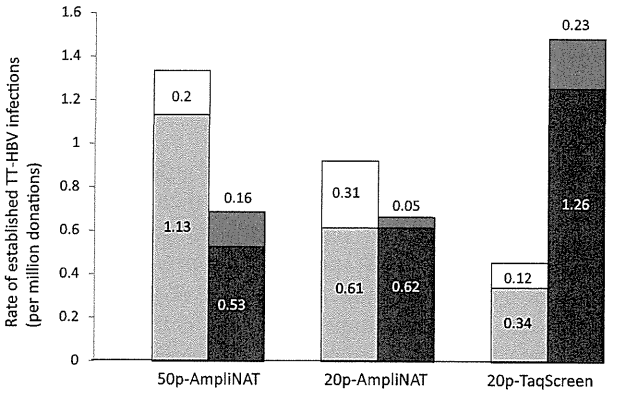


Fig. 2. Number of established TT-HBV infections grouped according to pool-based NAT screening systems. See Table 2 for intervals when indicated NAT systems were applied, sensitivities of NAT systems used, and actual yields for each category at each interval. (□, ▨) Infections caused by transfusion with ID-NAT–negative and -positive WP-derived components, respectively. (■, ▩) Infections caused by transfusion with ID-NAT–negative and -positive OBI-derived components, respectively.

lished TT-HBV infections relative to the three periods. The rate of infections caused by transfusion with a WP-derived component notably decreased with increasing NAT sensitivity, but that caused by transfusion with OBI-derived components rather increased despite the increased NAT sensitivity. Current NAT screening protocols indicated that TT-HBV infections occur more frequently due to transfusion with OBI- than with WP-related components (1.49/million vs. 0.46/million donations; Fig. 2). Nine TT-HBV infections occurred as a result of transfusion with blood components containing more than 10 mIU/mL anti-HBs during the past decade (Table 2). Two of them were caused by donations with negative ID-NAT.

The number of TT-HBV infections caused by transfusion with ID-NAT–negative components accounts for 15% (2/13) and 25% (1/4) of OBI- and WP-related TT-HBV infections, respectively, according to the current NAT system (Table 2). These infections involving ID-NAT–negative donations were determined as TTI by analyzing repository blood samples obtained before the index donation and/or by following up with the implicated donors after the index donation. Details of the clinical course of a typical TT-HBV infection caused by ID-NAT–negative OBI-related blood components are shown in Tables 3 and 4.

Impact of blood product on transmission rate

Table 5 shows the numbers of implicated donations by either ID-NAT negative or positive for groups categorized by the type of component and WP/OBI status. During the past decade, ID-NAT–positive donations have caused 79 TT-HBV infections. Transfusion with red blood cells (RBC), fresh-frozen plasma (FFP), and platelet concentrate (PC) was associated with infections in 42, 22, and 15 of them, respectively. Of 19 TT-HBV infections associated with ID-NAT–negative donations, 2, 4, and 13 were caused by transfusion with RBCs, FFP, and PC, respectively. Transfusion with blood components containing a larger plasma volume (FFP and PC, but not RBCs) caused more frequent TT-HBV infections among patients who received ID-NAT–negative donations (17/19, 89%) than among those who received ID-NAT–positive donations (37/79, 47%; $p < 0.01$), which could be a reflection of the plasma volume effect on infectivity.

Table 5 also shows that if ID-NAT had been implemented during the screening, 81% of established TT-HBV infections would have been avoided. The introduction of ID-NAT would have been the most (95%) and least (54%) effective for preventing TTI caused by RBC- and PC-related transfusions, respectively. Under the current 20p-TaqScreen system, 75 and 85% of TT-HBV infections arising from WP and OBI donations, respectively, are ID-NAT positive and will be interdicted by ID-NAT. In particular, the effect of ID-NAT will be 100% for OBI-related infections caused by RBC transfusion.

Outcomes of patients with TT-HBV infection

ALT levels during TT-HBV infection were determined in 68 transfusion recipients who developed TT-HBV infection. Table 6 shows the maximal ALT values relative to WP or OBI donations and ID-NAT–positive or -negative donations. Almost half (47%, 32/68) of the patients had maximal ALT values of more than 1000 IU/L. The proportion of patients with maximal ALT of more than 1000 IU/L was greater in OBI-related (61%, 19/31) than in WP-related (35%, 13/37; $p < 0.05$) infections. Total viral load in the implicated components did not significantly differ between patients with ALT values above and below 1000, which was true for both WP- and OBI-related infections. Although barely insignificant, total infused viral load tended to be lower in OBI- than in WP-related patients among groups with maximal ALT values of more than 1000.

Three patients with TT-HBV infection died of fulminant hepatitis after the introduction of NAT. One was caused by transfusion with PC derived from an ID-NAT–negative, WP-related donation (Genotype A with wild type precore region). The other two developed hepatitis after transfusion with RBCs derived from ID-NAT–positive, OBI-related donations (Genotype B with a G1898A precore mutation and Genotype C with a G1896A precore mutation).

ID-NAT screening trial in donations with low anti-HBc and anti-HBs titers

During a 6-month ID-NAT trial, 4742 (0.74%) of 640,628 blood donations at the Tokyo Blood Center with low anti-

HBc and anti-HBs titers were analyzed by ID-NAT. The number of donations analyzed by ID-NAT decreased as the anti-HBc titer increased (Fig. 3). HBV DNA was detected in 92 (1.94%) of the 4742 donations. Figure 4 shows the frequency of ID-NAT–positive donations relative to the anti-HBc titer. The frequency of ID-NAT positivity for HBV did not correlate with the anti-HBc titer and did not tend to increase with an increasing anti-HBc titer. The proportions of anti-HBs–positive (>10 mIU/mL) donations among those that were ID-NAT positive and negative were 77 and 75%, respectively, and did not significantly differ. The proportion of anti-HBs–positive donations increased with increasing anti-HBc S/CO values among ID-NAT–negative donations (67.5, 82.0, and 87.5% for anti-HBc S/CO 1.0-3.9, 4.0-7.9, and 8.0-11.9, respectively; $p < 0.01$ between any two groups). The frequency of ID-NAT positivity between males (1.8%) and females (2.4%) did not significantly differ. Eighty-three (90.2%) of the 92 ID-NAT–positive donors were at least 50 years of age. Fifteen had a viral load of less than 100 copies/mL, whereas quantitative NAT could not detect HBV DNA loads in samples from the remaining 77. The distribution of HBV genotypes among the ID-NAT–positive donations did not differ from that among the general Japanese population: Genotypes A, B, C, and D, n = 1, 24, 45, and 1, respectively (21 were undetermined).

TABLE 3. Representative TT-HBV infection caused by OBI-derived, ID-NAT–negative blood component: clinical course of a patient who received an implicated blood component.

Date	Clinical events and test results
Nov. 10, 2008	Surgery to treat head injury*
	HBsAg negative, anti-HBc negative, HBV DNA negative, preoperatively
	Transfused until Nov. 20 with 21 RBC units, 5 PCs, and 11 FFP† including one derived from the donation of Mar. 27, 2008, shown in Table 4
Mar. 05, 2009	AST 15, ALT 32
Mar. 25, 2009	AST 517, ALT 1273
Mar. 30, 2009	AST 1312, ALT 3110, HBsAg positive, IgM-anti-HBc positive
	Reported to JRC blood center
Mar. 31, 2009	AST 695, ALT 2396
Apr. 01, 2009	HBsAg negative, anti-HBs positive, HBV DNA positive

* Recipient was a teenage boy who was injured in a traffic accident.
† HBV DNA was not detected based on ID-NAT for the repository samples from these 37 blood components transfused. These results were obtained in the first lookback study performed in April 2009.

Estimation of current TT-HBV risk in Japan

From the frequency of ID-NAT–positive (1.94%) donations among those with low anti-HBc and anti-HBs titers (69,000/year or 13,000/million; see Table 1 and below), we calculated that 1339/year or 252/million donations should be ID-NAT positive among screening NAT-negative donations with low anti-HBc and anti-HBs titers. Using an infectivity rate of 3%[7] among components derived from OBI donations that were screening NAT negative and ID-NAT positive, we calculated that 40/year or 7.6/million OBI-related TT-HBV infections should arise. If TT-HBV infections related to OBI-derived ID-NAT-negative donations are taken into account, then the total number of TT-HBV infections should be 47/year or 8.9/million. This estimate was based on the observation that TT-HBV infection caused by ID-NAT–negative components during the

TABLE 4. Representative TT-HBV infection caused by OBI-derived, ID-NAT–negative blood component: HBV marker profile of blood donor responsible for the outcome shown in Table 3

Date of donation	Date of testing	Test results
Oct. 17, 2007*	Oct. 17, 2007 (screening)	Pool NAT negative, anti-HBc 2^4 (negative), anti-HBs negative
	Feb. 24, 2010 (repository sample tested in second lookback study)	ID-NAT negative
Mar. 27, 2008† (index donation)	Mar. 27, 2008 (screening)	Pool NAT negative, anti-HBc 2^4 (negative), anti-HBs negative
	Apr. 7, 2009 (repository sample tested in first lookback study)	ID-NAT negative (negative result reported to corresponding facility)
Feb. 05, 2010‡	Feb. 05, 2010 (screening)	Pool NAT negative, anti-HBc 15.4 S/CO§ (positive), anti-HBs negative
	Feb. 10, 2010 (donated blood sample tested in second lookback study)	ID-NAT positive (high probability of TT-HBV infection in Patient A reported to corresponding facility)

* RBCs derived from this donation were transfused to an HBsAg-negative patient. Patient continued to be HBsAg-negative until May 2008 when he died.
† FFP derived from this donation was transfused to patient shown in Table 3. Cocomponent (RBCs) processed from this donation was transfused to a patient who died of the primary disease soon after transfusion. Whether TT-HBV occurred remains unknown.
‡ This donation was rejected due to anti-HBc seroconversion and a second lookback study was conducted on the donation of October 17, 2007.
§ Because of very low HBV load in donated blood sample of February 5, 2010, HBV sequence was assessed in donor blood only at 193 bp (Nucleotides 475-667) of S region. HBV sequence in that region was identical except for nt. 654 between the blood samples from donor and patient on April 01, 2009.

TABLE 5. Blood components implicated in established TT-HBV infection*

	ID-NAT+/ID-NAT−					
	WP transmissions established			OBI transmissions established		
Screening period	RBCs	FFP	PC	RBCs	FFP	PC
50p-AmpliNAT	15/0	6/1	7/4	5/0	5/1	3/3
20p-AmpliNAT	8/2	0	4/4	7/0	5/1	0
20p-TaqScreen	2/0	0	1/1	5/0	6/1	0/1
Total	25/2	6/1	12/9	17/0	16/3	3/4

	WP plus OBI				All components		
	RBCs	FFP	PC	FFP + PC	WP	OBI	Total
50p-AmpliNAT	20/0	11/2	10/7		28/5	13/4	41/9
20p-AmpliNAT	15/2	5/1	4/4		12/6	12/1	24/7
20p-TaqScreen	7/0	6/1	1/2		3/1	11/2	14/3
	100%	86%	33%		75%	85%	82%
Total	42/2	22/4	15/13	37/17	43/12	36/7	79/19
	95%	85%	54%		78%	84%	81%

* Ratios (%) in the two bottom rows represent rates of ID-NAT–positive events or effectiveness of ID-NAT implementation.

