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REVIEW ARTICLE

Liver transplantation for patients with human immunodeficiency virus and hepatitis C virus co-infection: update in 2013

Susumu Eguchi · Mitsuhisa Takatsuki · Tamotsu Kuroki

Abstract Because of the progress of anti-retroviral therapy (ART) for human immunodeficiency virus (HIV), mortality due to opportunistic infection resulting in AIDS has been remarkably reduced. However, meanwhile, half of those patients have died of end-stage liver cirrhosis due to hepatitis C virus (HCV) with liver cirrhosis and early occurrence of hepatocellular carcinoma. Recently, in 2013, non-cirrhotic portal hypertension due to ART drugs or still unknown mechanisms have become problematic with early progression of the disease in this patient population. Liver transplantation (LT) could be one treatment of choice in such cases, but the indications for LT perioperative management, including both HIV and HCV treatments and immunosuppression, are still challenging. In this review, we update the literature on HIV/HCV co-infection and LT as well as recent effort for modifying allocation system for those patients.

Keywords Co-infection · Hepatitis C virus · HIV · Human immunodeficiency virus · Liver transplantation

Introduction

The causes of death of human immunodeficiency virus (HIV) infected patients have dramatically changed since 1995. A major background factor behind these trends is the improved HIV control achieved with anti-retroviral therapy (ART) [1]. Despite dramatic reduction of death due to acquired immunodeficiency syndrome (AIDS), co-infected hepatitis C virus (HCV)-related death due to liver failure or hepatocellular carcinoma (HCC) became a serious problem, not only in Japan but all over the world, including England [2]. In Japan, in the late 1980s, contaminated blood products for hemophilia caused co-infection by HIV and HCV. In such cases, liver transplantation (LT) is the only possible treatment option to achieve long-term survival, but several modifications of perioperative management are required recently for better outcome.

In this review, the outcome and the points of management of LT for HIV/HCV co-infected patients were reviewed to save relatively young patients with HIV/HCV co-infection bearing HCC [3, 4], non-cirrhotic portal hypertension (NCPH) [5–7], and decompensated liver cirrhosis [8, 9]. An updated critical review of the literature in 2013 was performed, and new information on problems and results for LT for HIV/HCV co-infection were included.

Upcoming topics regarding LT indications for HIV/HCV co-infection in 2013

Non-cirrhotic portal hypertension

In HIV/HCV coinfected patients, liver failure due to HCV hepatitis was enhanced by ART-related hepatotoxicity, especially manifesting as non-cirrhotic portal hypertension [5–7]. One of the ART drugs, Didanosin (DDI), has been suspected for serious morbidity. Thus, not only in cases with deteriorated liver function, such as in Child–Pugh B or C cases, but also even in Class A cases, patients' liver function can easily deteriorate abruptly [10, 11]. The actual natural course of pure NCPH is unknown, because it can be modulated with HCV or other causes and reported as only case series. However, an important study regarding "Non-cirrhotic portal hypertension in HIV mono-infected patients without HCV" was published in 2012 [12]. All five patients had portal hypertensive symptoms such as ascites or variceal bleeding after ART medication. We need to await their prognostic information, since it can be extrapolated into HIV/HCV co-infected patients after successful HCV eradication.

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Therefore, all HIV/HCV co-infected patients should be carefully followed up so as not to miss the opportunity for LT. Recently, in Japan, a scoring system was created for listing a deceased donor LT for those patients with HIV/HCV co-infection due to previous contaminated blood products.

Hepatocellular carcinoma

Recently it became evident that HCC in HIV/HCV co-infected patients develop HCC at a very early stage of life, such as in the 30s and 40s [3, 4]. The molecular mechanism of its development still remains unclear, but surveillance in those patients should be considered for HCC strictly. In Japan, HIV/HCV co-infected hemophilic patients have been undergoing periodic examination for liver-related disease on a research basis. Early detection could contribute to treatment choices such as liver resection or liver transplantation. Regardless of the infectious status of HIV, treatment strategy for HCC in HIV/HCV infected patients should be the same in HCV mono-infected patients. Namely, whether liver resection could be performed or not should be based on the liver functional reserve. Also radio frequency ablation and transarterial chemoembolization can be selected according to the location, size and number of HCC.

Current results of LT for HIV/HCV co-infected patients in 2013

Indications for LT

As HCV mono-infected patients, LT should be considered when patients develop deteriorated liver function as indicated by a Child–Pugh score of class B or C in co-infected patients. Recently, Murillas et al. reported that the Model for End-stage Liver Disease (MELD) score is the best prognostic factor in HIV-infected patients [13]. HIV/HCV co-infected patients might be considered for LT before their MELD score increases to achieve comparable results with HCV mono-infected patients. Several studies showed that aggressive fibrosis in HIV/HCV co-infected patients compared with HCV mono-infected patients [14, 15], but the mechanism of this aggressive fibrosis remains unclear. Recently, transient elastography or acoustic radiation force impulse (ARFI) imaging to check for liver stiffness has been introduced as an effective and noninvasive modality to determine patients' candidacy for LT [16, 17].

Regardless of the presence of hemophilia, the indications and methods for performing liver transplantation remains unchanged for patients with HIV/HCV co-infection. In fact, after a successful liver transplantation, hemophilia can normally be cured. Usually, the conditions for liver transplantation are as follows: (1) AIDS symptoms have not surfaced; (2) CD4+ T lymphocyte count is 150–200/μl or above; and (3) as a result of ART, the amount of HIV RNA in the blood by PCR method is below the level of sensitivity of the assay.

In HIV/HCV co-infected patients, current studies show that a count of more than 100/μl CD4+ T lymphocytes is acceptable [18, 19], because patients generally have portal hypertension, which can cause leukocytopenia. In such patients, the ratio of CD4/CD8 is reported to be a realistic marker to predict postoperative complications including opportunistic infections. When the ratio is less than 0.15, the incidence of infectious complications is significantly higher [20].

In 2013, based on the evidence of rapid progression of the liver cirrhosis and portal hypertension in patients with HIV/HCV co-infection, a ranking system for waiting list of deceased donor LT has been set up in Japan. Even HIV/HCV co-infected liver cirrhotic patients with Child–Pugh class A can be listed for LT as "point 3" because of NCPH nature. Also co-infected patients with Child–Pugh class B and C can be listed as "point 6" and "point 8" based on the data from our HIV/AIDS project team of the Ministry of Health, Labor, and Welfare of Japan, and world literatures [21–23]. It is basically considered for previous victims of contaminated blood products for hemophilia.

Results of LT for patients with HIV/HCV co-infection

In the United States and Europe, liver transplantation from deceased donors has been performed in HIV patients since the 1980s. At that time, the outcomes of LT were very poor [11]. Recent series of reports are listed in Table 1 [24–31]. The reality is that, in addition to those listed therein, there have been many sporadic reports, such as reviews, expectations for liver transplantation, and assessment of indications.

In general, most reports concluded that the results were 10% worse than in the cases with HCV mono-infection, with a 3-year survival of around 60–70%. Recently, a 5-year patient survival of around 50% was reported, and there is debate whether these results can be accepted for patients of a younger age and were co-infected through previous use of a contaminated blood product. In Japan, the Tokyo group reported six cases of living donor liver transplantation (LDLT) between 2001 and 2004 [32]. Terrault et al. reported that older donor age, combined kidney-liver transplantation, an anti-HCV positive donor, and a body mass index <21 kg/m^2 were independent predictors of graft loss [33]. After LT, several studies showed that acute cellular rejection was more frequent and more severe in HIV/HCV co-infected patients than in HCV mono-infected patients, possibly due to difficulties in achieving optimal immunosuppression because of interactions between antiretroviral agents and immunosuppression.

