inhalation of airborne spores, primary infection develops in the respiratory tract. Since the actions of neutrophils and phagocytes are the major host defense mechanisms against mucormycosis, patients with neutropenia or dysfunction of phagocytes are at high risk of developing the disease [7].

Clinical manifestation of mucormycosis can be divided into five clinical categories: (1) rhinocerebral, (2) pulmonary, (3) cutaneous, (4) gastrointestinal, and (5) disseminated. These forms of mucormycosis tend to occur in patients with specific risk factors. For example, those with diabetic ketoacidosis typically develop the rhinocerebral form [9], whereas pulmonary infection occurs more often in those with malignancy. Neutropenic patients are at high risk of developing the disseminated form of the disease [10], which is characterized by a high rate of mortality.

The diagnosis of mucormycosis is based on histopathological examination of surgical or biopsied specimens. Microbiological culture is usually negative, and there are no reliable serologic or PCR-based tests for diagnosis of mucormycosis. Biopsy may be the only positive diagnostic tool for most patients.

Cases of mucormycosis in Japan have been described sporadically. We report here eight patients with hematologic malignancies who developed mucormycosis and review the recent literature.

2 Patients and methods

The medical records of patients affected by hematologic malignancies with the diagnosis of mucormycosis between 1997 and 2008 were reviewed retrospectively. The diagnosis of mucormycosis was made on the basis of histopathological examination of tissue biopsy or autopsy specimens. Patient characteristics, type of hematologic disease, clinical symptoms and signs of infection, radiological findings, site of infection, results of treatment, and autopsy or biopsy findings were collected for the analysis of mucormycosis.

Statistical analysis was performed with Dr SPSS II software. The probability of overall survival (OS) was calculated according to the Kaplan–Meier method.

3 Results

3.1 Clinical profile

Among 3,082 patients with hematologic malignancies who had been treated with chemotherapy at Jikei University Hospital between March 1997 and December 2008, eight patients suffered from mucormycosis. The background of patients is listed in Table 1. Three patients had myelodysplastic syndrome (MDS), two patients had acute myeloid leukemia (AML), one had acute lymphoblastic leukemia (ALL), one had chronic myeloid leukemia (CML), and one had multiple myeloma (MM). None of them achieved complete remission (CR) at the time of the fungal infection. Two patients were under treatment of induction chemotherapy and six patients received salvage therapy or supportive care. Six of eight patients had a neutrophil count below 500/μl and four patients were treated under laminar air flow environment, five of them received corticosteroid, and four patients had concomitant hyperglycemia (Table 1). None of the patients received the iron chelator deferoxamine.

3.2 Clinical manifestations

Clinical forms of mucormycosis were classified into rhinocerebral (1 patient), pulmonary form (2 patient) or disseminated (5 patients) (Table 1). The most frequent symptom was fever. Five of eight patients complained of facial or oral pain, headache, or chest pain. Serum β-D-glucan was elevated in two patients (Case 2, 7) and serum galactomannan antigen was elevated in one patient (Case 6). In accordance with the criteria of the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) [11], definitive premortal diagnosis of proven mucormycosis was made in only one patient (Case 3) using sinus tissue collected by biopsy. The probable diagnosis of aspergillosis was made in three patients using host factor (neutropenia), clinical criteria (ethmoid infiltration on sinonasal CT in Case 2, or dense, well-circumscribed lesions on lung CT in Case 6 and 7), and mycological evidence (detection of galactomannan antigen or β-D-glucan) (Table 1). The possible diagnosis of aspergillosis was made in one patient (Case 1). The other three patients were clinically diagnosed with pneumonia (Case 4), cellulitis (Case 5), or bacterial sepsis (Case 8). In seven out of eight patients, autopsy or postmortem biopsy was the only positive diagnostic tool.

3.3 Outcome

Five patients who were clinically diagnosed with mucormycosis or aspergillosis were treated with amphotericin B or voriconazole, and the other three patients were treated with micafungin (Table 1). A sinus tissue biopsy enabled correct diagnosis of the disease in Case 3, who was treated with amphotericin B and surgical debridement resulting in a longer survival of 6 months. However, the prognosis of mucormycosis disease was poor in our patients and median survival was 23 days (Fig. 1). Although it seems that patients who were treated with amphotericin B survived longer than those with micafungin or voriconazole