TABLE 6. Maximal values for ALT in patients with TT-HBV infection and total viral load contained in implicated components

	ALT	
	<1000	>1000
WP* (n‡)	24 (7)§	13
OBI† (n‡)	12 (2)§	19
Total viral load (copies/bag)‖		
WP		
n‡	21	10
Min	40	100
Max	260,000	560,000
Median	1,400	9,100
Mean	20,460	74,790
OBI		
n‡	8	16
Min	60	40
Max	6,240	19,200
Median	630	1,470
Mean	1,440	3,750
ID-NAT status		
Positive‡	30	28
Negative‡	6	4
Component types		
RBCs‡	19	16
FFP‡	7	13
PC‡	10	3

* Patients transfused with WP-related components include 11, 8, and 18 patients with malignant hematologic disorder, solid tumor, and others, respectively.
† Patients transfused with OBI-related components include 7, 11, and 13 patients with malignant hematologic disorder, solid tumor, and others, respectively.
‡ Numbers of patients.
§ Numbers in parentheses, patients with maximal ALT values of less than 100 IU/L.
‖ Total viral load was calculated using viral concentrations in implicated donations and average plasma volume of each component type. When viral load was less than 100 copies/mL, total viral load in the component was calculated assuming that viral concentration is logarithmically distributed between 1 and 100 copies/mL.

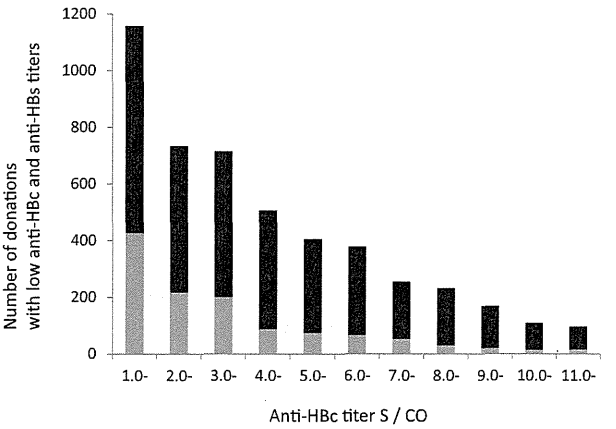


Fig. 3. Number of donations screened by ID-NAT trial categorized by anti-HBc titer. All donations tested had low anti-HBc (S/CO 1.0-11.9) and anti-HBs (<200 IU/L) titers and were qualified serologically based on algorithm applied at JRC blood centers. Donations verified to be ID-NAT-positive were disqualified. (■, ▨) donations with anti-HBs titers of more than and not more than 10 mIU/mL, respectively.

20p-TaqScreen period accounted for 15% (2/13) of all OBI-related infections (Table 2).

We estimated how many more WP-related TT-HBV infections would be prevented by introducing ID-NAT. The current screening NAT yield (30 donations/year or 5.7/million, Table 2) was multiplied by the ratio of the interval between ID-NAT and 20p NAT detection (11.2 days) to that between 20p NAT and HBsAg detection (9.7 days). We then deduced that 34.6/years or 6.6/million more viremic donations would be captured by ID-NAT. The number of ID-NAT–negative WP donations was calculated separately for each component type. Based on the plasma volume of each component (20, 200, 240, 450, and 120 mL for RBCs, PC, FFP-3, FFP-5, and FFP1.5,

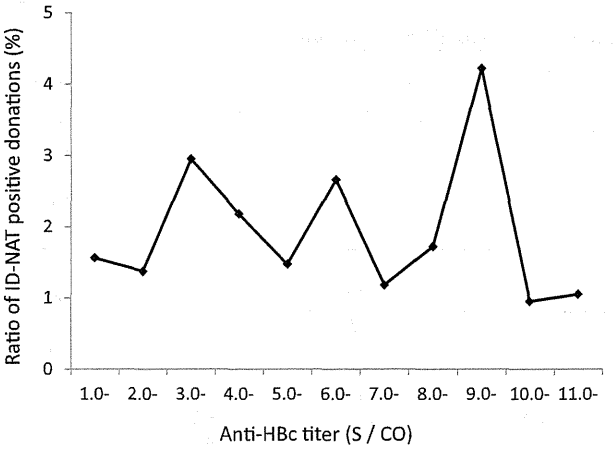


Fig. 4. Ratios (%) of ID-NAT–positive donations with low anti-HBc and anti-HBs titers relative to anti-HBc titer.

respectively), the deduced intervals between 1 copy/bag and ID-NAT detection were 16.3, 24.9, 25.5, 28, and 23 days, respectively. The ratio of the number of those components issued to hospitals is 6.3:2.2:2.1:0.7:0.1. The incidence of ID-NAT–negative WP donations calculated from these data was 59.5/year or 11.4/million. Adding ID-NAT–positive WP donations (34.6/year or 6.6/million), current risk related to WP donations amounts to 94.1/year or 18.0/million. The effect of ID-NAT on the reduction of all WP donation would be 37% (6.6/18.0). If the infectious risk (50%) of ID-NAT–positive, screening NAT-negative WP-related components is also applied to ID-NAT–negative WP-related components, the total number of WP-related TT-HBV infections would be 47.1/year or 9.0/million. Together, these estimates for WP- and OBI-related TT-HBV infections indicate that 94.1/year or 17.9/million TT-HBV infections are likely to occur in Japan.

DISCUSSION

Infection with HBV results in a wide spectrum of clinical manifestations ranging from asymptomatic liver dysfunction with only slightly elevated transaminase levels or acute self-limiting hepatitis to chronic hepatitis that in some patients progresses to cirrhosis, liver failure, or hepatic cell carcinoma. In rare cases, HBV infection can cause fulminant hepatitis that is associated with high mortality. Fulminant hepatitis in Japan is frequently associated with primary infection by HBV carrying precore or core-promoter mutations.[12,13] These HBV mutants are frequently found among chronic HBV carriers[14,15] who typically have an anti-HBc–positive serostatus. To prevent fulminant hepatitis arising as a result of blood transfusion,[16] the JRC incorporated anti-HBc testing into blood screening in 1989.

The agglutination method had been used for all serologic testing at JRC blood centers before 2008. Although this method was somewhat insensitive to HBsAg, it could semiquantify anti-HBc. Thus, the cutoff point for the anti-HBc titer had been set at 2^6, and donations with an anti-HBc titer of at least 2^6 and an anti-HBs titer of less than 200 mIU/mL were disqualified.[17] Although this anti-HBc testing had essentially prevented transfusion-transmitted fulminant hepatitis since 1989,[18] reports of fulminant or acute severe hepatitis continued for an additional 7 years. These conditions were attributed to transfusion with components with a 2^5 anti-HBc titer.[19] Consequently, the JRC lowered the anti-HBc cutoff from 2^6 to 2^5 in 1997. The agglutination method for serologic screening was replaced in 2008 with CLEIAs, which can also semiquantify anti-HBc. The policy described above is maintained in the algorithm for HBV screening with CLEIA; the range defined as a low anti-HBc titer includes S/CO values between 1.0 and 11.9, and donations with anti-HBc S/CO values within this range are currently accepted.

The highly sophisticated strategy of multiplex NAT was designed to decrease the incidence of TTI. Implementing HBV NAT into blood screening was important in Japan mainly because of the unsatisfactory sensitivity of the standard agglutination method to HBsAg. The JRC implemented multiplex NAT targeting HBV DNA, HIV RNA, and HCV RNA during 1999[6] and improved the sensitivity of the test at three points. The 98 infections described herein had been confirmed as TT-HBV since the introduction of 50p-AmpliNAT in 2000. The HBV genome was not detected in donor repository samples of 587 (74.4%) suspected TT-HBV infections. The JRC has informed the appropriate physicians of the ID-NAT results of viral detection that imply a low or high probability of TT-HBV infection.

In parallel with the increase in the screening NAT sensitivity, the incidence of WP-related TT-HBV infection has decreased as predicted, whereas that of OBI-related TT-HBV infection has not decreased (Fig. 2). To explain the increasing number of OBI-related TT-HBV infections, the increase in the sensitivity of NAT used in JRC laboratories for retrospective studies might have helped to identify TT-HBV infections, thus sustaining the number of OBI-related TT-HBV infections despite improvements in screening NAT sensitivity.[4]

This consideration could encourage the speculation that most of the 587 infection reports that had been excluded from established TTI (Fig. 1) based on negative results from repository samples might have been confirmed as TT-HBV had more sensitive NAT and a larger sample volume been analyzed. With regard to this notion, the outcomes of recent hemovigilance for TT-HBV are described below. During the 20p-Taqscreen period, the JRC received 61 clinical reports of possible HBV-TTI. Seventeen were determined as TTI, among which, three

repository samples were ID-NAT negative. Historical HBV infection was confirmed in 10 patients by retesting pretransfusion samples. Results from HBV tests of posttransfusion samples from five patients were false positive. The possibility of TT-HBV was ruled out in two patients related to ID-NAT–negative donations because repeated blood donations from two of two and three of three implicated donors were not sero- or NAT-converted. The remaining 27 patients related to ID-NAT–negative donations are inconclusive for TTI as follow-up studies have not yet been completed. Some of the 587 reported infections had been confirmed to be associated with passive anti-HBc transfer from infused components. Thus, it is unlikely that a considerable proportion of the infections excluded from the TTI category were real TT-HBV infections.

Among 19 patients with TT-HBV infections associated with ID-NAT–negative donations, 17 (89%) of them were caused by transfusion with FFP or PC that contained a larger plasma volume (120 to 450 mL) than RBCs (20 mL). In contrast, 37 (47%) were caused by FFP or PC among 79 infections associated with ID-NAT–positive donations (Table 5). This finding suggests that because HBV infectivity is extremely high, the relationship between infectivity and plasma volumes contaminated with HBV could only be established in the era of ID-NAT screening when the viral load in the donation is low enough to escape ID-NAT screening. This might explain why we could not previously establish such a relationship using viral loads around the sensitivity of the pool-based NAT system or serology.[7] If ID-NAT is introduced as routine screening, it will prevent 75 and 85% of WP- and OBI-related infections. In particular, all RBC-related TT-HBV could be prevented because of the small plasma volume involved. The finding also suggests that novel viral reduction technologies[20,21] could be an attractive strategy to decrease the incidence of TT-HBV because these technologies are presently more applicable to FFP or PC than to RBC.

The maximal ALT levels of patients with TT-HBV infection showed that transfusion with components harboring an extremely low HBV load that escaped NAT screening is not necessarily associated with mild clinical illness. This seems particularly true for OBI-related infection (Table 6). The frequency with which transfusion causes severe hepatitis (i.e., ALT > 1000) is significantly higher for OBI- than for WP-derived components. Moreover, OBI-derived components tend to cause severe hepatitis despite lower total viral loads compared with those in the WP-derived components. These findings should be further substantiated by analyzing samples from patients that are regularly obtained after transfusion because most of the maximal ALT values described in this article were found after occasional sampling.