Table 1 Updated outcome of liver transplantation for HIV positive recipients

Authors	Year	Country	*n*	Patient survival (%)			
				1 year	3 years	5 years	
Duclos-Vallee et al. [25]	2008	France	35	–	73	51	
Tsukada et al. [32]	2011	Japan	6	66	66	50	Only LDLT, only hemophilia
Terrault et al. [33]	2012	US	89	76	60	–	
Miro et al. [26]	2012	Spain	84	88	62	54	
Anadol et al. [27]	2012	Germany	32	90	65	60	
Harbell et al. [28]	2012	USA	125	91	67	–	
Baccarani et al. [31]	2012	Italy	32	–	79	69	
Di Benedetto et al. [46]	2012	Italy	30	75	65	50	with HCC
Ragni et al. [29]	2013	USA	15	71	38	–	only hemophilia

HCC hepatocellular carcinoma, *LDLT* living donor liver transplantation

Lowered outcome can be presumed from previous reports. Final mortality (graft loss) after LT was usually due to infection and multiorgan failure. As in Miro's report the causes due to the higher proportion of organs from donation after cardiac death (DCD) donors, higher rate of combined liver-kidney transplantation, increased rate of acute cellular rejection, HBV co-infection and infection. However, it was of note that there was no death due to infections related to HIV.

Preoperative management of HIV/HCV in liver transplantation

The number of HIV-RNA copies before LT is suggested as an independent risk factor of postoperative mortality, so that HIV should be controlled sufficiently before LT [30]. Accordingly, in patients who are under consideration to receive LT, ART can be safely stopped before LT, because HIV is generally well controlled for a long period by ART. Also ART can be toxic for the virgin graft, which underwent ischemia/reperfusion injury and liver resection in a donor. Once it is settled down after liver transplant, especially in LDLT cases, ART can be resumed with meticulous adjustment with calcineurin inhibitors.

Actually, after LT, ART should be restarted as soon as possible, because HIV-RNA appears at 3 to 30 days after ART is stopped [34], but the timing of restart of ART depends on the patient's condition, including liver function [35]. As long as the liver function has not fully recovered, or partial liver graft such as in LDLT has not yet sufficiently regenerated, ART cannot be started. Castells et al. reported in their case-control study that ART was started at a median of 8 days after LT (range 4–28 days) [36]. ART administered after LT should be the same as the preLT regimen, but the majority of ART drugs, including protease inhibitors and non-nucleoside reverse transcriptase inhibitors, have interactions with calcineurin inhibitors (CNI) or mammalian target-of-rapamycin (mTOR) [37], so that the monitoring of blood levels of immunosuppression is extremely important to avoid infectious complications or rejection. It can easily overshoot beyond the therapeutic level. Currently, a novel HIV-1 integrase inhibitor, raltegravir, is expected to be a feasible drug because it has no interactions with CNI, unlike other drugs [38, 39]. Therefore, the current recommended strategy in the light of LT could be to try raltegravir as ART before LT and see if HIV can be controlled with raltegravir. If it is the case, CNI could be used as usual after LT. However, if raltegravir cannot control HIV or cannot be applied due to other reasons, meticulous management of CNI (e.g. once a week administration with frequent trough monitoring) or Mycophenolate mofetil protocol should be considered. In fact, the novel protease inhibitor anti-HCV drug, telapreir, has the same character as ART drugs for HIV, and transplants team learn to overcome such drug interactions when post-LT HCV mono-infected patients are treated with telaprevir.

The treatment strategy for HCV in HIV/HCV co-infected patients is the same as in HCV mono-infected patients. Combination therapy of pegylated interferon (Peg-IFN) and ribavirin is the standard treatment both before and after LT in 2013. The treatment should be started as soon as possible, because in HIV/HCV co-infected patients, HCV recurrence may be accelerated in an immunocompromised state [40, 41]. As mentioned above, the novel protease inhibitor telaprevir is currently being introduced as an effective drug to achieve sustained viral response (SVR) of 70%, even in genotype 1b, with Peg-IFN/ribavirin in a non-transplant setting [42], but this drug is metabolized via cytochrome P450, as are CNI and various protease inhibitors of ART for HIV. Close monitoring of the CNI trough level should be performed, and although triple therapy with telaprevir/Peg-IFN/ribavirin or even without Peg-IFN is currently reported to be effective to prevent HCV recurrence after LT in HCV mono-infected cases, special attention should be paid when

this regimen is adapted for HIV/HCV co-infected patients. Additionally, mutational status of the IL28 B genotype should be investigated before interferon therapy for both donor and recipient.

Immunosuppression

Several reports have demonstrated both the in vitro and in vivo effectiveness of rapamycin in reducing HIV replication [43–45]. Di Benedetto et al. found that rapamycin monotherapy was significantly beneficial in long-term immunosuppression maintenance and HIV control after LT [46]. Mycophenolate mofetil is expected to be an effective immunosuppressive drug because of its efficacy in reducing HIV infection by both virological and immunological mechanisms. Mycophenolic acid, a selective inhibitor of the de novo synthesis of guanosine nucleotides in T and B lymphocytes, has been proposed to inhibit HIV replication in vitro by depleting the substrate (guanosine nucleotides) for reverse transcriptase. Using these drugs, a more effective regimen of immunosuppression with ART may be established. However, more information needs to be obtained to establish concrete immunosuppressive protocol.

As to steroids, several studies proposed that a steroid-free regimen can be safely applied and effective in LT for HCV cirrhosis. In HIV/HCV co-infected patients, a steroid-free protocol may play a beneficial role in preventing both HIV and HCV recurrence after LT [47, 48].

Hepatocellular carcinoma

Liver transplantation has been performed also for indication of HCC. The most updated study indicated that the existence of HCC did not change the outcome of LT provided that HCC was downstaged preoperatively for UCSF criteria [49]. Also for these cases sirolimus tended to be used as primary immunosuppressive agents. This encouraging result awaits further reports [50].

Conclusions

The above is an overview of liver transplantation performed to date in HIV/HCV- co-infected patients. Although, the results are 10% lower in patient survival after LT than those for HCV mono-infected patients, LT could be feasible in selected cases with HIV/HCV co-infection after careful evaluation within suitable stages of the disease. In light of the fact that most HIV/HCV co-infected patients in Japan are the victims of contaminated blood products, it is believed that the importance of liver transplantation will increase in the future in the context of medical relief as well. Our investigating team under the Ministry of Health, Labor, and Welfare of Japan has made all possible efforts to clarify the appropriate timing to put HIV/HCV co-infected patients on a waiting list for LT.

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Conflict of interest None declared.