Table 1 Clinical characteristic and outcome

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Age/sex	30/M	81/M	67/M	71/M	66/M	33/M	28/M	59/F
Disease (status)	ALL (nonCR)	MDS (RCMD)	MDS (RCMD)	AML (nonCR)	MDS (RAEB-1)	AML (nonCR)	CML (BC)	MM (nonCR)
Type of treatment	Induction	Supportive care	Supportive care	Supportive care	Induction	Salvage	Salvage	Salvage
Neutropenia (<500/μl)	Yes	No	No	Yes	Yes	Yes	Yes	Yes
Steroid exposure	Yes	Yes	No	No	No	Yes	Yes	Yes
Hyperglycemia	No	No	No	Yes	Yes	Yes	Yes	No
Elevation of B-D glucan or GM	No	Yes	No	No	No	Yes	Yes	No
Patterns of mucormycosis	Pulmonary	Dissemianted	Rhinocerebral	Dissemianted	Dissemianted	Dissemianted	Pulmonary	Dissemianted
Symptom of pain	No	Head	Face	No	Oral	Chest	Chest	No
Diagnosis derived	Postmorten biopsy	Autopsy	Surgery	Autopsy	Autopsy	Autopsy	Postmorten biopsy	Autopsy
Therapy	AMPH	AMPH	AMPH	MCFG	MCFG	VRCZ	AMPH	MCFG
Survival (days)	28	34	193	14	18	8	68	23
Mixed infection		Candida				Aspergillus Bacterial	Aspergillus	Bacterial
Autopsy (site of mucormycosis)	Not done	Brain, cardiac ethmoid sinus lung, kidney	Not done	Brain, cardiac, lung, kidney, liver	Brain, cardiac, lung, kidney, liver, gut, aorta, cellutis	Brain, cardiac, lung, liver, aorta	Not done	Lung, gut

M male, *F* female, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* myelodysplastic syndrome, *RCMD* refractory cytomenia with multillineage dysplasia, *RAEB* refractory anemia with excess of blasts, *CML* chronic myeloid leukemia, *MM* multiple myeloma, *CR* complete remission, *BC* blastic crisis, *GM* galactomannan, *AMPH* amphotericin B, *MCFG* micafungin, *VRCZ* voriconazole

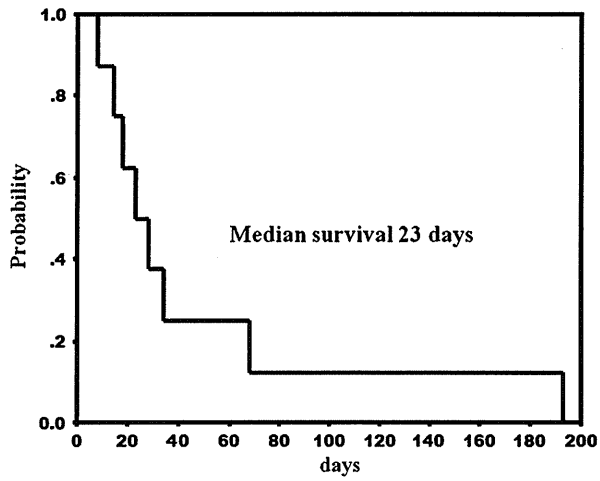

Fig. 1 Probability of survival after episode of mucormycosis. Survival was measured from initiation of uncontrollable fever to death. Estimated median survival was 23 days

(Table 1), all patients eventually died of the progressive disease of mucormycosis.

Radiographic examination demonstrated rapid progression of the disease. One patient (Case 4) had an infiltrate in the right lower lobe although there was no evidence of infiltration 10 days beforehand (Fig. 2a, b). One patient (Case 5) did not have any lesions in the central nervous system (CNS) upon the occurrence of loss of consciousness, but CT scan revealed that marked infiltrate appeared 4 days later (Fig. 2c, d).

Autopsy or postmortem biopsy was carried out on seven patients. The main site of involvement of mucormycosis was lung. Other sites of infection were brain, ethmoid sinus, heart, kidney, liver, and large bowel (Table 1). Although three patients (Case 2, 6, 7) were clinically diagnosed with the probable aspergillosis, autopsy or postmortem biopsy revealed that two patients (Case 6, 7) had both aspergillosis and mucormycosis and one patient (Case 2) had the coexistence of intestinal candidiasis but no evidence of aspergillosis. The infiltrative lesions of lung in Case 6, 7 should be caused by both aspergillosis and mucormycosis, but ethmoid lesion in Case 2 must be caused by mucormycosis. Finally, four patients in the present series revealed the coexistence of other fungus and/or bacterial infection (Table 1). An autopsy specimen obtained from a patient (Case 5) revealed many microscopic hyphae of mucormycosis in an artery (Fig. 3).

4 Discussion

Mucormycosis is a rare but emerging group of life-threatening opportunistic fungal infections. Because the actions of neutrophils and phagocytes are the major host defense mechanisms against mucormycosis, patients with neutropenia or dysfunction of phagocytes are at high risk of developing the disease [7]. Macrophages kill intracellular spores by oxidative mechanisms, whereas neutrophils can damage fungal hyphae by extracellular mechanisms [12]. Although the exact mechanisms involved remain unknown, hyperglycemia and acidosis impair the ability of phagocytes to defend an organism. Corticosteroid exposure also negatively affects the ability of macrophages to prevent germination of the spores in a murine model [13]. As such, the disease typically occurs in patients with hematologic malignancies, recipients of organ or hematopoietic stem cell transplantation, and those with diabetes mellitus. In fact, all our patients had hematologic malignancies, none of them obtained CR, five of them received corticosteroid, and four of them had hyperglycemia. But there must be important mechanisms that initiate the disease, because only a few immunocompromised patients develop mucormycosis. For example, inhaled hyphae may need to undergo some essential process to convert into the infectious spores.