Three patients died of TT-HBV fulminant hepatitis caused by transfusion with blood that had escaped NAT screening. Two of them were notably caused by transfusion of OBI-derived RBCs, and the other was caused by an ID-NAT–negative WP donation. Although a larger plasma volume might generally be required to establish TT-HBV infection under the NAT screening system, plasma volume or the total infused viral load might not be determining factors in fulminant hepatitis. Although viral genome mutations such as those in precore or core-promoter regions are frequently associated with the development of fulminant hepatitis in Japan,[12,13] other crucial factors have not clearly been demonstrated despite considerable investigation.

The JRC accepted 5.3 million donations in 2010, of which 4.9% (261,000) was anti-HBc reactive (Table 1), 0.19% (10,000) was rejected because of high anti-HBc and low anti-HBs titers. Another 3.4% (182,000) was accepted because of high anti-HBs titers (≥200 IU/L). The notion that blood components with an anti-HBs titer of more than 100 IU/L are not infectious is generally accepted.[22-25] The relationship between anti-HBs titer and TT-HBV infection will be discussed elsewhere (manuscript in preparation). Importantly, 1.3% of donations (69,000) with low anti-HBc and anti-HBs titers were accepted, and this category included all donations to which OBI-related TT-HBV infections were attributed. Our ID-NAT trial verified that 1.94% of the donations with low anti-HBc and anti-HBs titers were HBV DNA positive.[26] Accordingly, an estimated 1339/year or 252/million viremic OBI donors and 47/year or 8.9/million TT-HBV infections caused by OBI-derived components would be missed by the current screening algorithm. When estimates for WP-related TT-HBV infections are included, the calculated number of TT-HBV infections was 94.1/year or 17.9/million. Whole blood withdrawn from donors in Japan is split into RBCs and FFP, and the total number of components processed averages 23% more than the number of donations. However, because of outdating and rejection by testing or processing problems, the number of components finally issued by JRC becomes almost the same as the number of donations. Therefore, the calculated number of TT-HBV infections was not significantly influenced by the issue of splitting.

The considerable discrepancy between the estimated and established TT-HBV incidence per million (8.9 to 1.49 and 9.0 to 0.46 for OBI-related and WP-related infections, respectively) might be due to the following factors. A clinical manifestation of HBV infection is often unclear in patients transfused with blood components harboring a low viral load and low proliferative ability. Physicians might thus be likely to overlook infection under such circumstances. Medical practitioners are not compliant with national guidelines for lookback investigations. Indeed, only 30% to 40% of transfused patients were reportedly traced for TTI even after the guidelines were established.[27] A considerable proportion of patients who receive blood

transfusions die before TTI evaluation.[28] In fact, when we inquired about the outcomes of transfusions with components containing verified HBV at medical facilities, 99 (42%) of 238 patients who had been transfused with such components had already died (JRC data from 2009 to 2010). The transmissibility of ID-NAT–positive donations might require reevaluation because of the low numbers of patients analyzed in the previous study[7] (30 and 22 for OBI- and WP-related cases, respectively). The fact that a large proportion of elderly patients are immune to HBV due to prior infection might also contribute to the low figure for established TT-HBV and, finally, anti-HBs in cotransfused components neutralizes HBV. Classified WP donation that is anti-HBs positive and could be attributed to possible vaccine breakthrough infection or anti-HBc–negative chronic OBI could also be a factor influencing infectivity. However, we have not encountered any implicated WP donations with anti-HBs among established TT-HBV infections.

Because of the high probability of a residual risk of TT-HBV, novel strategies that reinforce the safety of blood components but do not damage the blood supply should be implemented. Transfusion with ID-NAT–negative infectious components currently cause 15 and 25% of OBI- and WP-related TT-HBV infections, respectively (Table 2), and screening with ID-NAT would interdict 85 and 75% of these infections, respectively (Table 5). With respect to this, the ID-NAT screening of only donations with low anti-HBc and anti-HBs titers that are currently qualified has been suggested.[29] However, screening with ID-NAT might not be as effective as expected. For example, the variability in viral load in individuals with OBI might allow persistent OBI-related TT-HBV infection; some individuals might have an intermittently elevated viral load.[30-33] Such donations could be identified as HBV positive only when the viral load exceeds the detection threshold of ID-NAT screening. Alternatively, the detection of intermittent viremia might reflect the stochastic phenomenon inherent in NAT technology, particularly at very low viral concentrations. Moreover, one report describes a donor in whom viral load increased in blood samples over a period of several years.[34] Nine among 48 blood donations from this donor were ID-NAT positive, and two of four ID-NAT–positive and three ID-NAT–negative blood transfusions had caused TT-HBV infections. The diverse fluctuation of viremia described above has supposedly hindered the efficient detection of viremic donations by pool-based NAT screening,[35] which is predictable even in the event of ID-NAT screening. Table 5 shows that ID-NAT is not sensitive enough in 16% of established OBI-related transmission events although most of those events are caused by FFP or PC transfusions and ID-NAT screened RBC transfusions are relatively safe. Moreover, although viremia is considered undetectable in most individuals with OBI, this assumption might be dependent on the sensitivity of the NAT used; a considerable number of donations might have viremia with a viral load below the ID-NAT detection limit.

Another strategy that might increase the safety of OBI-derived donations could be to accept only those OBI-derived donations with a profile that is safer than the current standards, if such a profile can be found and systematically applied. We initially expected to find that OBI donations with a very low anti-HBc titer would be safer based on ID-NAT. However, the finding from the ID-NAT trial was that the frequency of viremia does not correlate with anti-HBc titers in the range of S/CO 1.0 to 11.9. Therefore, we concluded that the risk of TT-HBV infection will not be mitigated by implementing a strategy that qualifies only donations with very low anti-HBc titers such as S/CO between 1.0 and 3.0.

We speculated during 2003 that more than 4% of donations would be disqualified if the anti-HBc cutoff were set at 2^1, that is, if all donations with low anti-HBc and anti-HBs titers are rejected. We thought that the loss of so many donations would cause catastrophic damage to the blood inventory and thus that cutoff was not implemented. However, based on current data, the number of donations received in 2010 with low anti-HBc and anti-HBs titers was 69,000, which accounts for 1.31% of all donations in Japan. Given this ratio, we consider that to eliminate all donations with low anti-HBc and anti-HBs titers is feasible. We verified that severe hepatitis is caused more often by OBI- than WP-derived blood. The fact that two patients died of fulminant hepatitis related to OBI-related donations is also serious. Rejecting this category of donations would eliminate nearly all those harboring a risk of OBI-related infection.[26] However, a slight, but distinct risk of TT-HBV infection might persist because a small fraction of OBI donors have an anti-HBc titer of less than 1.0 S/CO, and these donors as well as NAT WP donors present a TT-HBV risk.[36] A committee of the Ministry of Health, Labour and Welfare of the Japanese government has just discussed and authorized the implementation of a new policy in which all donations with low anti-HBc and anti-HBs titers would be rejected.

In conclusion, ID-NAT screening of donations with low anti-HBc and anti-HBs titers revealed that nearly 2% of these donations were associated with low-level viremia and that viremia was identified over the entire range of anti-HBc titers. Importantly, anti-HBc titer did not correlate with the frequency of viremia. The elimination of all donations with low anti-HBc and anti-HBs titers would be important to any strategy aimed at preventing OBI-related TT-HBV infections in countries such as Japan that have a slightly elevated HBV prevalence in blood donations. If this strategy is implemented, the only acceptable donors with OBI in Japan will be those with high anti-HBs titers (≥200 IU/L).

ACKNOWLEDGMENT

All data presented herein were obtained by the staff at Japanese Red Cross blood centers who are engaged in blood testing, quality assurance, or liaison with medical facilities.

CONFLICT OF INTEREST

This research did not receive support in the form of grants, equipment, or drugs. The authors have no conflicts of interest regarding this article.

REFERENCES

1. Tanaka J, Koyama T, Mizui M, Uchida S, Katayama K, Matsuo J, Akita T, Nakashima A, Miyakawa Y, Yoshizawa H. Total numbers of undiagnosed carriers of hepatitis C and B viruses in Japan estimated by age- and area-specific prevalence on the national scale. Intervirology 2011;54:185-95.
2. Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, Suzuki K, Miyakawa Y, Yoshizawa H. Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995-2000. Intervirology 2004;47:32-40.
3. Ohnuma H, Tanaka T, Yoshikawa A, Murokawa H, Minegishi K, Yamanaka R, Iizuka HY, Miyamoto M, Satoh S, Nakahira S, Tomono T, Murozuka T, Takeda Y, Doi Y, Mine H, Yokoyama S, Hirose T, Nishioka K; Japanese Red Cross NAT Screening Research Group. The first large-scale nucleic acid amplification testing (NAT) of donated blood using multiplex reagent for simultaneous detection of HBV, HCV, and HIV-1 and significance of NAT for HBV. Microbiol Immunol 2001;45:667-72.
4. Allain JP. Occult hepatitis B virus infection: implications in transfusion. Vox Sang 2004;86:83-91.
5. Raimondo G, Allain JP, Brunetto MR, Buendia MA, Chen DS, Colombo M, Craxì A, Donato F, Ferrari C, Gaeta GB, Gerlich WH, Levrero M, Locarnini S, Michalak T, Mondelli MU, Pawlotsky JM, Pollicino T, Prati D, Puoti M, Samuel D, Shouval D, Smedile A, Squadrito G, Trépo C, Zoulim F. et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. J Hepatol 2008;49:652-7.
6. Mine H, Emura H, Miyamoto M, Tomono T, Minegishi K, Murokawa H, Yamanaka R, Yoshikawa A, Nishioka K; Japanese Red Cross NAT Research Group. High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. J Virol Methods 2003;112:145-51.
7. Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K. Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. Transfusion 2007;47:1197-205.
8. Pharmaceutical and Food Safety Bureau, Blood and Blood Products Division. Guidelines for lookback study for blood products. Tokyo, Japan: Japanese Ministry of Health, Labour and Welfare; 2005. p. 2-26.
9. Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH; NHLBI-REDS NAT Study Group. A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45:254-64.
10. Satake M. Infectious risks associated with the transfusion of blood components and pathogen inactivation in Japan. Int J Hematol 2004;80:306-10.
11. Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K; Japanese Red Cross NAT Screening Research Group. HBV NAT positive [corrected] blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77-86.
12. Kosaka Y, Takase K, Kojima M, Shimizu M, Inoue K, Yoshiba M, Tanaka S, Akahane Y, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. Gastroenterology 1991;100:1087-94.
13. Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, Tsuda F, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann Intern Med 1995;122:241-8.
14. Okamoto H, Yotsumoto S, Akahane Y, Yamanaka T, Miyazaki Y, Sugai Y, Tsuda F, Tanaka T, Miyakawa Y, Mayumi M. Hepatitis B viruses with precore region defects prevail in persistently infected hosts along with seroconversion to the antibody against e antigen. J Virol 1990;64:1298-303.
15. Kurosaki M, Enomoto N, Asahina Y, Sakuma I, Ikeda T, Tozuka S, Izumi N, Marumo F, Sato C. Mutations in the core promoter region of hepatitis B virus in patients with chronic hepatitis B. J Med Virol 1996;49:115-23.
16. Hoofnagle JH, Seeff LB, Bales ZB, Zimmerman HJ. Type B hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. N Engl J Med 1978;298:1379-83.
17. Iizuka H, Ohmura K, Ishijima A, Satoh K, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M. Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. Vox Sang 1992;63:107-11.
18. Japanese Red Cross Non-A, Non-B Hepatitis Research Group. Effect of screening for hepatitis C virus antibody and hepatitis B virus core antibody on incidence of post-transfusion hepatitis. Lancet 1991;338:1040-1.
19. Nozawa Y, Ohto H. An autopsy case of post-transfusion fulminant hepatitis B due to screened blood for anti-hepatitis B core. Kanzo 1993;34:433-6.