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ORIGINAL ARTICLE

Combination chemotherapy with S-1 plus cisplatin for gastric cancer that recurs after adjuvant chemotherapy with S-1: multi-institutional retrospective analysis

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Abstract
Background It is unclear whether S-1 plus cisplatin is effective for patients with recurrent gastric cancer after adjuvant S-1 chemotherapy.
Methods We retrospectively evaluated the efficacy of S-1 plus cisplatin in patients whose gastric cancer recurred after adjuvant S-1 chemotherapy.
Results In the 52 patients evaluated, the median duration of adjuvant S-1 chemotherapy was 8.1 months, and the median recurrence-free interval (RFI) since the last administration of adjuvant S-1 was 6.4 months. Among the 36 patients with measurable lesions, 7 achieved a complete or partial response, and 13 were evaluated as having stable

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disease, for an overall response rate of 19.4% and a disease control rate of 55.6%. For all patients, the median progression-free survival (PFS) was 4.8 months, and the median overall survival (OS) was 12.2 months. Compared with patients with an RFI of <6 months ($n = 25$), patients with an RFI of ≥6 months ($n = 27$) had a significantly higher response rate (5.0 vs. 37.5%, respectively), longer PFS (2.3 vs. 6.2 months, respectively), and longer overall survival (7.3 vs. 16.6 months, respectively). According to a multivariate Cox model including performance status (PS) and reason for discontinuation of adjuvant S-1, an RFI of 6 months was still significantly associated with PFS and OS. *Conclusions* S-1 plus cisplatin is effective for patients with gastric cancer that recurs after adjuvant S-1 chemotherapy, especially for those with an RFI of ≥6 months.

Keywords Adjuvant chemotherapy · Gastric cancer · Recurrence · S-1

Introduction

Gastric cancer is the fourth most common malignancy in the world (988,602 cases in 2008, 7.8% of total malignancy cases) and the second leading cause of cancer death (737,419 deaths, 9.7% of total) [1]. The prognosis of patients with advanced or recurrent gastric cancer remains poor; chemotherapy confers only a minimal survival advantage, with a median survival of approximately 1 year. The most commonly used regimens are combination chemotherapy consisting of a fluoropyrimidine [5-fluorouracil (5-FU) or oral fluoropyrimidine] plus a platinum agent with or without docetaxel or anthracyclines [2–6].

S-1 is an oral anticancer drug composed of the 5-FU prodrug tegafur and two 5-FU modulators; it has achieved high response rates in patients with gastric cancer in phase II studies [7, 8]. In the Japan Clinical Oncology Group (JCOG) 9912 trial, which compared S-1, cisplatin plus irinotecan, and 5-FU, S-1 demonstrated non-inferiority compared to 5-FU [9]. In another phase III trial that compared S-1 alone to S-1 plus cisplatin (SPIRITS trial), S-1 plus cisplatin showed a significantly higher response rate (54 vs. 31%), longer progression-free survival (PFS; 6.0 vs. 4.0 months), and longer overall survival (OS; 13 vs. 11 months) [4]. Also, in a large, non-Japanese, phase III trial (the First-Line Advanced Gastric Cancer Study; FLAGS trial), S-1 plus cisplatin was associated with fewer toxic effects and demonstrated non-inferiority compared with 5-FU plus cisplatin by exploratory analysis [6]. Therefore, S-1 plus cisplatin is now considered to be one of the standard regimens for metastatic or recurrent gastric cancer.

In addition, the ACTS-GC trial has demonstrated that S-1 is also effective as adjuvant chemotherapy for Japanese patients who have undergone curative gastrectomy for locally advanced gastric cancer [10]. However, approximately 30% of patients still develop recurrence after curative resection followed by adjuvant S-1 [10]. As few patients who received adjuvant chemotherapy were included in the phase III trials described above [4, 7, 9], it is unclear whether patients who develop recurrence after adjuvant S-1 could achieve efficacy with S-1 plus cisplatin similar to that achieved in patients without adjuvant chemotherapy. To address this issue, we conducted the following multi-institutional retrospective analysis.

Patients and methods

Patients

This retrospective study was designed to evaluate the efficacy of first-line chemotherapy with S-1 plus cisplatin for recurrence in patients with gastric cancer who had undergone curative gastrectomy followed by adjuvant S-1 chemotherapy. Patients with histopathologically proven recurrent gastric adenocarcinoma after gastrectomy and lymph node dissection with no residual tumor were eligible for analysis. Additional eligibility criteria were: (1) previous adjuvant S-1 chemotherapy at a planned standard dose and schedule (80 mg/m^2 for 28 consecutive days followed by a 14-day rest; 42-day cycles to be repeated for 1 year); (2) Eastern Cooperative Oncology Group performance status (ECOG PS) 0–2; (3) adequate bone marrow, hepatic, and renal function to be treated with S-1 plus cisplatin; (4) evaluable lesions according to Response Evaluation Criteria in Solid Tumors (RECIST ver. 1.1); and (5) treated with a standard regimen of S-1 plus cisplatin (S-1 80 mg/m^2 for 21 consecutive days followed by a 14-day rest; cisplatin 60 mg/m^2 intravenous infusion on day 8; 35-day cycles to be repeated) [4]. Written informed consent for treatment was obtained from each patient prior to treatment initiation. The Institutional Review Board of each participating center approved the study.

Evaluation of treatment and statistical analysis

The tumor response was assessed objectively according to RECIST ver. 1.1, and the best overall response was recorded as the antitumor effect for that patient. The disease control rate (DCR) represented the percentage of patients with a complete response (CR), partial response (PR), or stable disease (SD). PFS was measured from the date of initiation of S-1 plus cisplatin to the date of progressive disease or death from any cause. Time to treatment failure

(TTF) was measured from the date of initiation of S-1 plus cisplatin to the date of last administration of S-1. OS was estimated from the date of initiation of S-1 plus cisplatin to the date of death or last follow-up visit, using the Kaplan–Meier method. The interval from the last administration of adjuvant S-1 to recurrence was defined as the recurrence-free interval (RFI).

The Cox proportional hazards model was used to estimate the impact of the RFI on TTF, PFS, and OS, with adjustment for other factors that were shown to be significant with a univariate log-rank test. *P* values for testing differences between proportions and response rates were calculated with χ^2 tests for homogeneity or for trend, or with Fisher's exact test. Results were considered to be statistically significant when the *P* value was <0.05. All reported *P* values are two-sided. In particular, we compared the response rate, DCR, time to progression (TTP), PFS, and OS between patients with RFIs of ≥6 and <6 months, because several clinical trials in the first-line setting set this interval of ≥6 months as an inclusion criterion [5, 9, 11].

Results

Patient characteristics

A total of 406 patients with recurrent gastric cancer after adjuvant S-1 chemotherapy had received chemotherapy at 18 institutions until October 2010. Among them, 57 patients (14.0%) had received S-1 plus cisplatin as first-line chemotherapy for recurrence. After the exclusion of 5 patients (1 patient with a non-evaluable lesion and 4 patients with insufficient data), 52 patients were included in the final

Table 1 Patient characteristics

Characteristic	All ($n = 52$)	RFI <6 months ($n = 25$)	RFI ≥6 months ($n = 27$)	*P* value
Age, years				
Median (range)	61 (32–77)	59 (32–77)	62 (32–77)	
Gender, *n* (%)				
Male	30 (58)	15 (60)	15 (56)	0.75
Female	22 (42)	10 (40)	12 (44)	
ECOG PS at recurrence, *n* (%)				
0	32 (62)	11 (44)	21 (78)	*0.012*
1	20 (38)	14 (56)	6 (22)	
Histological type[a], *n* (%)				
wel or mod	27 (52)	10 (40)	17 (63)	0.1
por or sig	24 (46)	15 (60)	9 (33)	
Other	1 (2)	–	1 (4)	
Pathological stage[a], *n* (%)				
Stage I or II	8 (15)	4 (16)	4 (15)	0.57
Stage IIIA	17 (33)	6 (24)	11 (41)	
Stage IIIB	15 (29)	8 (32)	7 (26)	
Stage IV	12 (23)	7 (28)	5 (19)	
Site of recurrence, *n* (%)				
Peritoneum	21 (40)	7 (28)	14 (52)	0.08
Lymph node	25 (48)	13 (52)	12 (44)	0.59
Liver	14 (27)	10 (40)	4 (15)	*0.041*
Lung	4 (8)	3 (12)	1 (4)	0.262
Bone	6 (12)	1 (4)	5 (19)	0.102
Local	2 (4)	1 (4)	1 (4)	0.96
Number of recurrence sites, *n* (%)				
1	38 (73)	18 (72)	20 (74)	0.87
2 or more	14 (27)	7 (28)	7 (26)	