Iron overload should also play a role in the development of mucormycosis [2]. Not only the release of free iron allows rapid fungal growth, but also the iron chelator deferoxamine induces marked increase of mucormycosis [7]. The use of deferoxamine should not be recommended to treat iron overload in patients with a high risk of mucormycosis. On the other hand, a new iron chelator deferasirox did not allow the organism to take up iron and did not support the growth of mucormycosis because it has a higher affinity constant for iron and, as a result, deprives the fungi of iron, inhibiting their growth [14]. In the present cases, none of the patients received deferoxamine. However, it seems that transfusional iron overload might affect the incidence of the disease because patients for whom serum iron was evaluated showed high ferritin levels (median 4,821 ng/ml, range 791–9,349 ng/ml).

Roden et al. [6] reviewed 929 cases of mucormycosis and reported that diabetes type II was the most common underlying disease, followed by no primary underlying disease, and third, hematologic malignancy. The primary site of infection at the time of initial diagnosis varied as a function of the host population. Patients with diabetes mellitus were associated with rhinocerebral mucormycosis, and cutaneous mucormycosis constituted one-half of infections in patients with no underlying disease; patients receiving deferoxamine had a tendency to develop disseminated disease [6, 9]. Pulmonary mucormycosis has been found to occur in patients with hematologic malignancies [6], and pulmonary disease in neutropenic patients has the highest incidence of dissemination [7]. In our patients, affected sites of mucormycosis were rhinocerebral (one patient), pulmonary

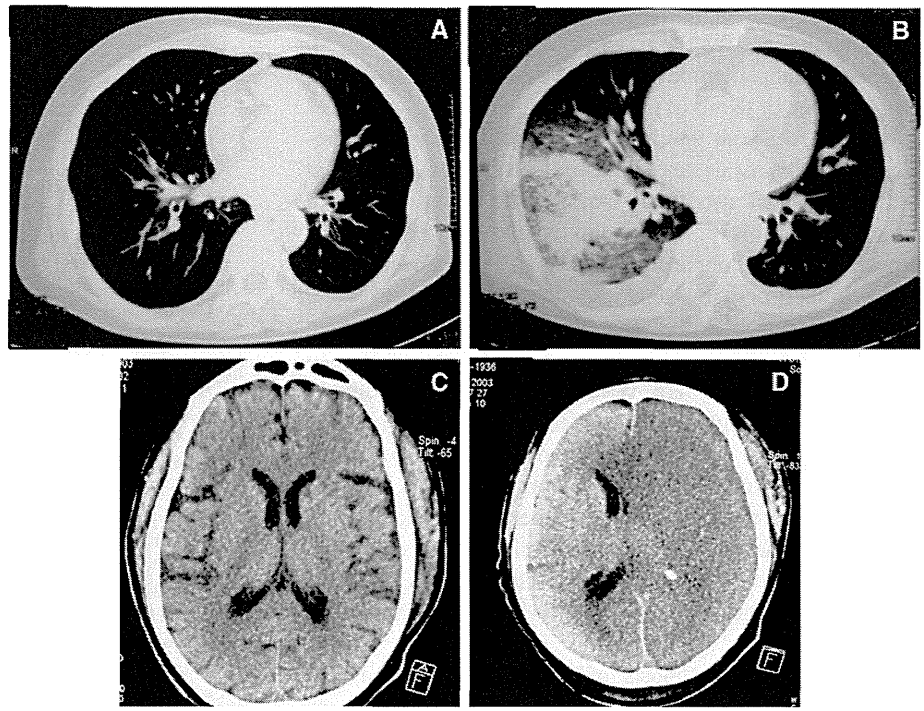

Fig. 2 Computed tomography (*CT*) scan of patient No. 4 shows a cavitated consolidation in the right lung (**b**), although there was no evidence of the infiltration 10 days beforehand (**a**). CT scan of patient No. 5 shows marked infiltrate with edema in the brain (**d**), but the patient did not have any lesions in the central nervous system at an occurrence of loss of consciousness (**c**)