20. Klein HG, Anderson D, Bernardi MJ, Cable R, Carey W, Hoch JS, Robitaille N, Sivilotti ML, Smaill F. Pathogen inactivation: making decisions about new technologies. Report of a consensus conference. Transfusion 2007;47:2338-47.
21. McCullough J. Pathogen inactivation: a new paradigm for preventing transfusion-transmitted infections. Am J Clin Pathol 2007;128:945-55.
22. Allain JP, Hewitt PE, Tedder RS, Williamson LM. Evidence that anti-HBc but not HBV DNA testing may prevent some HBV transmission by transfusion. Br J Haematol 1999;107: 186-95.
23. Grob P, Jilg W, Bornhak H, Gerken G, Gerlich W, Günther S, Hess G, Hüdig H, Kitchen A, Margolis H, Michel G, Trepo C, Will H, Zanetti A, Mushahwar I. Serological pattern "anti-HBc alone": report on a workshop. J Med Virol 2000; 62:450-5.
24. Gerlich WH, Wagner FF, Chudy M, Harritshoj LH, Lattermann A, Wienzek S, Glebe D, Saniewski M, Schüttler CG, Wend UC, Willems WR, Bauerfeind U, Jork C, Bein G, Platz P, Ullum H, Dickmeiss E. HBsAg non-reactive HBV infection in blood donors: transmission and pathogenicity. J Med Virol 2007;79:S32-S36.
25. Dreier J, Kröger M, Diekmann J, Götting C, Kleesiek K. Intermittent HBV viremia in an anti-HBc and anti-HBs-positive blood donor. Transfus Med 2005;15:65-6.
26. Stramer SL, Zou S, Notari EP, Foster GA, Krysztof DE, Musavi F, Dodd RY. Blood donation screening for hepatitis B virus markers in the era of nucleic acid testing: are all tests of value? Transfusion 2012;52:440-6.
27. Kino S, Tomoda Y, Itoh Y, Karasaki H, Kasai S. Implementation of pre- and post-transfusion viral marker tests at Asahikawa medical college hospital. Jpn J Transfus Cell Ther 2009;55:21-8.
28. Hollinger FB, Dodd RY. Hepatitis B virus traceback and lookback: factors to consider. Transfusion 2009;49:176-84.
29. Yang MH, Li L, Hung YS, Hung CS, Allain JP, Lin KS, Tsai SJ. The efficacy of individual-donation and minipool testing to detect low-level hepatitis B virus DNA in Taiwan. Transfusion 2010;50:65-74.
30. Weber B, Melchior W, Gehrke R, Doerr HW, Berger A, Rabenau H. Hepatitis B virus markers in anti-HBc only positive individuals. J Med Virol 2001;64:312-9.
31. Bréchot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? Hepatology 2001;34:194-203.
32. Alhababi F, Sallam TA, Tong CY. The significance of "anti-HBc only" in the clinical virology laboratory. J Clin Virol 2003;27:162-9.
33. Levicnik-Stezinar S, Rahne-Potokar U, Candotti D, Lelie N, Allain JP. Anti-HBs positive occult hepatitis B virus carrier blood infectious in two transfusion recipients. J Hepatol 2008;48:1022-5.
34. Inaba S, Ito A, Miyata Y, Ishii H, Kajimoto S, Tanaka M, Maruta A, Saito S, Yugi H, Hino M, Tadokoro K. Individual nucleic amplification technology does not prevent all hepatitis B virus transmission by blood transfusion. Transfusion 2006;46:2028-9.
35. Candotti D, Allain JP. Transfusion-transmitted hepatitis B virus infection. J Hepatol 2009;51:798-809.
36. Liu CJ, Lo SC, Kao JH, Tseng PT, Lai MY, Ni YH, Yeh SH, Chen PJ, Chen DS. Transmission of occult hepatitis B virus by transfusion to adult and pediatric recipients in Taiwan. J Hepatol 2006;44:39-46.

［報告］

Occult HBV carrier からの輸血による急性B型肝炎が強く疑われた１例

香川県赤十字血液センター[1]，関東甲信越ブロック血液センター[2]，
日本赤十字社血液事業本部中央血液研究所[3]
本田豊彦[1]，小河敏伸[1]，佐藤美津子[1]，濱岡洋一[1]，百瀬俊也[2]，内田茂治[3]

The blood transfusion from an occult HBV carrier caused acute hepatitis B virus infection

Kagawa Red Cross Blood Center[1], *Japanese Red Cross Kanto-Koshinetsu Block Blood Center*[2], *Central Blood Institute, Blood Service Headquarters, Japanese Red Cross Society*[3]
Toyohiko Honda[1], Toshinobu Ogo[1], Mitsuko Sato[1], Yoichi Hamaoka[1], Shunya Momose[2] and Shigeharu Uchida[3]

抄　録

Occult HBV carrierが原因と考えられる輸血後急性B型肝炎の１例を報告する。受血者は30代男性。27歳で特発性門脈圧亢進症と診断された。今回，食道離断術と摘脾術を受け，赤血球製剤３本，新鮮凍結血漿10本，血小板製剤１本の輸血を受けた。術前にHBV-DNAは検出されず，HBs抗体・HBc抗体ともに陰性であった。輸血後213日目の検査で急性B型肝炎と診断された。当該事例で使用された血液製剤全ての保管検体のHBV個別NATを施行した結果，新鮮凍結血漿の１検体が陽性であった。受血者血液と供血者血液のHBV-DNA解析により，両者の相同性は高いと判断された。両者のHBV GenotypeはCで，受血者のHBV CP/PreC領域はwild typeであった。この供血者は，当該献血の前後690日間に４回の献血（前１回，後３回）をしているが，当該献血以外は個別NATが陰性であり，HBc抗体弱陽性のoccult HBV carrierと考えられた。当該献血者の新鮮凍結血漿以外の血液製剤からのB型肝炎感染は認められなかった。

Key words: occult HBV carrier, acute hepatitis B,
transfusion-transmitted HBV infection

はじめに

Occult HBV carrier[1]とは，HBs抗原陰性，HBV-DNA陽性で，かつ，ウイルス量が200IU/mL以下の低濃度の感染状態である[2]。Occult HBV carrierからの輸血によるHBV感染は，1979年に初めて報告されている[3]。今回，Occult HBV carrierが原因と考えられる輸血後急性B型肝炎の１例を経験したので報告する

論文受付日：2013年９月６日
掲載決定日：2013年11月19日

症　例

受血者は30歳代男性。27歳時に特発性門脈圧亢進症と診断された。今回食道離断術と摘脾術を受け，赤血球製剤３本，新鮮凍結血漿10本，血小板製剤１本の輸血を受けた。表１に示すように，輸血前にHBV-DNAは検出されず，HBs抗体・HBc抗体ともに陰性であった。輸血後１カ月目までは肝機能異常は認めなかった。輸血199日後の検査で肝機能異常を認めた。輸血213日後の検査では，HBV-DNAは6.8 log copy/mLで，HBc抗体およびIgM-HBc抗体と，HBe抗原が陽性であった（表１）。この時点で急性B型肝炎と診断され，エンテカビルの内服を開始した。

感染経路を特定するために，使用された14本の血液製剤の保管検体でHBV個別NATを施行したところ，新鮮凍結血漿の１検体が個別NAT陽性であった。しかし，この検体ではウイルス量が少なく，コバスTaqMan法で定量下限値（20IU/mL）以下の陽性であり，また，ウイルスのDNA解析もできなかった。

当該献血者（60歳代男性）の遡及調査結果を表２に示す。当該献血の前後690日間に，個別NAT陽性の当該献血を含めて５回の献血があったが，当該献血以外は個別NAT陰性であった。HBc抗体価は，1.3から9.6 C.O.I.と1.0以上12.0未満であった。今回の遡及調査では，該当する献血日が2012年８月以前のため，HBc抗体価は12.0以上が陽性である。輸血によるB型肝炎を減少させるために，2012年８月からは，HBc抗体価は1.0以上が陽性となった。そのため，HBc抗体価1.0以上12.0未満の場合は，従来の陽性と区別して弱陽性とした。HBs抗体価は，CLEIA法で2.0から9.5mIU/mLと低値であった。HBs抗体価は200mIU/mL以上が陽性である。

個別NAT陽性となった当該献血から製造された赤血球製剤は，すでに使用されていたが，この受血者の輸血17カ月後のHBs抗原検査は陰性であった。また，個別NAT陰性となった当該以外の献血からの血液製剤によるHBV感染は認められなかった。

個別NAT陽性の保管検体では，ウイルス量が少なく，HBV-DNAの解析ができなかった。そのため，検体量が確保できる500日後に献血された新鮮凍結血漿製剤を用いてHBV-DNAの解析を行

表１　受血者検査結果

	輸血前	37日後	199日後	213日後
ALT（IU/L）	17	14	73	677
HBV-DNA	陰性	N.T.	N.T.	6.8 log copy/mL
HBs抗体	陰性	N.T.	N.T.	陰性
HBc抗体	陰性	N.T.	N.T.	陽性
IgM-HBc抗体	陰性	N.T.	N.T.	陽性
HBe抗原	陰性	N.T.	N.T.	陽性
HBe抗体	陰性	N.T.	N.T.	陰性

（N.T.検査せず）

表２　遡及調査結果

献血日	個別NAT	RCC	FFP	原料血漿	HBc抗体（C.O.I.）	HBs抗体（mIU/mL）
190日前	陰性	使用済		送付済	1.3	9.5
当該献血	陽性	使用済	当該製品		1.4	4.7
106日後	陰性	使用済		送付済	9.6	7.9
202日後	陰性	使用済		送付済	8.1	4.6
500日後	陰性	使用せず	PCR実施		5.6	2.0