P values shown in italics indicate significant differences

RFI Recurrence-free interval, *PS* performance status, *ECOG* Eastern Cooperative Oncology Group, *wel* well-differentiated adenocarcinoma, *mod* moderately differentiated adenocarcinoma, *por* poorly differentiated adenocarcinoma, *sig* signet-ring-cell-like carcinoma

[a] According to the Japanese classification

analysis (Table 1). The median duration of adjuvant S-1 chemotherapy was 8.1 months (range 0.7–37.4 months), and the median RFI since the last administration of adjuvant S-1 was 6.4 months (range 0–81.3 months). Thirty of the 52 patients (57.7%) completed the planned duration of adjuvant S-1 therapy. In contrast, 14 patients discontinued S-1 due to disease recurrence, and 8 patients stopped therapy due to toxicity or patient refusal. Other than PS and liver metastasis, characteristics did not differ significantly between patients with an RFI of ≥6 months ($n = 27$) and those with an RFI of <6 months ($n = 25$) (Table 1).

Treatment results and efficacy

The median TTF was 4.1 months (95% confidence interval [CI] 2.5–5.1 months), with a median duration of follow-up of 32 months. Forty-four patients discontinued S-1 plus cisplatin due to disease progression ($n = 40$, 90.9%) or toxicity ($n = 4$, 9.1%). Of the 36 patients with measurable lesions, 7 achieved a CR ($n = 3$) or a PR ($n = 4$), and 13 were evaluated as having SD, for an overall response rate of 19.4% (95% CI 7.0–37.0%) and a DCR of 55.6% (95% CI 38.1–72.1%). The median PFS was 4.8 months (95% CI 3.9–6.2 months), and the median OS of all patients was 12.2 months (95% CI 10.2–16.6 months) (Fig. 1). Of the 44 patients who had discontinued S-1 plus cisplatin, 31 (70.4%) received second-line or third-line chemotherapy, including taxanes ($n = 25$) or irinotecan ($n = 17$).

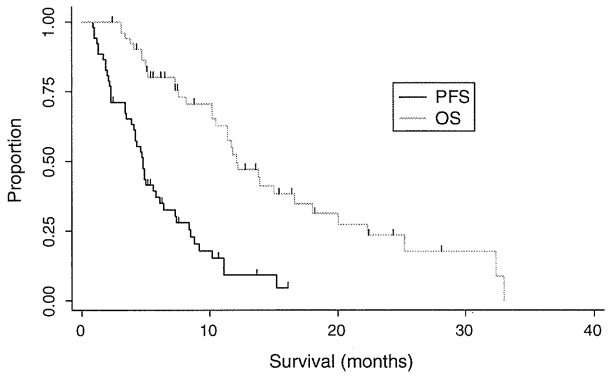

Fig. 1 Progression-free survival (*PFS*) and overall survival (*OS*) in all patients. The median PFS was 4.8 months (95% confidence interval [CI] 3.9–6.2 months), and the median OS was 12.2 months (95% CI 10.2–16.6 months). *PFS* progression-free survival, *OS* overall survival

Significance of the RFI

The response rate was significantly better in patients with an RFI of ≥6 months (37.5%; 95% CI 14–61%) than that in patients with an RFI of <6 months (5.0%; 95% CI 0–15%, $P = 0.014$, Table 2). In addition, compared with patients with an RFI of <6 months, patients with an RFI of ≥6 months had a significantly longer TTF (2.5 vs. 5.1 months, respectively, $P = 0.025$), longer PFS (2.3 vs. 6.2 months, respectively, $P < 0.001$, Fig. 2), and longer OS (7.3 vs. 16.6 months, respectively, $P = 0.003$, Fig. 2). According to a multivariate Cox model including PS and reason for discontinuation of adjuvant S-1, an RFI of 6 months was still significantly associated with PFS (hazard ratio [HR] 0.35, 95% CI 0.16–0.77, $P = 0.009$) and OS (HR 0.21, 95% CI 0.08–0.54, $P = 0.001$), although the association with TTF was not significant (HR 0.55, 95% CI 0.27–1.12, $P = 0.1$). When we divided the patients into two groups based on an RFI of 12 months, no significant difference between the groups was found in response rate, TTP, PFS, or OS.

Discussion

In the ACTS-GC study, adjuvant S-1 chemotherapy significantly improved the survival of patients who had undergone curative gastrectomy for locally advanced gastric cancer [10]. On the other hand, several small studies have suggested that patients with recurrence after adjuvant S-1 were refractory to S-1-containing regimens or had a worse prognosis compared with that of patients without adjuvant chemotherapy [12–14]. Although these reports never precluded the use of adjuvant S-1 chemotherapy, they raised the issue of how to treat recurrent disease after adjuvant S-1.

In the present retrospective study, we evaluated the efficacy of S-1 plus cisplatin in patients whose gastric cancer recurred after adjuvant chemotherapy with S-1. The response rate of 19.4% and PFS of 4.8 months were

Table 2 Objective response rates in patients with measurable lesions

	n	CR	PR	SD	PD	NE	ORR (%)	95% CI (%)
All	36	3	4	13	14	2	18.8	7–32
RFI <6 months	20	0	1	6	13	0	5.0	0–15
RFI ≥6 months	16	3	3	7	1	2	37.5	14–61

CR Complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *NE* not evaluable, *ORR* objective response rate, *CI* confidence interval

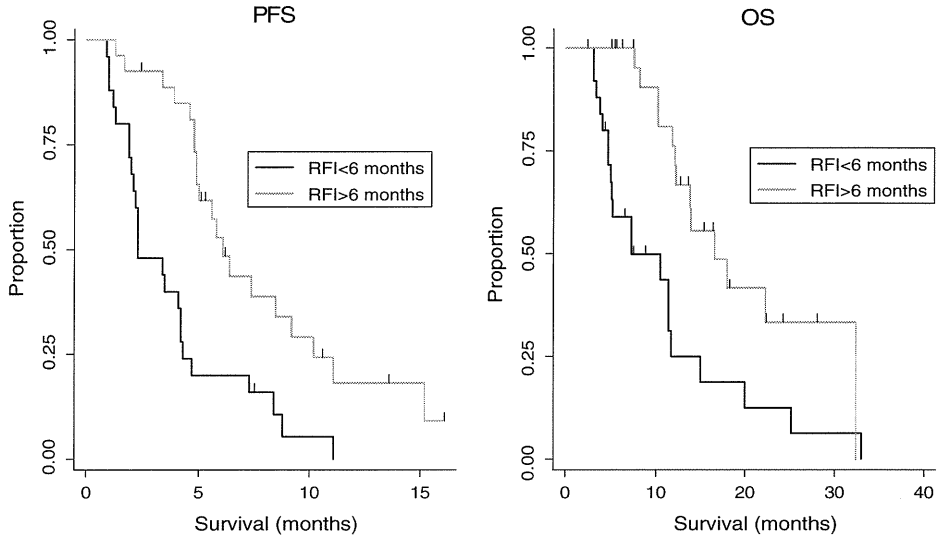

Fig. 2 Progression-free survival (*PFS*) and overall survival (*OS*) according to the length of the recurrence-free interval (*RFI*). Patients with an RFI of ≥6 months had a significantly longer median PFS (6.2 vs. 2.3 months, $P < 0.001$) and OS (16.6 vs. 7.3 months, $P = 0.003$) than patients with an RFI of <6 months. *RFI* recurrence-free interval, PFS progression-free survival, OS overall survival