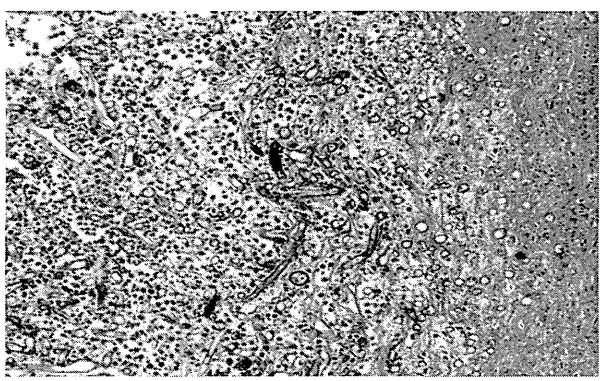

Fig. 3 Hyphae of mucormycosis occupied aortic intima and aortic media on Masson trichrome stain (patient No. 5). Non-septate hyphae of mucormycosis were unbranched

(2 patients), and disseminated (5 patients) (Table 1). Because a postmortem autopsy was not carried out in three patients (Case 1, 3, 7) with rhinocerebral or pulmonary mucormycosis, it is possible that their diseases had already developed into disseminated mucormycosis.

Since there are no specific symptoms and radiographic findings and no reliable serologic or PCR-based tests for mucormycosis, its diagnosis is generally difficult and can only be made pathologically with biopsy specimens of infected tissues. In our experience, the most frequent physical finding was fever and five out of eight patients complained of facial pain, headache, or chest pain; the symptom of pain with uncontrollable fever may be a suggestive but not specific symptom of mucormycosis. We could make correct diagnosis for only one patient (Case 3) using a collected biopsy specimen. The other seven patients were diagnosed with mucormycosis by postmortem needle biopsy or autopsy. Given the difficulty of making premortal diagnosis, there may still be a possibility that the incidence of mucormycosis has been underestimated. Unexpectedly, four patients were found to have dual infection. Findings from our patients suggested that, even when patients are diagnosed with bacterial or fungal infection other than mucormycosis, we should suspect mucormycosis if they do not respond to treatments with antifungal agents and/or antibiotics.

The success of treatment of mucormycosis may require the following four steps: (1) early diagnosis, (2) removal of risk factors such as neutropenia, hyperglycemia, and administration of deferoxamine, (3) surgical debridement, and (4) antifungal therapy [15]. Surgical debridement of pulmonary disease improved survival more than that in patients treated with antifungal therapy alone [16–19]. However, patients with neutropenia usually develop disseminated disease in the early stage and multiple diseases do not allow for surgical resection. For success of surgical treatment, one of the most important issues is early diagnosis because focal lesions can be excised before they progress to disseminate. Therefore, aggressive biopsy should be undertaken.

There have been no prospective randomized trials to define the optimal antifungal therapy for mucormycosis.

However, as amphotericin B remains the only licensed antifungal agent for the treatment of mucormycosis [15], liposomal amphotericin B in the highest tolerable dosage must be one of the most recommended antifungal therapies in Japan. Although the optimal dose of liposomal amphotericin B remains unclear, doses in the range 10–15 mg/kg/day have been used [20]. Another attractive agent for mucormycosis is posaconazole, which is a new broad-spectrum triazole not available in Japan [21]. In neutropenic mice model, posaconazole was statistically less effective than amphotericin B [22]. Therefore, posaconazole has been considered to be a reasonable option for patients with mucormycosis who are refractory to or intolerant of amphotericin B. Itraconazole has limited activity against *Absidia* species, and fluconazole and voriconazole do not have reliable activity against mucormycosis [15, 23–25]. Micafungin and caspofungin, members of the echinocandins, were synergized with amphotericin B lipid complex in treating murine mucormycosis [26]. These agents may have a role as a second agent, especially in combination with the polyene.

In our series, prognosis of mucormycosis disease in patients with hematologic malignancies was poor, and estimated median survival was 23 days. Only one patient who survived longer than 6 months received surgical debridement and administration of amphotericin B at the dosage of 0.5 mg/kg/day. Although patients who were treated with amphotericin B survived longer than those with micafungin or voriconazole, all patients died of the progressive disease. The reason for our poor results was that most patients could not be treated with aggressive surgical debridement, and we should have treated the patients with high dosage of amphotericin B or liposomal amphotericin B.

Mucormycosis is a rare fungal infection, but the mortality rate remains high. Premortal diagnosis was difficult because the biopsy of infected tissues was the only diagnostic tool and some patients revealed dual infection. We conclude that, even when patients are diagnosed with bacterial or fungal infection other than mucormycosis, we should consider the possibility of mucormycosis, in particular if patients have the symptoms of pain with uncontrollable fever. High dosages of liposomal amphotericin B should be given and surgical debridement should be attempted promptly in cases that are highly indicative of mucormycosis.