HBc抗体価は12以上が陽性。HBs抗体価は200以上が陽性。

い，受血者と献血者由来のHBV-DNAを比較した。新鮮凍結血漿製剤検体もウイルス量は少なく，PreS/S領域を含むP領域の前半部1,550bp (nt. 2,333-3,215/1-667) はPCRで増幅できなかったので，S領域内の193bp (nt.475-667) について解析・比較した（図１）。新鮮凍結血漿製剤検体５mLから核酸を抽出・濃縮しS領域の増幅を試みたところ，12回行ったうちの１回から増幅産物が得られた。この領域の献血者由来HBV-DNAと受血者由来HBV-DNAの塩基配列 (193bp) を比較したところ，２カ所で相違が認められた。その１カ所がコドン122の，サブタイプ特異抗原基であった。この点突然変異により，サブタイプが両者で異なっているが，サブタイプ自体容易に変異することが報告されている[4]。また，図２に示すように，データベース上で高い相同性を示した８株と塩基配列を比較したところ，献血者由来HBV-DNAと受血者由来HBV-DNAの両者にのみ特徴的な塩基配列が，nt.507，nt.547，nt.554の３カ所に認められた。以上より，当該献血者血液による輸血後B型肝炎であることが強く疑われた。両者のHBV-DNAはGenotype Cであった。献血者検体のCP/PreC領域はPCRで増幅できなかった。受血者由来HBV-DNAのCP/PreC領域の塩基配列はWild typeであった。

考　察

本事例の献血者は，HBc抗体弱陽性のHBV感染既往者である。輸血によるB型肝炎を認めた献血時の保管検体の個別NATで，コバスTaqMan法で定量下限以下の低濃度のHBVを保有していた。そして，上記献血の500日後に献血された新鮮凍結血漿製剤を用いてHBV-DNAの解析を行い，受血者のHBV-DNAとの相同性が高いと判断された。この500日後の献血時の保管検体は個別NATが陰性であったにもかかわらず，多量の検体から核酸を抽出・濃縮して解析を行い，献血者HBV-DNAの一部を同定し得た。以上より，本事例は，HBs抗原陰性，HBV-DNA陽性のoccult HBV carrierが感染源であることが強く疑われた。これはB型肝炎の流行地で多くみられるパターンで，慢性のHBV carrierで，HBs抗原陰性化し，HBc抗体のみ陽性となった“anti-HBc alone”の状態である[5]。

Occult HBV carrierからの輸血でHBVが感染する頻度は低い。急性B型肝炎のウインドウ期の感染率は81％で，occult HBV carrierからの感染率は19％とする報告がある[6]。受血者が免疫不全状態にあると感染のリスクが高いと報告されている[7]が，本事例では免疫不全状態ではなかった。並存するHBs抗体が低値であったことが，HBV感染成立に関与したと思われる[8]。

本事例では，個別NAT陰性の献血血液からHBV-DNAの解析が行われ，受血者のそれと相同性が高いと判断された。すなわち，個別NAT陰性であっても，HBVが存在することが示された。花田らは，個別NAT陰性のoccult HBV carrierからの献血血液を介したHBV感染が疑われる症例

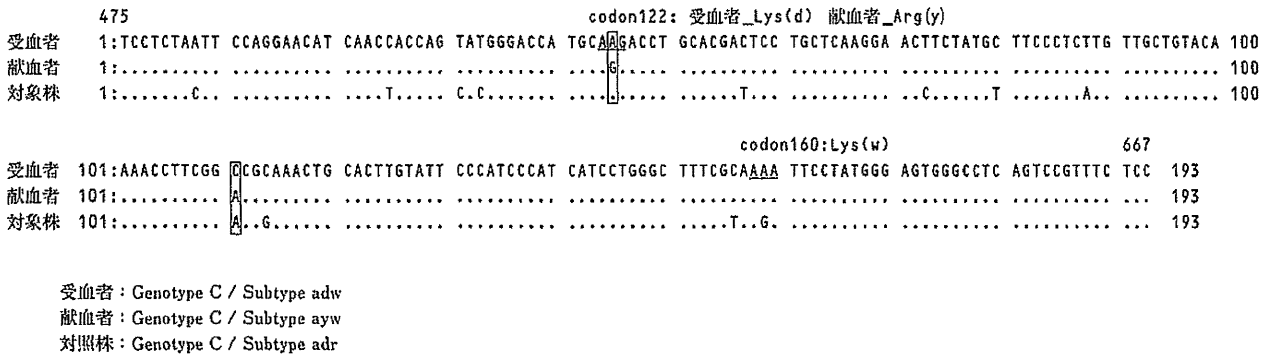
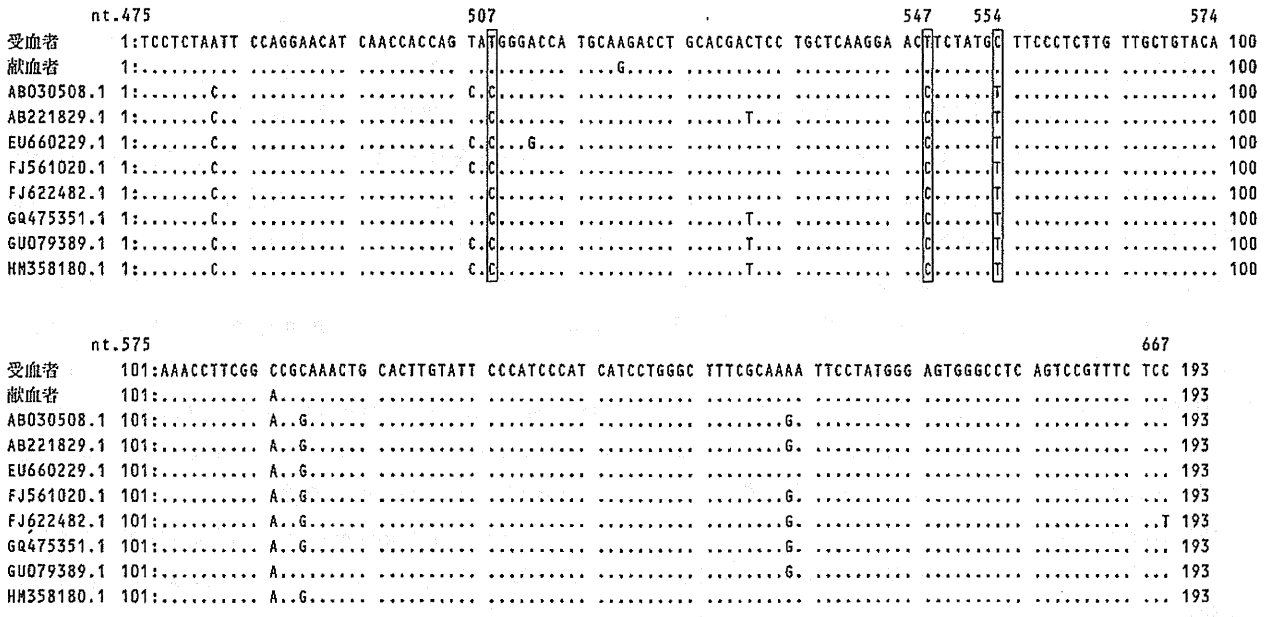


図１　S領域内の193塩基長の配列



AB030508.1～HM358180.1　DDBJデータベースで高い相同性を示した株

図２　データベースで高い相同性を示した８株とのS領域内193塩基長の配列の比較

を報告している[9]。しかし，本事例では，個別NAT陰性の赤血球製剤からのB型肝炎感染は認められなかった。また，個別NAT陽性の赤血球製剤からの感染も認めなかった。B型肝炎ウイルスの感染リスクは，輸血された血漿量すなわち輸血されたウイルス総量に比例すると考えられる[6]。

日本赤十字社ではHBVの更なる安全対策として，2012年８月からHBc抗体の基準を厳しくし，HBc抗体価1.0（C.O.I.）以上かつHBs抗体価200mIU/mL未満のHBV感染既往献血者の血液を排除することとした。これにより，本事例のような既感染者由来occult HBV carrierからの輸血によるHBV感染は，さらに減少すると思われるが，引き続き医療機関と協力して，自発報告・遡及調査等の情報を共有しつつ，感染の拡大を防止することが重要である。

本論文の要旨は，第61回日本輸血・細胞治療学会総会（横浜市，2013年５月）に於いて報告した。

文　献

1) Zeinab Nabil Ahmed Said: An overview of occult hepatitis B virus infection, World J Gastroenterol, 17: 1927-1938, 2011
2) Raimondo G *et al.*: Statements from the Taormina expert meeting on occult hepatitis B virus infection, J Hepatol, 49: 652-657, 2008
3) Tabor E *et al.*: Studies of donors who transmit posttransfusion hepatitis, Transfusion, 19: 725-731, 1979
4) 岡本宏明：肝炎ウイルスの分子医学的研究とその応用，モダンメディア，55：28-35，2009
5) Allain JP: Occult hepatitis B virus infection, Transfus Clin Biol, 11: 18-25, 2004
6) Candotti D, Allain JP: Transfusion-transmitted hepatitis B virus infection, J Hepatol, 51: 798-809, 2009
7) 梶本昌子ほか：Occult HBV carrierによる感染事例から得られた知見について，日本輸血細胞治療学会誌，52：599-606，2006

8) Satake M *et al.*: Infectivity of blood omponents with low hepatitis B virus DNA levels identified in a lookback program, Transfusion, 47: 1197-1205, 2007

9) 花田大輔ほか：Occult HBV carrierから供血された血液の輸血によりB型肝炎ウイルス感染が強く疑われた１例，日本輸血細胞治療学会誌，58：463-466，2012

Clinical Study

Oncology

Oncology 2013;84:108–114
DOI: 10.1159/000342650

Received: May 21, 2012
Accepted after revision: August 10, 2012
Published online: November 9, 2012

Efficacy, Safety, and Survival Factors for Sorafenib Treatment in Japanese Patients with Advanced Hepatocellular Carcinoma

Masahito Nakano[a] Masatoshi Tanaka[b] Ryoko Kuromatsu[a] Hiroaki Nagamatsu[c] Kenji Sakata[d] Satoru Matsugaki[e] Masahiko Kajiwara[f] Kunitaka Fukuizumi[g] Nobuyoshi Tajiri[h] Norito Matsukuma[i] Terufumi Sakai[j] Noriyuki Ono[k] Yoichi Yano[l] Hironori Koga[a] Junichi Kurogi[a] Akio Takata[a] Shuji Sumie[a] Manabu Satani[a] Shingo Yamada[a] Takashi Niizeki[a] Hajime Aino[a] Hideki Iwamoto[a] Takuji Torimura[a] Michio Sata[a] for the Kurume Liver Cancer Study Group of Japan

[a]Division of Gastroenterology, Department of Medicine, Kurume University School of Medicine, and [b]Kurume University Medical Center, Kurume, [c]Yame General Hospital, Yame, [d]Ōmuta City Hospital, Ōmuta, [e]Tobata Kyouritsu Hospital, Kitakyushu, [f]Asakura Medical Association Hospital, Asakura, [g]Kyushu Medical Center, Fukuoka, [h]Social Insurance Tagawa Hospital, Tagawa, [i]Kurume Daiichi Social Insurance Hospital, and [j]St. Mary's Hospital, Kurume, [k]Chikugo City Hospital, Chikugo and [l]Saga Social Insurance Hospital, Saga, Japan