relatively worse compared with those in the SPIRITS study [4]. However, our results also suggested that patients with an RFI of ≥6 months who received S-1 plus cisplatin had a significantly better response rate, longer PFS, and longer OS compared to patients with an RFI of <6 months. The efficacy of S-1 plus cisplatin for patients with an RFI of ≥6 months in this study was almost compatible with that of patients in the SPIRITS trial in terms of PFS and OS, although these results should be interpreted cautiously due to the heterogeneity of the characteristics of the patients in the two studies. Although no prospective study has evaluated any chemotherapy specifically for patients who have failed adjuvant S-1, Kang and colleagues [15] conducted a phase II study of capecitabine plus cisplatin for 32 patients with gastric cancer that recurred after adjuvant chemotherapy with doxifluridine or 5-FU-containing regimens. They reported a response rate of 28% and a median TTP of 5.8 months, and concluded that capecitabine plus cisplatin was effective as first-line treatment in patients with recurrent gastric cancer after fluoropyrimidine-based adjuvant chemotherapy. In their report, the response rates (21 vs. 39%, $P = 0.427$), TTF (8.3 vs. 5.4 months, $P = 0.072$), and OS (14.1 vs. 9.3 months, $P = 0.075$) tended to be better in patients with an RFI of >6 months ($n = 13$) than in patients with an RFI of ≤6 months ($n = 19$), although the differences did not reach statistical significance [15]. These results were also consistent with those of previous studies in patients with other types of cancer, which suggested the importance of the RFI or treatment-free interval as a predictive marker of responsiveness to similar types of chemotherapy after recurrence [16–18]. Additionally, in the present study, the RFI cut-off value of 6 months was better than that of 12 months for predicting better outcomes and this finding may support the use of the conventional exclusion criteria in clinical trials in the first-line setting, which excluded patients who experienced disease recurrence within 6 months after the last adjuvant chemotherapy [5, 9, 11]. Therefore, selected patients with an RFI of ≥6 months with sufficient organ function may be adequately treated as chemo-naïve patients with standard chemotherapies such as S-1 plus cisplatin.

In contrast to the results for patients with an RFI of ≥6 months, the response rate in patients with an RFI of <6 months in the present study seemed to be worse than that of commonly used second-line chemotherapy regimens such as irinotecan and taxane combinations, which have a reported response rate of approximately 20% for patients with gastric cancer who received prior chemotherapy with fluoropyrimidines alone [18–23]. Based on these results, it may be suggested that the evaluation of chemotherapy regimens other than S-1 plus cisplatin might be warranted for the initial treatment of gastric cancer recurrence after adjuvant S-1. The response rate of 5.0% in our subset of patients with an RFI of <6 months was also lower than that reported previously by Kang et al. for capecitabine plus cisplatin after adjuvant chemotherapy (21%) [15]. The exact reasons for this difference are unknown. One possible reason is that Kang and colleagues did not use the same fluoropyrimidine (capecitabine after doxifluridine or 5-FU), and this choice might have contributed to a higher response in regard to early recurrence, although rechallenge with different types of fluoropyrimidine after the failure of another drug is still controversial in several types of cancer [24–28]. Second, the planned dose intensity of cisplatin as another key drug for gastric cancer was higher in their capecitabine plus cisplatin regimen (60 mg/m^2 every 3 weeks) [15] than that in the S-1 plus cisplatin regimen (60 mg/m^2 every 5 weeks). The efficacy of capecitabine plus cisplatin compared with other

chemotherapy (irinotecan, taxane or irinotecan plus cisplatin) for recurrence after adjuvant S-1 should be evaluated in future clinical trials.

It is important to note the limitations of the present study. First, it was retrospective, and treatment after recurrence was selected by each physician individually. Considering the low proportion of patients who received S-1 plus cisplatin after recurrence (14.0%), the selected population may have been biased toward patients with good performance status (PS) and low tumor burden. Second, toxicity was not evaluated in this study, although the proportion of patients who discontinued S-1 plus cisplatin due to toxicity was low. Third, human epidermal growth factor receptor 2 (HER2) status was not evaluated. Trastuzumab, a humanized monoclonal antibody against HER2, has recently been shown to improve the prognosis of HER2-positive advanced gastric cancer [29], and the HER2 status of all gastric cancer types should be evaluated, even in this setting of recurrent disease. Fourth, the moderate sample size in a single-country study is another limitation; therefore, it would be better to validate the significance of the RFI after adjuvant failure on the PFS in other cohorts as well.

In conclusion, this is the first report to have evaluated the efficacy of chemotherapy with S-1 plus cisplatin in patients with gastric cancer that recurred after adjuvant chemotherapy with S-1. S-1 plus cisplatin was effective in such patients, especially in those with an RFI of $\geq$6 months. Further well-defined, prospective trials in this important patient population are required to identify optimal treatment regimens.

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Conflict of interest None of the authors have financial or personal conflicts of interest to disclose.

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CLINICAL TRIAL

High expression of ATP-binding cassette transporter ABCC11 in breast tumors is associated with aggressive subtypes and low disease-free survival

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Abstract ATP-binding cassette (ABC) transporters are membrane proteins that efflux various compounds from cells, including chemotherapeutic agents, and are known to affect multidrug resistance. Recent reports disagree on whether ABCC11 is a risk factor for breast tumorigenesis, but its expression in breast cancer is poorly investigated. We hypothesized that both frequency and expression levels of ABC transporters in breast tumors would vary by cancer subtype, and be associated with prognosis. Here, we constructed a tissue microarray breast tumor samples from 281 patients, and analyzed expressions of ABCB1, ABCC1, ABCC11, and ABCG2 immunohistochemically. Breast cancer subtypes were determined by immunohistochemistry of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2 (HER2). Protein expression was correlated to clinicopathological characteristics, clinical follow-up, and pathological complete response to neoadjuvant chemotherapy. The tissue microarray comprised 191 luminal A (68.0 %), 17 luminal B (6.0 %), 27 HER2 (9.6 %), and 46 triple-negative (16.4 %) samples. ABCC1 and ABCC11 expressions were associated with significantly shorter disease-free survival ($P = 0.027$ and $P = 0.003$, respectively). ABCC1, ABCC11, and ABCG2, but not ABCB1, were expressed significantly more, and more frequently, in aggressive subtypes. Patients with HER2+ and triple-negative tumor subtypes that expressed high levels of ABCC11 had significantly worse disease-free survival ($P = 0.017$ and $P < 0.001$, respectively). We have shown, for the first time, that ABCC1, ABCC11, and ABCG2 are highly expressed in aggressive breast cancer subtypes, and that tumor ABCC11 expression is associated with poor prognosis.