References

1. Rees JR, Pinner RW, Hajjeh RA, Brandt ME, Reingold AL. The epidemiological features of invasive mycotic infections in the San Francisco Bay area, 1992–1993: results of population-based laboratory active surveillance. Clin Infect Dis. 1998;27:1138–47.
2. Maertens J, Demuynck H, Verbeken EK, Zachée P, Verhoef GE, Vandenberghe P, et al. Mucormycosis in allogeneic bone marrow transplant recipients: report of five cases and review of the role of iron overload in the pathogenesis. Bone Marrow Transplant. 1999;24:307–12.
3. Gleissner B, Schilling A, Anagnostopolous I, Siehl I, Thiel E. Improved outcome of mucormycosis in patients with hematological diseases? Leuk Lymphoma. 2004;45:1351–60.
4. Greenberg RN, Scott LJ, Vaughn HH, Ribes JA. Zygomycosis (mucormycosis): emerging clinical importance and new treatments. Curr Opin Infect Dis. 2004;17:517–25.
5. Pagano L, Offidani M, Fianchi L, Nosari A, Candoni A, Piccardi M, et al. Mucormycosis in hematologic patients. Haematologica. 2004;89:207–14.
6. Roden MM, Zaoutis TE, Buchanan WL, Knudsen TA, Sarkisova TA, Schaufele RL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. Clin Infect Dis. 2005;41:634–53.
7. Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. Clin Microbiol Rev. 2005;18:556–69.
8. Boelaert JR, Van Cutsem J, de Locht M, Schneider YJ, Crichton RR. Deferoxamine augments growth and pathogenicity of Rhizopus, while hydroxypyridinone chelators have no effect. Kidney Int. 1994;45:667–71.
9. Peterson KL, Wang M, Canalis RF, Abemayor E. Rhinocerebral mucormycosis: evolution of the disease and treatment options. Laryngoscope. 1997;107:855–62.
10. Nosari A, Oreste P, Montillo M, Carrafiello G, Draisci M, Muti G, et al. Mucormycosis in hematologic malignancies: an emerging fungal infection. Haematologica. 2000;85:1068–71.
11. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46:1813–21.
12. Shoham S, Levitz SM. The immune response to fungal infections. Br J Haematol. 2005;129:569–82.
13. Waldorf AR, Ruderman N, Diamond RD. Specific susceptibility to mucormycosis in murine diabetes and bronchoalveolar macrophage defense against Rhizopus. J Clin Invest. 1984;74:150–60.
14. Symeonidis AS. The role of iron and iron chelators in zygomycosis. Clin Microbiol Infect. 2009;Suppl 5:26–32.
15. Spellberg B, Walsh TJ, Kontoyiannis DP, Edwards J Jr, Ibrahim AS. Recent advances in the management of mucormycosis: from bench to bedside. Clin Infect Dis. 2009;48:1743–51.
16. Kontoyiannis DP, Wessel VC, Bodey GP, Rolston KV. Zygomycosis in the 1990s in a tertiary-care cancer center. Clin Infect Dis. 2000;30:851–6.
17. Pavie J, Lafaurie M, Lacroix C, Marie Zagdanski A, Debrosse D, Socié G, et al. Successful treatment of pulmonary mucormycosis in an allogenic bone-marrow transplant recipient with combined medical and surgical therapy. Scand J Infect Dis. 2004;36:767–9.
18. Reid VJ, Solnik DL, Daskalakis T, Sheka KP. Management of bronchovascular mucormycosis in a diabetic: a surgical success. Ann Thorac Surg. 2004;78:1449–51.
19. Tedder M, Spratt JA, Anstadt MP, Hegde SS, Tedder SD, Lowe JE. Pulmonary mucormycosis: results of medical and surgical therapy. Ann Thorac Surg. 1994;57:1044–50.
20. Rogers TR. Treatment of zygomycosis: current and new options. J Antimicrob Chemother. 2008;61 Suppl 1:i35–40.
21. Kwon DS, Mylonakis E. Posaconazole: a new broad-spectrum antifungal agent. Expert Opin Pharmacother. 2007;8:1167–78.

22. Sun QN, Najvar LK, Bocanegra R, Loebenberg D, Graybill JR. In vivo activity of posaconazole against *Mucor* spp. in an immunosuppressed-mouse model. Antimicrob Agents Chemother. 2002;46:2310–2.
23. Sun QN, Fothergill AW, McCarthy DI, Rinaldi MG, Graybill JR. In vitro activities of posaconazole, itraconazole, voriconazole, amphotericin B, and fluconazole against 37 clinical isolates of zygomycetes. Antimicrob Agents Chemother. 2002;46:1581–2.
24. Marty FM, Cosimi LA, Baden LR. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. N Engl J Med. 2004;350:950–2.
25. Imhof A, Balajee SA, Fredricks DN, Englund JA, Marr KA. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. Clin Infect Dis. 2004;39:743–6.
26. Ibrahim AS, Gebremariam T, Fu Y, Edwards JE Jr, Spellberg B. Combination echinocandin-polyene treatment of murine mucormycosis. Antimicrob Agents Chemother. 2008;52:1556–8.