Key Words
Sorafenib · Hepatocellular carcinoma · Japanese

Abstract
Background: Sorafenib, an oral multikinase inhibitor, was approved for the treatment of advanced hepatocellular carcinoma (HCC), but has not been adequately evaluated for safety and effectiveness in Japanese patients with advanced HCC. ***Aims:*** The purpose of this study was to prospectively assess the efficacy, safety, and risk factors for survival in patients with advanced HCC treated with sorafenib. ***Methods:*** Between May 2009 and December 2010, 96 Japanese patients with advanced HCC (76 male, 20 female, mean age: 70.4 years) were treated with sorafenib. Eighty-eight patients had Child-Pugh class A, and 8 patients had Child-Pugh class B liver cirrhosis. Barcelona Clinic Liver Cancer stage B and C were found in 64 and 32 patients, respectively. ***Results:*** Twelve patients demonstrated partial response to sorafenib therapy, 43 patients had stable disease, and 33 patients had progressive disease at the first radiologic assessment. The most frequent adverse events leading to discontinuation of sorafenib treatment were liver dysfunction (n = 8), hand-foot skin reaction (n = 7), and diarrhea (n = 4). The median survival time and time to progression were 11.6 and 3.2 months, respectively. By multivariate analysis, des-γ-carboxy prothrombin serum levels and duration of treatment were identified as independent risk factors for survival. ***Conclusions:*** This study showed that sorafenib was safe and useful in Japanese patients with advanced HCC. In addition, this study demonstrated that sorafenib should be administered as a long-term treatment for advanced HCC regardless of therapeutic effect and dosage.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world [1–3]. Recent advances in imaging have enabled an increased detection rate for early-stage HCC. By detecting HCC at an early stage, curative therapies, such as hepatic resection, liver transplantation, and radiofrequency ablation, are possible,

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0030–2414/13/0842–0108$38.00/0

Accessible online at:
www.karger.com/ocl

Masahito Nakano, MD
Division of Gastroenterology, Department of Medicine
Kurume University School of Medicine
67 Asahi-machi, Kurume, Fukuoka 830-0011 (Japan)
E-Mail nakano_masahito@kurume-u.ac.jp

which improve patient survival rates [4, 5]. In Japan, transarterial chemoembolization is an important locoregional treatment for patients with unresectable HCC [6]. However, long-term survival remains limited due to high rates of recurrence, even after these curative therapies [7, 8]. In particular, the development of advanced HCC with macroscopic vascular invasion or extrahepatic metastasis greatly reduces survival rates as effective systemic therapies have not been developed to date [9–11].

Recently, sorafenib, an oral multikinase inhibitor, has become available as a new molecular targeted therapy for advanced HCC. The magnitude of the benefit obtained with sorafenib (25–35% decreased risk of death) is similar to that observed with trastuzumab in breast cancer, bevacizumab in colon cancer, or erlotinib in lung cancer [12–14]. Sorafenib has been shown to suppress tumor growth and angiogenesis by inhibiting the Raf/MEK/ERK signaling pathway and by inhibiting receptor tyrosine kinases, such as vascular endothelial growth factor receptor (VEGFR)-1, VEGFR-2, and VEGFR-3, and platelet-derived growth factor receptor-β (PDGFR-β) [15].

The introduction of sorafenib has changed the standard systemic therapy for advanced HCC, as demonstrated by the recent positive results from randomized controlled trials, and this new treatment was approved in Japan in May 2009 [16, 17]. These results, proving the efficacy of molecular targeted therapies for liver cancer, have triggered the search for additional molecular agents to further improve patient survival. However, concerns regarding the development and approval of new molecular targeted therapies, including sorafenib, include the inclusion and exclusion criteria for the trials and frequent adverse events. The SHARP trial was conducted at 121 centers in 21 countries in Europe, North America, South America, and Australasia [16], and 23 centers in China, South Korea, and Taiwan were enrolled in the Asia-Pacific study [18], but no trials have been performed in Japan. Moreover, these studies did not primarily include patients infected with hepatitis C virus (HCV). In Japan, >70% of HCC cases are related to chronic liver disease with HCV infection. Therefore, in this study, we prospectively assessed the efficacy and safety of sorafenib and identified the factors associated with improved survival in Japanese patients with advanced HCC primarily due to HCV infection.

Patients and Methods

Patients

Eligibility criteria for this study were as follows: (1) Eastern Cooperative Oncology Group (ECOG) performance status of 0–1; (2) measurable disease using the Response Evaluation Criteria in Solid Tumors (RECIST); (3) Child-Pugh class A or B; (4) leukocyte count ≥2,000/mm^3; (5) platelet count ≥50 × 10^9/l; (6) hemoglobin level ≥8.5 g/dl; (7) serum creatinine level <1.5 mg/dl, and (8) no ascites or encephalopathy. Between May 2009 and December 2010, 96 patients diagnosed with advanced HCC were included in this study. HCC was either confirmed on histology or diagnosed using noninvasive criteria according to the European Association for the Study of Liver. Included patients were treated with sorafenib at 1 of the 12 experienced member institutions of the Kurume Liver Cancer Study Group of Japan: Asakura Medical Association Hospital, Chikugo City Hospital, Kurume Daiichi Social Insurance Hospital, Kurume University Medical Center, Kurume University School of Medicine, Kyushu Medical Center, Ōmuta City Hospital, Saga Social Insurance Hospital, Social Insurance Tagawa Hospital, St. Mary's Hospital, Tobata Kyouritsu Hospital, or Yame General Hospital. The primary outcome of this study was overall survival time. Overall survival time was defined as the time from sorafenib initiation to the date of death or the patient's last follow-up. Relevant data from the patients' clinical records, including history, laboratory results, radiologic findings, histologic results, and survival data, as well as the dosage and adverse events associated with sorafenib therapy, were prospectively collected. The study protocol was approved by University hospital Medical Information Network (UMIN) Center (No. UMIN000007427) and conformed to the guidelines of the 1975 Declaration of Helsinki. Patients were given full information regarding the details of the clinical study, and they provided written informed consent prior to participation in the study.

Diagnosis of Intrahepatic Lesions and Extrahepatic Metastasis

Intrahepatic lesions and vascular invasion were diagnosed using a combination of contrast-enhanced computed tomography (CT), magnetic resonance imaging (MRI), ultrasonography, and digital subtraction angiography. In addition, determination of α-fetoprotein (AFP), *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3), and des-γ-carboxy prothrombin (DCP) serum levels was performed up to 1 month prior to treatment. Intra-abdominal metastases were detected on abdominal CT, MRI, and ultrasonography, which were performed to evaluate intrahepatic lesions. Pulmonary lesions were detected on chest radiography or chest CT, which were routinely performed up to 1 month prior to treatment. Additional examinations, such as bone scintigraphy and brain CT or MRI, were indicated when symptoms attributable to extrahepatic metastasis appeared. These examinations were also undertaken when AFP, AFP-L3, or DCP were elevated, and the elevation could not be accounted for by the status of the intrahepatic lesions [11]. Tumor stage was classified according to the Barcelona Clinic Liver Cancer (BCLC) staging classification [19].

Sorafenib Treatment

An initial sorafenib dose of 400 mg was orally administered twice daily. Discontinuation and dose reduction were based on tolerance. Side effects of sorafenib were determined via the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE), version 3.0 [20]. Treatments were discontinued upon development of grade 3 or higher adverse events according to CTCAE classification with the exception of platelet counts and leukocyte counts of <25 × 10^9/l and <1,500/mm^3, respectively.

Table 1. Baseline clinical characteristics

Patient characteristics	n
Age, <70/≥70 years	39/57
Sex, male/female	76/20
Etiology, HBV/HCV/both negative	20/59/17
Child-Pugh class, A/B	88/8
BCLC stage, B/C	64/32
AFP, <1,000/≥1,000 ng/ml	62/34
DCP, <1,000/≥1,000 mAU/ml	49/47

HBV = Hepatitis B virus.

Assessment of Tumor Response

To assess tumor response, 4 weeks after beginning the administration of sorafenib and every 4–6 weeks thereafter, an imaging study was performed. Tumor response was evaluated according to the RECIST criteria, version 1.1 [21] as follows: complete response, all measurable lesions disappeared for >4 weeks; partial response (PR), the sum of the diameters of the largest target lesions decreased by >30% and there was no development of a new lesion for >4 weeks; progressive disease (PD), the sum of the largest diameters increased by >20% or a new lesion appeared, and stable disease, neither PR nor PD was seen [22]. Cancer in patients who died before their first radiographic assessment was classified as PD. The time to radiologic progression was defined as the time from sorafenib initiation to disease progression. Data from patients who died without tumor progression were censored. The disease control rate was defined, on the basis of independent radiologic review, as the percentage of patients whose best-response RECIST rating of complete response, PR, and stable disease was maintained for at least 30 days after the first demonstration of that rating.

Statistical Analysis

Baseline patient characteristics were analyzed using descriptive statistical methods. Survival curves were calculated via the Kaplan-Meier method. Univariate survival curves were compared using the log-rank test. A p value <0.05 was considered statistically significant. All analyses were performed using the statistical software package SPSS (IBM, Armonk, N.Y., USA). The Cox proportional hazards model was used to evaluate the interaction between baseline characteristics and the effect of sorafenib on overall survival.

Results

Patient Characteristics

There were 76 male (79%) and 20 female (21%) patients, with a mean age of 70.4 (range 33–87) years (table 1). Chronic HCV infection was the predominant cause of liver disease (n = 59; 61%), followed by chronic hepatitis B virus infection (n = 20; 21%). Eighty-eight (92%) patients had Child-Pugh class A, and 8 (8%) patients had Child-Pugh class B liver cirrhosis. With respect to tumor stage, 64 (67%) patients had stage B disease and 32 (33%) patients had stage C disease, according to the BCLC staging classification [19]. The most frequent sites of extrahepatic metastases were the lung (n = 41), bone (n = 14), and lymph nodes (n = 12). Prior to sorafenib therapy, 88 (92%) patients had been treated with surgical, loco-regional, or pharmacologic therapies. Of these 88 patients, 48 received transcatheter arterial infusion chemoembolization, 34 received hepatic arterial infusion chemotherapy, 25 underwent hepatic resection, and 23 patients underwent radiofrequency ablation.

Overall Response and Efficacy

The mean duration of oral treatment was 4.2 (range 0.1–16.2) months, and the mean follow-up duration was 6.4 (range 0.1–16.2) months. Forty (42%) patients died during the observation period, whereas 56 (58%) patients were alive at the end of the follow-up period. At the first radiologic assessment, 12 (13%) patients showed PR, 43 (45%) patients showed stable disease, and 33 (34%) patients showed PD; 8 (8%) patients had no follow-up radiologic evaluation and were not included in further analysis.

Treatment Compliance

Performance status was used to determine initial sorafenib dose at the discretion of each chief physician. Fifty-eight patients with a performance status of 0 started treatment with 800 mg sorafenib daily and 38 patients with a performance status of 1 began with a 400-mg daily dose of sorafenib. Dose reduction was necessary in 40 patients during treatment. By December 2010, the end of the follow-up period, 71 patients had discontinued treatment. The reasons for discontinuation were adverse events (36 patients), radiologic and symptomatic progression (27 patients), and deterioration in performance status (8 patients). The mean duration of treatment, prior to discontinuation, was 3.5 (range 0.1–15.5) months.