Keywords Breast cancer · ATP-binding cassette transporters · ABCC11 · Tissue microarray · Subtype

Introduction

Breast cancer is a heterogeneous disease [1]. DNA microarray profiling studies on breast cancer have identified distinct subtypes: luminal A, luminal B, human epidermal

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growth factor receptor 2 (HER2)-enriched, and triple-negative (which is sometimes further subdivided into the core-basal and five-negative subtypes) [2]. These subtypes are reportedly associated with differences in resistance to chemotherapy [3–5] and subsequent outcomes [6, 7]. Several mechanisms affect how cancer cells become resistant to cytotoxic drugs, which include efflux of the drug compound from cancer cells, and others such as mutation, overexpression of the drug's targets, and drug inactivation [8].

The ATP-binding cassette (ABC) transporters are transmembrane proteins that use ATP to transport various molecules across extra- and intra-cellular membranes. This function is thought to have evolved as a xenobiotic protective mechanism [9]. Of the 49 human ABC transporters so far identified (which have been classified into seven subfamilies), ABCA2, ABCB1, ABCC1–6, ABCC11, and ABCG2 have been associated with chemoresistance in breast cancer [8]. Unfortunately, all clinical trials that have targeted ABC transporters failed to improve outcomes [10]. One explanation for this is that they all targeted ABCB1 [also known as MDR1, permeability glycoprotein 1 (P-glycoprotein or Pgp), and cluster of differentiation 243 (CD243)]. This led us to hypothesize that other ABC transporters may be more important for drug resistance.

ABCC11 is a member of the ABCC1 (also known as MDR-associated protein) sub-family. A single nucleotide polymorphism (SNP) in the *ABCC11* gene was shown to be responsible for "wet earwax" in humans [11]. Reports as to whether *ABCC11* is a risk factor for breast tumorigenesis conflict; although this gene was originally shown to be a risk factor for development of breast cancer among Japanese women [12], it is reportedly not the case in Caucasian women [13, 14]. There has been no investigation of ABCC11 protein expression levels in breast tumors or their association with cancer subtype and prognosis. We hypothesized that both frequency and expression levels of ABC transporters (ABCB1, ABCC1, ABCC11, and ABCG2) in breast tumors would differ by cancer subtype and be associated with prognosis. Here, utilizing a tissue microarray newly constructed from 281 breast cancer samples, we analyzed the expression of these transporters in light of breast cancer subtype and prognosis, as well as investigating the effects of neoadjuvant chemotherapy.

Methods

Tissue sources and clinical characteristics

Tissues for this study were obtained from 281 patients treated in Yokohama City Medical Center, Japan, between 2006 and 2008, involving all stages of breast cancer. This study was approved by the Institutional Review Board of Yokohama City University, Kanagawa, Japan, and the patients gave their informed consent before their inclusion in the study. Core biopsy samples taken prior to treatment were obtained from 50 patients who received neoadjuvant chemotherapy (35 patients received anthracycline followed by taxane; 14 received anthracycline alone; and one received taxane alone). One hundred and eight patients received adjuvant chemotherapy after surgery (45 received anthracycline followed by taxane; 38 received anthracycline alone; 15 received taxane alone; and 10 received other regimens) and 208 patients received adjuvant hormonal therapy (tamoxifen and luteinizing hormone-releasing hormone-agonist for 61 premenopausal patients; tamoxifen or aromatase inhibitor for 147 postmenopausal patients). None of the tissues described here was obtained after any treatment. All the patients were followed up at least every 3 months after surgery. The mean observation period was 49 months (range: 28–60 months). The clinical characteristics are presented in Table 1.

Table 1 Patients' characteristics

	N	%
Age		
<65	197	70.1
65≦	80	28.5
	4	1.4
Menstruation states		
Pre menopause	87	31.0
Post menopause	154	54.8
NA	40	14.2
Estrogen receptor		
Positive	210	74.8
Negative	71	25.2
NA	0	0.0
Progesterone receptor		
Positive	162	42.7
Negative	119	57.3
NA	0	0.0
HER2 overexpression		
Present	44	15.7
Absent	237	84.3
NA	0	0.0
Basal markers		
Basal	34	12.1
Non basal	235	83.4
NA	12	4.5
Subtype		
Luminal A	191	68.0
Luminal B	17	6.0
HER2	27	9.6

Table 1 continued

	N	%
Triple negative	46	16.4
Core basal	26	9.3
Five-negative	20	7.1
Tumor stage		
T1	123	43.8
T2	122	43.4
T3	11	3.9
T4	19	6.8
NA	6	2.1
Node		
N0	150	53.4
N1	83	29.5
N2	23	8.2
N3	11	3.9
NA	14	5.0
Metastases		
M0	259	92.2
M1	6	2.1
NA	16	5.7
TNM stage		
1	106	37.8
2	122	43.4
3	31	11.0
4	6	2.1
NA	16	5.7
Observation time (days)	1458 ± 509[a]	

[a] Expressed as mean ± standard deviation

Tissue microarray

The tissue microarray was constructed by taking 3.0-mm cores from representative areas of surgical specimens from patients using a KIN-2 tissue arrayer (Azumaya, Tokyo, Japan), and re-embedding these cores into a gridded paraffin block. Tissue cores were excluded from the tissue microarray if they fail to adhere to the glass slide, did not include invasive carcinoma, or were a non-interpretable specimen.

Immunohistochemistry

Formalin-fixed paraffin-embedded tissue blocks were sliced into 5-μm sections. The sections were baked at 60 °C, deparaffinized in xylene, and gradually rehydrated in ethanol. Sections were boiled in antigen retrieval solution (Funakoshi, Japan) for 30 min. Activity of endogenous peroxidase was blocked by 20 min of quenching in 0.3 % H_2O_2 and methanol; the sections were then incubated in 5 % rabbit serum for ABCB1 and ABCC1, or goat serum for ABCC11 and ABCG2. Immunohistochemical reactions were performed overnight at 4 °C using monoclonal mouse antibodies against ABCB1 (C219; 1:100; Abcam, UK), monoclonal rat antibodies against ABCC1 (MRPr1; 1:40; Monosan, The Netherlands), polyclonal rabbit antibodies against ABCC11 (1:500) [15], or monoclonal mouse antibodies against ABCG2 (BXP-21; 1:100; Abcam). For the triple-negative subtype, cytokeratin 5/6 (D5/16 B4; Dako, Denmark) and epidermal growth factor receptor (EGFR; Roche Diagnostics K.K, Japan,) were used for subdivision into the core-basal or non-basal (five-negative) subtypes. After washing, the slides were incubated with biotinylated antibodies (15 min, room temperature) and streptavidin-biotinylated peroxidase complex (5 min, room temperature). 3,3′-diaminobenzidine (Dako Japan, Tokyo, Japan) was used as the chromogen. All sections were counterstained with Meyer's hematoxylin.

Evaluation of staining

Staining results were assessed by two pathologists independently, using a 4-point scoring system as shown in Fig. 1: 0 = invasive tumor cells present in the tissue core with no staining; 1 = invasive tumor cells present with weak staining intensity; 2 = invasive tumor cells present with strong staining intensity and <30 % of tumor cells stained or intermediate staining intensity in ≥30 % of tumor cells; and 3 = invasive tumor cells present with strong staining in ≥30 % of tumor cells. To evaluate positivity, both membranous and/or cytoplasmic staining scoring 2 or above was considered positive (high expression). CK5/6 and EGFR were considered positive when cytoplasmic and/or membranous staining of invasive carcinoma cells was observed, regardless of intensity.

Genotyping

Genotyping of ABCC11 by the SmartAmp method was performed as previously reported [12].