Scandinavian Journal of Infectious Diseases, 2012; 44: 60–64

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SHORT COMMUNICATION

Correlation between serum linezolid concentration and the development of thrombocytopenia

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Abstract
We evaluated the possible association between trough linezolid (LZD) concentrations and platelet counts using a dose–response curve with a logit model equation. We demonstrated that trough LZD concentrations correlated with platelet counts. A significant decrease in platelet count was observed in patients with trough LZD concentrations higher than 22.1 μg/ml.

Keywords: *Dose–response curve, linezolid, logit analysis, renal dysfunction, thrombocytopenia*

Introduction

Linezolid (LZD) is a novel synthetic oxazolidinone antimicrobial agent with a unique mechanism of action compared with other existing agents [1]. Notably, LZD has proven effective for the treatment of infections caused by multidrug-resistant Gram-positive cocci, including vancomycin-resistant Staphylococcus aureus (VRSA), vancomycin-resistant Enterococcus (VRE), and penicillin-resistant Streptococcus pneumoniae (PRSP), for which many current antimicrobial therapies are inadequate [2–4]. Although LZD treatment has been associated with similar or higher clinical response rates than vancomycin for methicillin-resistant S. aureus pneumonia [5], its use is not without risk. A high incidence of reversible thrombocytopenia has been reported in LZD-treated patients, particularly in those treated for 2 weeks or more [6–8]. Notably, Wu et al. [6] performed a survival analysis for development of thrombocytopenia or death and detected significant differences ($p < 0.001$) between patients with end-stage and non-end-stage renal disease. Our group also detected high trough concentrations of LZD in patients with renal dysfunction, leading us to speculate that these patients have delayed elimination of LZD, which may be a factor in the development of thrombocytopenia [9]. However, no association between trough LZD concentrations and the rate of decrease in platelet (PLT) counts has been reported to date.

The present prospective study was conducted to investigate the relationship between trough LZD concentrations and PLT counts using logit model analysis and a bootstrapping method. In addition, we assessed the effect of renal dysfunction on trough LZD concentration and PLT counts.

Methods

Patients

All subjects provided informed consent to participate in this study prior to the first administration of LZD

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(Received 23 January 2011; accepted 21 July 2011)

ISSN 0036-5548 print/ISSN 1651-1980 online
DOI: 10.3109/00365548.2011.608712

for the treatment of pneumonia. Patients with disseminated intravascular coagulation (DIC) and multiple organ failure (MOF), undergoing dialysis, or with a disease that might affect PLT counts, such as sepsis, were excluded from this study. In addition, patients who received a blood transfusion or drugs that could affect PLT counts, before or during LZD administration, were also excluded from this study.

Medications warranting exclusion

Patients who underwent treatment with the following agents were excluded from the present study: heparin, enoxaparin, valproic acid, gold drug, penicillin, cephalosporin antibiotics, sulfonamides, alpha-interferon, digoxin, digitoxin, procaine amide, cimetidine, and ranitidine.

LZD treatment and blood collection

Patients received LZD 600 mg by intravenous infusion over 60 to 120 min every 12 h. Blood was collected immediately before LZD administration for measurement of serum LZD concentration on or after the 4th day following drug initiation (at which time the LZD level was assumed to have reached a steady state) and subsequently on arbitrary days during the treatment period. The same blood samples were used to determine serum LZD and creatinine (SCr) concentrations and PLT counts.

Measurement of serum LZD concentrations

The serum LZD levels were measured by high-performance liquid chromatography (HPLC), as previously described [10]. Briefly, blood samples were first deproteinized using an equivalent volume of acetonitrile and then centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was subjected to HPLC, employing the absolute calibration method. For the measurement, 20-µl samples were loaded onto an octadecyl silane (ODS) Hypersil column (4.6 mm ID × 150 mm; Thermo Scientific Co., Yokohama, Japan). The HPLC system (CBM-20A, Shimadzu Co., Kyoto, Japan) consisted of an LC-20AT flow pump and SPD-10AV VP ultraviolet (UV) detector (Shimadzu Co.). As the mobile phase, a solution of 1% orthophosphoric acid, 30% methanol, and 2 g/l heptane sulfonic acid (adjusted to pH 5 with 10 M sodium hydroxide) was used at a flow rate of 1.0 ml/min. LZD in the samples was measured at a wavelength of 254 nm, and the lower limit of detection in this analysis was 0.1 µg/ml.

Determination of SCr levels and creatinine clearance rate

The creatinine clearance rate (CLCr) for each of the patients included in the study was estimated from the SCr concentration using the Cockcroft–Gault equation [11]. Patients with a CLCr of less than 60 ml/min were judged to have renal dysfunction.

Measurement of serum PLT counts

The PLT count measured before the first administration of LZD was determined to be the PLT baseline. The PLT count that displayed the greatest degree of deterioration from the baseline during the administration of LZD was considered the PLT minimum value. The rate of decrease in PLT counts (Y) was calculated using the following equation: $Y = 1 - (B/A)$, where A is the PLT baseline, and B is the PLT minimum value.