Treatment-Related Toxicities

Hand-foot skin reaction (HFSR) was the most troublesome adverse event in our series, occurring in 49 (51%) patients. Other frequent toxicities included diarrhea (n = 23; 24%), alopecia (n = 13; 14%), liver dysfunction (n = 13; 14%), and fatigue (n = 11; 11%). The most frequent adverse events leading to discontinuation of sorafenib treatment were HFSR (n = 7; 7%), diarrhea (n = 4; 4%), and liver dysfunction [n = 8; 8%; 7 patients with Child-Pugh class A disease (8%) and 1 with Child-Pugh class B (13%)]. In par-

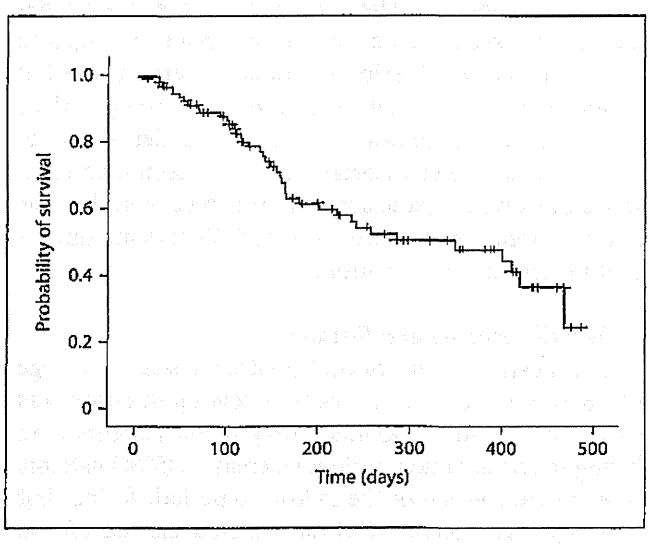


Fig. 1. Cumulative survival of 96 patients with advanced HCC treated with sorafenib. The MST of these patients was 11.6 months. The 1-year survival rate was 48%.

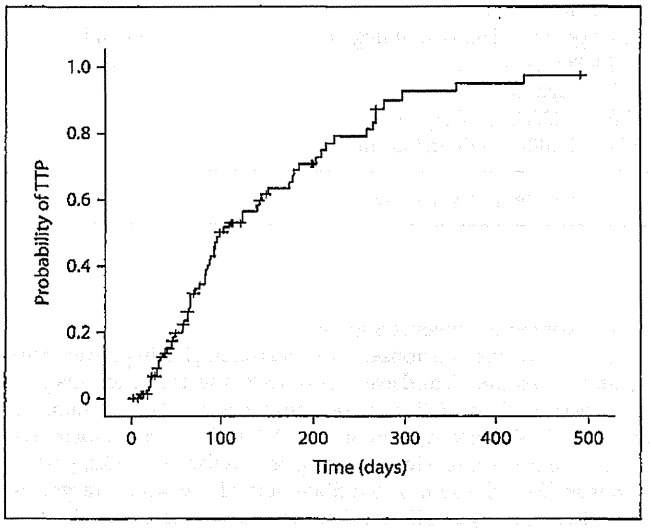


Fig. 2. Cumulative progression of 96 patients with advanced HCC treated with sorafenib. The median TTP of these patients was 3.2 months.

Table 2. Univariate and multivariate analyses of survival in patients with HCC

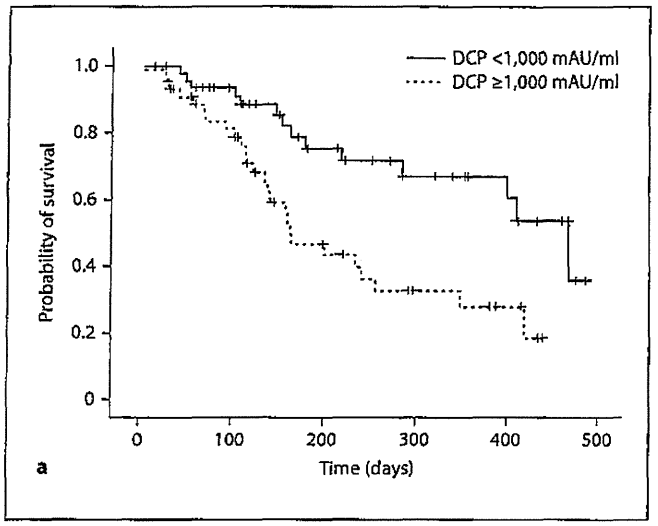
	Univariate		Multivariate	
	HR (95% CI)	p value	HR (95% CI)	p value
Age (≥70 years)	1.091 (0.581–2.050)	0.786		
Sex (male)	0.670 (0.320–1.403)	0.288		
Child-Pugh class (B)	2.273 (0.868–5.952)	0.094		
AFP (≥1,000 ng/ml)	1.953 (1.046–3.647)	0.036		
DCP (≥1,000 mAU/ml)	2.723 (1.394–5.316)	0.003	2.722 (1.369–5.412)	0.004
Daily average dosage (≥400 mg)	0.970 (0.503–1.870)	0.927		
Daily average dosage (≥600 mg)	1.042 (0.556–1.954)	0.898		
Duration of treatment (≥30 days)	0.403 (0.199–0.816)	0.012	0.407 (0.196–0.848)	0.016
Therapeutic effect (PD)	1.876 (0.991–3.549)	0.053		

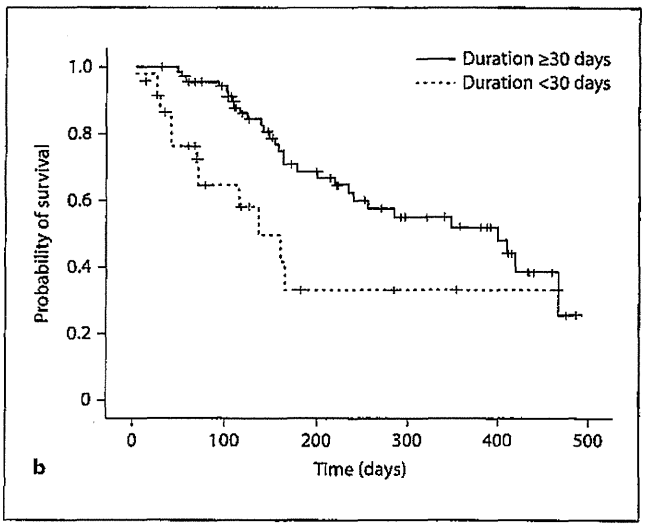
HR = Hazard ratio; 95% CI = 95% confidence interval.

ticular, interstitial pneumonia (n = 1; 1%) and tumor lysis syndrome (n = 1; 1%) were serious adverse events. The single case of interstitial pneumonia resulted in death.

Survival and Factors Associated with Outcome

The cumulative survival curve of 96 patients is shown in figure 1. The median survival time (MST) was 11.6 (range 0.1–16.2) months, with a 1-year survival rate of 48%. The median time to progression (TTP) was 3.2 (range 0.1–16.2) months (fig. 2). Cox proportional hazards regression analysis was performed to identify independent factors associated with survival (table 2). The results of univariate analysis showed that AFP serum level (≥1,000 ng/ml, p = 0.036), DCP serum level (≥1,000 mAU/ml, p = 0.003), and duration of treatment (>30 days, p = 0.012) were significant risk factors adversely impacting survival. Multivariate analysis showed that DCP serum level (≥1,000 mAU/ml, HR 2.722, 95% CI 1.369–5.412, p = 0.004) and duration of treatment (>30 days, HR 0.407, 95% CI 0.196–0.848, p = 0.016) were independent risk factors for decreased survival. Cumulative survival curves, plotted for DCP serum level and duration of treatment, are shown in figure 3.





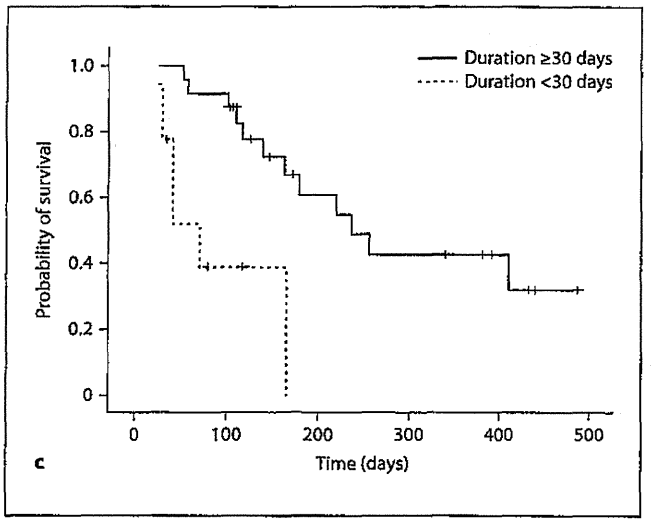


Fig. 3. **a** Cumulative survival of patients grouped by serum DCP levels. The MSTs of the group with DCP >1,000 and <1,000 mAU/ml were 5.4 and 15.6 months, respectively (p = 0.0023). **b** Cumulative survival of patients grouped by duration of treatment. The MSTs of >30 and <30 days of treatment were 13.3 and 4.5 months, respectively (p = 0.0091). **c** Cumulative survival of patients with PD grouped by duration of treatment. The MSTs with >30 and <30 days of treatment were 7.8 and 2.4 months, respectively (p = 0.0008).

Discussion

Sorafenib, an oral multikinase inhibitor, has recently become available as a new molecular targeted therapy for advanced HCC. A significant survival benefit and good tolerance was demonstrated with sorafenib treatment for patients with advanced HCC in 2 randomized phase III placebo-controlled trials [16, 18]. Consequently, sorafenib has become the standard treatment for advanced HCC in the United States, Europe, and many other countries, including Japan. This study prospectively assessed the efficacy and safety of sorafenib and identified the factors associated with survival in Japanese patients with advanced HCC. In this study, the TTP and MST of Japanese patients receiving sorafenib were 3.2 and 11.6 months, respectively. TTP in this study was shorter than that observed in the SHARP trial (5.5 months) and was similar to that observed in the Asia-Pacific study (2.8 months) [16, 18]. However, the MST in the current study was longer than that observed in the Asia-Pacific study (6.5 months) and was similar to that observed in the SHARP trial (10.7 months) [16, 18]. Compared with these 2 previous studies, the time between TTP and MST was longer in the current study, though the reason for this is unclear.