Statistical analysis

Statistical analysis used SPSS 19.0 for Windows software (SPSS Inc., Chicago, IL). Correlations among the clinicopathologic parameters and each transporter were evaluated by the Pearson χ^2 test, the Fisher exact test, and the Mann–Whitney test. Tukey-type multiple comparison analyses with the χ^2 test and Mantel test were carried out to compare expression of each transporter among the subtypes. Patient outcomes were assessed by disease-free survival. Survival distributions were estimated by the Kaplan–Meier method; differences were compared using the log-rank test. The multivariate Cox proportional hazard regression method

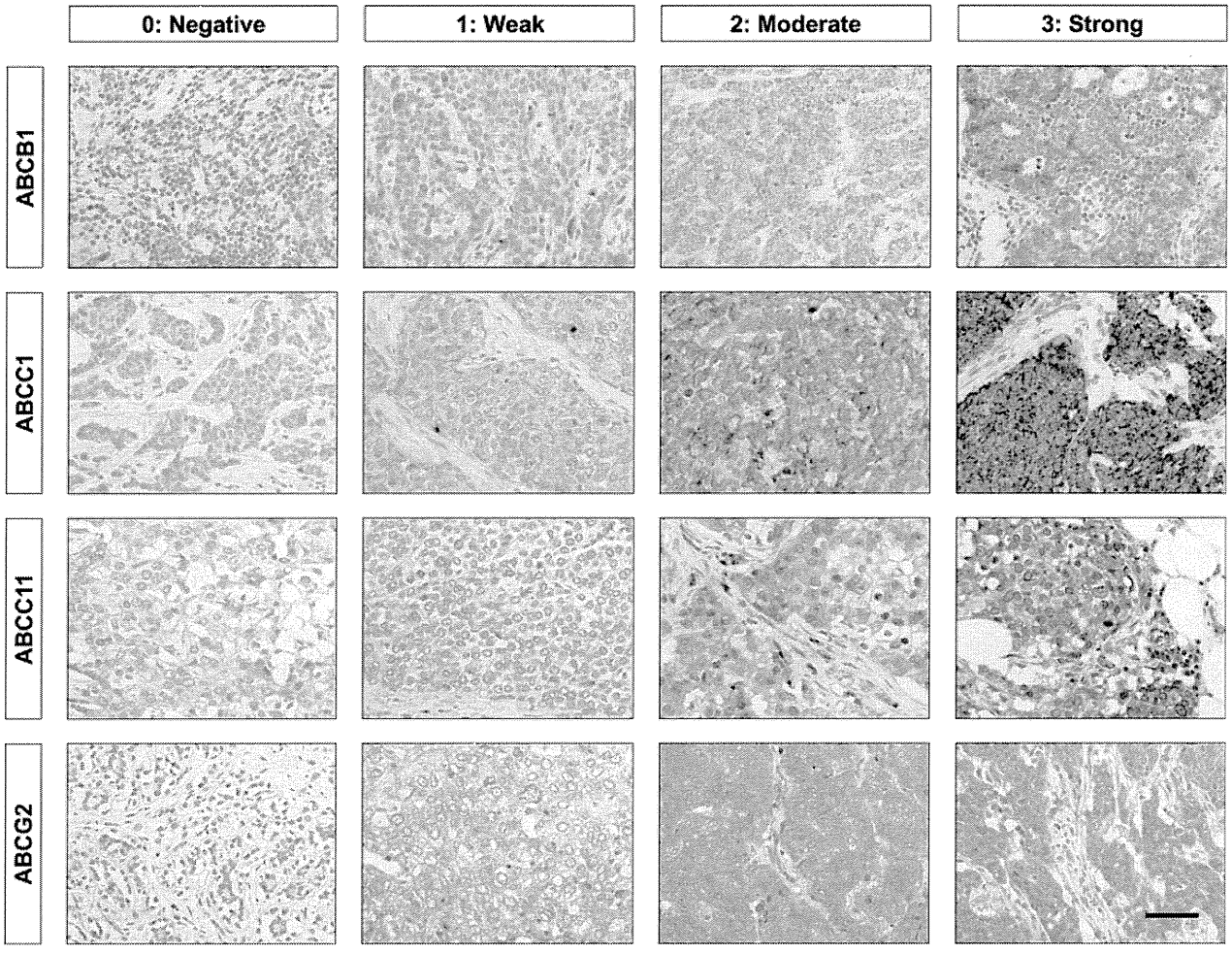


Fig. 1 4-Point scoring system for ABCB1, ABCC1, ABCC11, and ABCG2 protein expression. Our tissue microarray contained 281 breast tumor tissues, and was stained with antibodies against ABCB1 (1:100), ABCC1 (1:40), ABCC11 (1:500), and ABCG2 (1:100). Stain intensity was graded as negative (0), weak (1), moderate (2), or strong (3). Representative images are shown under high magnification. *Scale bar*: 50 μm

was used to determine the independent prognostic value. $P < 0.05$ was considered statistically significant.

Results

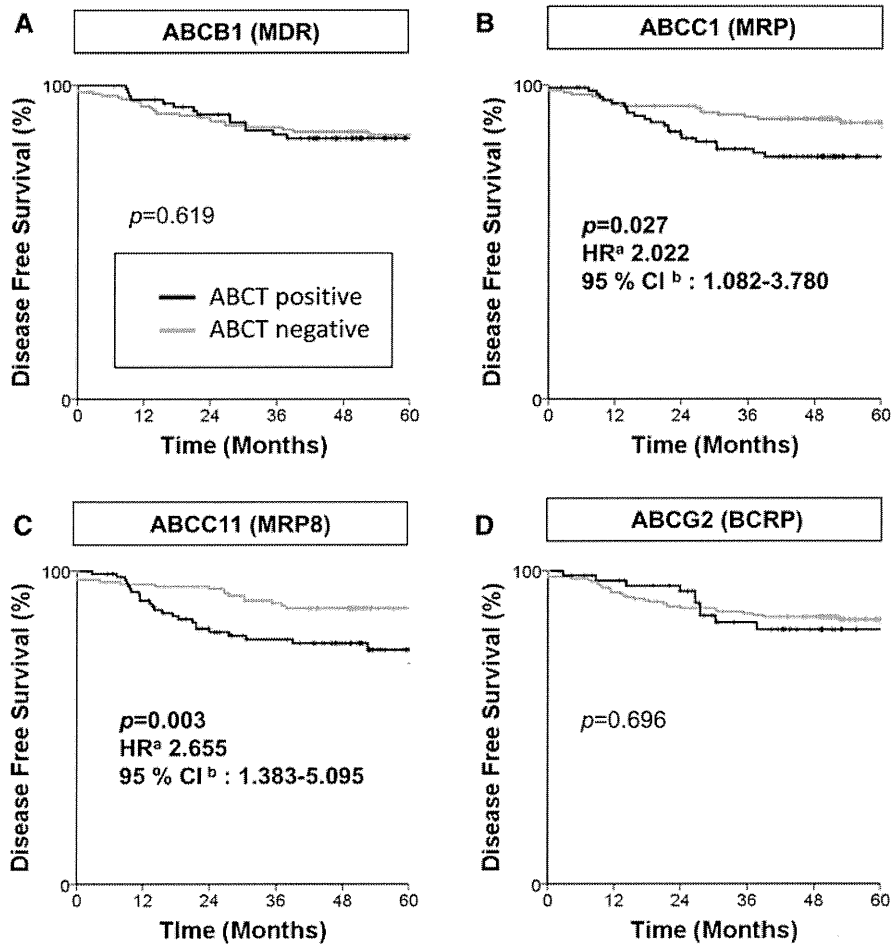
Characteristics of samples used for the tissue microarray

Subtypes of the 281 samples on the tissue microarray were determined using immunohistochemistry for the estrogen receptor (ER), progesterone receptor (PgR), and HER2, as previously reported [5, 16]. Patients' and tumor characteristics used for the tissue microarray are summarized in Table 1. The numbers of cases of the respective subtypes were: luminal A (ER+ and HER2−): 191 (68.0 %); luminal B (ER+ and HER2+): 17 (6.0 %); HER2 (ER− and HER2+): 27 (9.6 %); and triple-negative (ER− and HER2−): 46 (16.4 %). Triple-negative tumors were further sub-divided into two groups, core-basal (CK5/6+ and/or EGFR+) and five-negative (CK5/6− and EGFR−). The core-basal subtype constituted 56.5 % (26/46) of triple-negative tumors.