Dose–response curve determination using the logit model

We evaluated the association between LZD concentration and the decrease in PLT counts through dose–response curve determination using the logit model, in which the independent and dependent variables were the natural logarithm (ln) of the LZD concentration (x) and the decrease in PLT (y), respectively. The data analysis was performed using R software and the generalized linear model (GLM) function (family = binomial) [12]. To estimate the 95% confidence intervals (CI), and α and β values used in the non-parametric bootstrapping method [13], we utilized the following logit model equation:

$$p = \frac{1}{1 + exp(-a - \beta x)}$$

where p is the probability of a binary outcome, x is a continuous stimulus or exposure variable (trough LZD concentration), α determines the location of the curve on the x-axis, and β represents the slope of the curve.

Extrapolation was performed based on the dependent variable (PLT decrease). For all values greater than 0.7, the rate of decrease in PLT counts was considered to be 1.0. We also calculated the 50% hazard ratio of the dose–response curve using the equation: exp (α/β).

Statistical analysis

Statistical differences were assessed using the Mann–Whitney U-test with the significance level

set at $p < 0.05$. Statistical analysis was performed using SPSS analysis software v. 18 (SPSS, Inc., Tokyo, Japan).

Ethics

This study was approved by the ethics committee of the National Hospital Organization Kumamoto Medical Center, and was conducted in accordance with the ethics guidelines of the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The study was explained to the patients in writing, and written informed consent was obtained from each study participant.

Results

Eight patients with pneumonia (3 males and 5 females) participated in the study. Five of the patients had normal renal function and a mean ± standard deviation (SD) age of 65.2 ± 15.1 y, body weight of 49.0 ± 9.4 kg, SCr of 0.56 ± 0.2 mg/dl, CLCr of 94.5 ± 49.8 ml/min, and LZD therapy duration of 14.4 ± 15.5 days. The other 3 patients had renal dysfunction and a mean ± SD age of 63.7 ± 4.7 y, body weight of 64.7 ± 6.8 kg, SCr of 3.4 ± 2.2 mg/dl, CLCr of 28.9 ± 27.1 ml/min, and LZD therapy duration of 14 ± 1.7 days. No significant differences were detected in the duration of therapy between patients who had normal renal function and those who exhibited renal dysfunction (Table I). The individual patient characteristics, including therapy duration, trough LZD concentrations, and PLT counts, are summarized in Table I.

We determined the mean trough LZD concentrations and compared the baseline PLT counts and the PLT minimum value during LZD administration. In total, 21 samples were collected from the 8 patients. The mean trough LZD concentration of all 8 patients was 30.4 ± 21.4 μg/ml (range 7.5–90.9 μg/ml), but was significantly different between the patients with and without renal dysfunction, with mean values of 43.5 ± 25.5 and 19.7 ± 8.2 μg/ml, respectively ($p = 0.0402$). A decrease in the PLT count of greater than 50% was observed for 5 of 8 patients, which included the 3 patients with impaired renal function. The mean PLT counts of the patients without renal dysfunction before and after administration were $197.2 \pm 53.7 \times 10^3$/μl and $189.2 \pm 130.4 \times 10^3$/μl, respectively ($p = 0.68$), compared with $274.3 \pm 70.0 \times 10^3$/μl and 69.7 ± 29.3. $\times 10^3$/μl, respectively ($p = 0.05$), in the patients with renal dysfunction.

Finally, to examine the association between LZD trough concentrations and PLT counts, the values determined for all 8 patients were examined by logit model analysis. In addition, the 95% CIs were estimated using a bootstrapping method. A dose–response curve was obtained by convergence of the logit model, in which the estimated α and β values were −12.7 (97.5% CI − 11.60–14.10, $p < 0.0001$) and 4.1 (97.5% CI 4.54–3.75, $p < 0.0001$), respectively, while Akaike's information criterion (AIC) was 250.4 (Figure 1). Our analysis revealed that the trough LZD concentrations and PLT counts for each patient fit the dose–response curve. From the logit regression analysis, we determined that the 50% hazard ratio for the development of thrombocytopenia correlated to a trough LZD concentration of 22.1 μg/ml.

Discussion

In this study, we hypothesized that high trough LZD concentrations, which develop as a result of delayed elimination of LZD, particularly in patients with

Table I. Characteristics, linezolid trough concentrations, and platelet counts of the 8 patients with infective pneumonia treated with linezolid.