An exploratory multivariate analysis with the use of a Cox proportional hazards model identified 2 baseline patient characteristics that were prognostic indicators for overall survival: duration of treatment and serum DCP

level. In contrast, therapeutic effect and dosage of sorafenib were not significant risk factors adversely affecting survival in this study. In the SHARP trial and the Asia-Pacific study, administration of sorafenib was continued until the occurrence of both radiologic and symptomatic progression, or the occurrence of either unacceptable adverse events or death [16, 18]. In the current study, neither radiologic nor symptomatic progression were criteria for discontinuation. The difference in the discontinuation criteria may explain the gap between TTP and MST in this study. Even with tumor progression, the patients who continued on sorafenib may have had better survival potential compared to the patients in whom sorafenib was discontinued (fig. 3c). Therefore, this study suggests that sorafenib should be administered long-term in patients with advanced HCC independent of therapeutic effect and dosage.

Previous studies reported that for patients with HCC, high serum DCP levels are associated with vascular invasion, metastasis, and tumor recurrence [23]. Hypoxia has been reported to induce epithelial mesenchymal transition or cytoskeletal changes. Indeed, hypoxic stimulation induced hepatoma cell lines (HepG2 or PLC/PRF/5 cells) to undergo epithelial-to-fibroblastoid conversion or epithelial mesenchymal transition, and these cells produced DCP [23]. Therefore, DCP as an HCC tumor marker is more useful in larger tumors which are likely to be exposed to hypoxia during tumor development [23]. Thus, it is suggested that higher serum DCP levels represent a more advanced state of HCC, and, as a result, lead to reduced survival rates.

In this study, disease classification at the first radiologic assessment was PR for 12 (13%) patients, stable disease for 43 (45%) patients, and PD for 33 (34%) patients. Notably, the proportion of patients with PR in our study was higher compared to the SHARP trial (2%) and the Asia-Pacific study (3.3%). It is not clear why there appears to be a higher rate of PR in Japanese patients. Previous studies suggested that there may be racial differences in terms of gene mutations that may affect sorafenib treatment [24, 25]. Lynch et al. [26] reported that patients with non-small-cell lung cancer have specific mutations in the *EGFR* gene, which correlate with clinical responsiveness to the tyrosine kinase inhibitor gefitinib. Therefore, it is suggested that Japanese patients with advanced HCC may be more sensitive to sorafenib than Western and other Asian populations. To investigate the possible differences in the therapeutic effects of sorafenib, further studies with larger patient populations will be needed.

Treatment-related adverse events were a substantial issue impacting the continuation of treatment with sorafenib. In this study, although the overall incidence of treatment-related adverse events was high (90%), events were primarily controlled with medical treatment and/or sorafenib dose reductions. Adverse events leading to discontinuation of treatment included liver dysfunction (8%), HFSR (7%), and diarrhea (4%), which are commonly associated with sorafenib [27, 28]. However, in the SHARP trial, the overall incidence of treatment-related adverse events was 80% in the sorafenib group, and the most frequent adverse events leading to discontinuation of sorafenib treatment were gastrointestinal events (6%), fatigue (5%), and liver dysfunction (5%) [16]. HFSR is particularly well known as an early adverse event [29–31] associated with sorafenib therapy and the severity of HFSR depends on the duration of treatment, dosage, and accumulation of the drug [32]. Further effort put towards the effective control of adverse effects and management of sorafenib dosing, with a priority given to facilitating long-term administration, will lead to the most effective therapy for patients with HCC. Moreover, hepatic reserve is important for hepatic extraction and metabolism of sorafenib. In this study, liver dysfunction necessitating suspension or discontinuation of sorafenib occurred with similar frequency in patients with Child-Pugh class B and Child-Pugh class A disease. This result suggests that sorafenib can be used in patients with Child-Pugh class B, as well as in patients with Child-Pugh class A disease.

In conclusion, sorafenib was a safe and effective therapy in Japanese patients with advanced HCC. In addition, duration of treatment and serum level of DCP were independent risk factors negatively impacting survival in this study. The results of this study indicate that sorafenib should be administered as a long-term treatment for advanced HCC in patients regardless of therapeutic effect and dosage.

References

1 Parkin DM, Bray F, Ferlay J, Pisani P: Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2 El-Serag HB, Mason AC: Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–750.
3 Sherman M: Hepatocellular carcinoma: epidemiology, risk factors, and screening. Semin Liver Dis 2005;25:143–154.
4 Takayama T, Makuuchi M, Hirohashi S, Sakamoto M, Yamamoto J, Shimada K, Kosuge T, Okada S, Takayasu K, Yamasaki S: Early hepatocellular carcinoma as an entity with a high rate of surgical cure. Hepatology 1998;28:1241–1246.

5 Zhang BH, Yang BH, Tang ZY: Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol 2004; 130:417–422.
6 Kudo M, Imanaka K, Chida N, Nakachi K, Tak WY, Takayama T, Yoon JH, Hori T, Kumada H, Hayashi N, Kaneko S, Tsubouchi H, Suh DJ, Furuse J, Okusaka T, Tanaka K, Matsui O, Wada M, Yamaguchi I, Ohya T, Meinhardt G, Okita K: Phase III study of sorafenib after transarterial chemoembolisation in Japanese and Korean patients with unresectable hepatocellular carcinoma. Eur J Cancer 2011;47:2117–2127.
7 Nagasue N, Uchida M, Makino Y, Takemoto Y, Yamanoi A, Hayashi T, Chang YC, Kohno H, Nakamura T, Yukaya H: Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. Gastroenterology 1993;105:488–494.
8 Yang Y, Nagano H, Ota H, Morimoto O, Nakamura M, Wada H, Noda T, Damdinsuren B, Marubashi S, Miyamoto A, Takeda Y, Dono K, Umeshita K, Nakamori S, Wakasa K, Sakon M, Monden M: Patterns and clinicopathologic features of extrahepatic recurrence of hepatocellular carcinoma after curative resection. Surgery 2007;141:196–202.
9 Llovet JM, Burroughs A, Bruix J: Hepatocellular carcinoma. Lancet 2003;362:1907–1917.
10 Bruix J, Sherman M, Practice Guidelines Committee, American Association for the Study of Liver Diseases: Management of hepatocellular carcinoma. Hepatology 2005; 42:1208–1236.
11 Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodes J, EASL Panel of Experts on HCC: Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421–430.
12 Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE Jr, Davidson NE, Tan-Chiu E, Martino S, Paik S, Kaufman PA, Swain SM, Pisansky TM, Fehrenbacher L, Kutteh LA, Vogel VG, Visscher DW, Yothers G, Jenkins RB, Brown AM, Dakhil SR, Mamounas EP, Lingle WL, Klein PM, Ingle JN, Wolmark N: Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005;353:1673–1684.
13 Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F: Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–2342.
14 Shepherd FA, Rodrigues Pereira J, Ciuleanu T, Tan EH, Hirsh V, Thongprasert S, Campos D, Maoleekoonpiroj S, Smylie M, Martins R, van Kooten M, Dediu M, Findlay B, Tu D, Johnston D, Bezjak A, Clark G, Santabárbara P, Seymour L, National Cancer Institute of Canada Clinical Trials Group: Erlotinib in previously treated non-small-cell lung cancer. N Engl J Med 2005;353:123–132.
15 Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M: Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther 2008;7:3129–3140.
16 Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J, SHARP Investigators Study Group: Sorafenib in advanced hepatocellular carcinoma. N Engl J Med 2008;359:378–390.
17 Furuse J, Ishii H, Nakachi K, Suzuki E, Shimizu S, Nakajima K: Phase I study of sorafenib in Japanese patients with hepatocellular carcinoma. Cancer Sci 2008;99:159–165.
18 Cheng AL, Kang YK, Chen Z, Tsao CJ, Qin S, Kim JS, Luo R, Feng J, Ye S, Yang TS, Xu J, Sun Y, Liang H, Liu J, Wang J, Tak WY, Pan H, Burock K, Zou J, Voliotis D, Guan Z: Efficacy and safety of sorafenib in patients in the Asia-Pacific region with advanced hepatocellular carcinoma: a phase III randomised, double-blind, placebo-controlled trial. Lancet Oncol 2009;10:25–34.
19 Forner A, Reig ME, de Lope CR, Bruix J: Current strategy for staging and treatment: the BCLC update and future prospects. Semin Liver Dis 2010;30:61–74.
20 Trotti A, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, Langer C, Murphy B, Cumberlin R, Coleman CN, Rubin P: CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. Semin Radiat Oncol 2003;13: 176–181.
21 Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009;45: 228–247.
22 Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000;92:205–216.
23 Murata K, Suzuki H, Okano H, Oyamada T, Yasuda Y, Sakamoto A: Hypoxia-induced des-gamma-carboxy prothrombin production in hepatocellular carcinoma. Int J Oncol 2010;36:161–170.
24 Kudo M, Ueshima K: Positioning of a molecular-targeted agent, sorafenib, in the treatment algorithm for hepatocellular carcinoma and implication of many complete remission cases in Japan. Oncology 2010;78(suppl 1):154–166.
25 Kim R, Aucejo F: Radiologic complete response with sirolimus and sorafenib in a hepatocellular carcinoma patient who relapsed after orthotopic liver transplantation. J Gastrointest Cancer 2011;42:50–53.
26 Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J, Haber DA: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. N Engl J Med 2004;350:2129–2139.
27 Abou-Alfa GK, Schwartz L, Ricci S, Amadori D, Santoro A, Figer A, De Greve J, Douillard JY, Lathia C, Schwartz B, Taylor I, Moscovici M, Saltz LB: Phase II study of sorafenib in patients with advanced hepatocellular carcinoma. J Clin Oncol 2006;24: 4293–4300.
28 Ratain MJ, Eisen T, Stadler WM, Flaherty KT, Kaye SB, Rosner GL, Gore M, Desai AA, Patnaik A, Xiong HQ, Rowinsky E, Abbruzzese JL, Xia C, Simantov R, Schwartz B, O'Dwyer PJ: Phase II placebo-controlled randomized discontinuation trial of sorafenib in patients with metastatic renal cell carcinoma. J Clin Oncol 2006;24:2505–2512.
29 Lee WJ, Lee JL, Chang SE, Lee MW, Kang YK, Choi JH, Moon KC, Koh JK: Cutaneous adverse effects in patients treated with the multitargeted kinase inhibitors sorafenib and sunitinib. Br J Dermatol 2009;161:1045–1051.
30 Lacouture ME, Wu S, Robert C, Atkins MB, Kong HH, Guitart J, Garbe C, Hauschild A, Puzanov I, Alexandrescu DT, Anderson RT, Wood L, Dutcher JP: Evolving strategies for the management of hand-foot skin reaction associated with the multitargeted kinase inhibitors sorafenib and sunitinib. Oncologist 2008;13:1001–1011.
31 Anderson R, Jatoi A, Robert C, Wood LS, Keating KN, Lacouture ME: Search for evidence-based approaches for the prevention and palliation of hand-foot skin reaction (HFSR) caused by the multikinase inhibitors (MKIs). Oncologist 2009;14:291–302.
32 Vincenzi B, Santini D, Russo A, Addeo R, Giuliani F, Montella L, Rizzo S, Venditti O, Frezza AM, Caraglia M, Colucci G, Del Prete S, Tonini G: Early skin toxicity as a predictive factor for tumor control in hepatocellular carcinoma patients treated with sorafenib. Oncologist 2010;15:85–92.