Associations between ABC transporter expression and clinical features of the tumors are shown in Table 2. ABCB1 was detected in 32.4 % (91/277) of the tumors, ABCC1 in 39.1 % (110/279), ABCC11 in 40.2 % (113/259), and ABCG2 in 24.2 % (68/278). There was no association between ABCB1 expression and any clinical features. ABCG2 was more frequently highly expressed in young premenopausal patients. High expressions of ABCC1 and ABCG2 were significantly more frequent in ER− tumors than in ER + ones ($P = 0.001$ and $P = 0.006$, respectively). There was no association between HER2 expression and ABC transporter expression.

Table 2 The expression of ABC transporters and clinical features

	ABCB1			*p* Value	ABCC1			*p* Value	ABCC11			*p* Value	ABCG2			*p* Value
	Negative	Positive	NA		Negative	Positive	NA		Negative	Positive	NA		Negative	Positive	NA	
N (%)	186 (66.2 %)	91 (32.4 %)	4 (1.4 %)		169 (60.2 %)	110 (39.1 %)	2 (0.7 %)		146 (52.0 %)	113 (40.2 %)	22 (7.8 %)		210 (74.7 %)	68 (24.2 %)	3 (1.1 %)	
Age																
<65	125 (63.5 %)	68 (34.5 %)	4 (2 %)	0.14	119 (60.4 %)	76 (38.6 %)	2 (1 %)	0.54	97 (49.2 %)	83 (42.1 %)	17 (8.7 %)	0.13	138 (70.0 %)	56 (28.4 %)	3 (1.6 %)	<0.01
65≦	58 (72.5 %)	22 (27.5 %)	0		49 (61.2 %)	31 (38.8 %)	0		47 (58.8 %)	28 (35.0 %)	5 (6.2 %)		69 (86.3 %)	11 (13.7 %)	0	
Menstruation status																
Pre menopause	55 (63.2 %)	29 (33.3 %)	3 (3.5 %)	0.40	51 (58.6 %)	36 (41.4 %)	0	0.32	41 (47.2 %)	37 (42.5 %)	9 (10.3 %)	0.35	54 (62.1 %)	31 (35.6 %)	2 (2.3 %)	<0.01
Post menopause	104 (67.5 %)	49 (31.8 %)	1 (0.7 %)		95 (61.7 %)	57 (37.0 %)			81 (52.6 %)	63 (40.9 %)	10 (6.5 %)		81 (52.6 %)	63 (40.9 %)	10 (6.5 %)	
Estrogen receptor																
Negative	50 (70.4 %)	21 (29.6 %)	0	0.61	30 (42.3 %)	39 (54.9 %)	2 (2.8 %)	<0.01	37 (52.1 %	27 (38.0 %)	7 (9.9 %)	0.65	43 (60.6 %)	27 (38.0 %)	1 (1.4 %)	<0.01
positive	135 (64.5 %)	70 (33.5 %)	4 (2.0 %)		139 (66.5 %)	70 (33.5 %)	0		108 (51.7 %)	86 (41.1 %)	15 (7.2 %)		166 (79.4 %)	41 (19.6 %)	2 (1.0 %)	
Progesterone receptor																
Negative	78 (65.5 %)	38 (31.8 %)	3 (2.7 %)	0.78	63 (52.9 %)	54 (45.4 %)	2 (1.7 %)	0.06	57 (47.9 %)	49 (41.2 %)	13 (10.9 %)	0.55	81 (68.0 %)	35 (29.4 %)	3 (2.6 %)	0.15
Positive	107 (66.5 %)	53 (32.9 %)	1 (0.6 %)		106 (65.8 %)	55 (34.2 %)	0		88 (54.7 %)	64 (40.0 %)	9 (5.3 %)		128 (79.5 %)	33 (20.5 %)	0	
HER2 expression																
Absent	155 (66.0 %)	77 (32.8 %)	3 (1.2 %)	1.00	146 (62.1 %)	88 (37.4 %)	1 (0.5 %)	0.18	123 (52.4 %)	97 (41.2 %)	15 (6.4 %)	1.00	179 (76.1 %)	55 (23.4 %)	1 (0.5 %)	0.33
Present	179 (76.1 %)	55 (23.4 %)	1 (0.5 %)		22 (50.0 %)	21 (47.7 %)	1 (2.3 %)		21 (47.7 %)	16 (36.4 %)	7 (15.9 %)		29 (65.9 %)	13 (29.5 %)	2 (4.6 %)	



[a] *HR* hazard ratio, [b] *CI* confidence interval

Fig. 2 Kaplan–Meier disease-free survival curves according to expression of ABCB1 (**a**), ABCC1 (**b**), ABCC11 (**c**), and ABCG2 (**d**). The *thick bold line* indicates positivity; and the *light gray line* indicates negativity, for the respective transporters. Only the ABCC1+ and ABCC11+ groups showed significantly improved survival ($P = 0.027$ and $P = 0.003$, respectively)

Expression of ABCC1 and ABCC11 is associated with poor patient survival

We compared expression of each transporter and patient disease-free survival (Fig. 2). In the entire study group, patients with ABCC1+ or ABCC11+ tumors had significantly shorter disease-free survival compared to patients with corresponding ABCC1− or ABCC11− tumors ($P = 0.027$ or $P = 0.003$, respectively).

ABC transporters are more frequently highly expressed in aggressive subtypes of breast cancer

Because breast cancer subtypes are associated with different clinical behaviors [2], we further analyzed clinical outcomes according to cancer subtype and ABC transporter expression. Expression of each transporter according to breast cancer subtype is shown in Fig. 3. The percentage of patients whose tumors expressed ABCB1 did not differ among the subtypes. ABCC1 and ABCG2 were more frequently highly expressed in triple-negative subtype, especially in the core-basal subtype, compared with the luminal A subtype, whereas highly expressed ABCC11 was more common in HER2-enriched, core-basal, and luminal A subtypes. Although core-basal tumors tended to express ABC transporters more often than five-negative tumors did, only ABCC11 showed significantly more frequent high expression in the core-basal subtype. Semi-quantification of ABC transporters expression is shown in Fig. 3b. ABCC1, ABCC11, and ABCG2 were more highly expressed in HER2-enriched and/or the core-basal subtypes, which is consistent with frequency data shown in Fig. 3a.

Patients whose tumors expressed high levels of ABCC11 tended towards decreased pathological complete responses to neoadjuvant chemotherapy

We next investigated whether there was any association between the "wet earwax" genotypes and ABCC11 expression. Figure 4a and b show the relationship between *ABCC11* genotypes and ABCC11 expression in breast