	Patient	Age (y)	Weight (kg)	CLCr (ml/min)	Therapy duration (days)	Mean LZD trough concentration[a] (μg/ml)	Day 0[b] PLT count ($\times 10^3$/μl)	Minimum PLT count ($\times 10^3$/μl)	Day of minimum PLT count
Normal renal function	1	46	39.8	64.3	8	31.7 ± 12.9	272	377	7
	2	58	60.0	179.5	9	7.5 ± 0.7	190	255	7
	3	61	52.0	99.0	7	16.5 ± 1.0	169	169	6
	4	80	54.4	62.9	6	22.8 ± 1.2	224	92	6
	5	81	38.6	66.8	42	18.3 ± 2.5	131	53	12
Renal dysfunction	6	60	67.4	59.8	16	65.2 ± 18.6	204	65	8
	7	62	69.7	17.9	13	20.5 ± 6.6	275	43	7
	8	69	56.9	9.0	13	48.8 ± 2.3	344	101	8

CLCr, creatinine clearance rate; LZD, linezolid. PLT, platelet.
[a]Mean LZD trough concentrations are shown as the mean ± standard deviation.
[b]Day 0 = before the first administration of LZD.

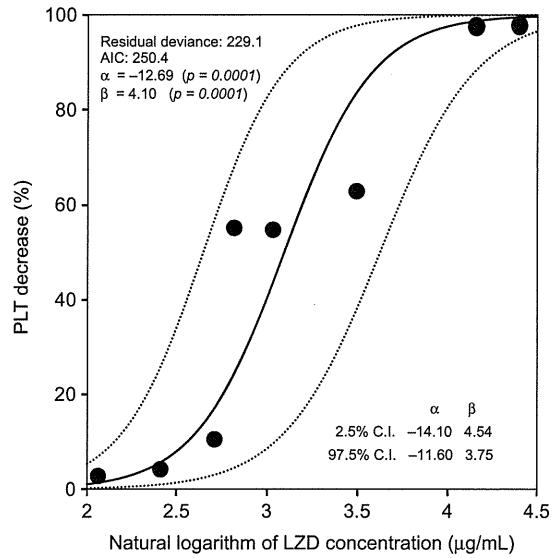

Figure 1. Logit analysis for the natural logarithm of the trough linezolid (LZD) concentration (μg/ml) and the rate of decrease in platelet (PLT) count (%) from baseline (before the first LZD administration) levels. The model adaptability was evaluated by the bootstrapping method ($n = 1000$). The continuous line depicts the predicted logit (p) and the dotted lines 95% confidence interval (95% CI). AIC, Akaike's information criterion; α, location of the curve on the x-axis; β, slope of the curve; •, measured value.

renal dysfunction, can lead to thrombocytopenia. By analyzing the association between trough LZD concentrations (independent variable, x) and PLT counts (dependent variable, y) using the logit model [13], we determined that the 50% hazard ratio for the development of thrombocytopenia correlated to a trough LZD concentration of 22.1 μg/ml. The PLT count also decreased as the trough LZD concentration increased in patients with normal renal function. As the 3 patients with renal dysfunction had LZD levels that exceeded 22.1 μg/ml, our preliminary results suggest that thrombocytopenia may develop with increased trough LZD concentrations in patients with delayed LZD elimination, and thrombocytopenia necessitates LZD treatment discontinuation.

The elevation in serum LZD concentration appeared to correlate with the PLT count, as all 3 patients with renal dysfunction exhibited decreases in PLT counts greater than 50% following LZD treatment, whereas only 2 patients with normal renal function had a marked reduction of PLT counts. Notably, however, the reduction in the latter 2 patients was not as severe as that observed in the patients with renal dysfunction, and 1 of the patients with normal renal function had received LZD treatment approximately 1 month longer than all other patients.

In general, dose adjustment of LZD is considered unnecessary, even for patients with mild to moderate renal or hepatic disorders, as LZD is predominantly metabolized through oxidation of its morpholine ring to an inactive form by non-enzymatic oxidative reactions [4]. However, in patients with renal dysfunction, Lin et al. [14] reported that thrombocytopenia often develops when the duration of LZD administration is longer than 14 days. Furthermore, Tsuji et al. [9] and Matsumoto et al. [15] suggested that the rise in the LZD concentration and in the area under the plasma LZD concentration–time curve over 24 h (AUC24h) are factors in the development of thrombocytopenia in patients with renal dysfunction. Our study provides additional support for the association between elevated LZD trough concentrations and decreases in PLT counts in patients with renal dysfunction.

Several limitations of the study warrant mention. First, due to the limited number of patients given LZD at our institution without exclusion criteria, only 8 patients were included in this study, thus limiting the significance of the findings. Second, blood collection was not performed to a defined schedule because the blood samples used in the study were those collected during the routine care of the patients. Finally, we only investigated the relationship between PLT counts and LZD concentration in this study. Data on confounding factors that can decrease PLT counts, such as co-morbidities and severity of illness, were not collected; thus, we cannot eliminate the possibility that PLT counts were influenced by factors aside from LZD concentrations.

In conclusion, we found that elevated LZD trough concentrations in patients with renal dysfunction were associated with low PLT counts. Although these findings are preliminary, our logit regression analysis suggests that a trough LZD concentration that exceeds 22 μg/ml may lead to the development of thrombocytopenia. Therefore, PLT levels should be monitored more closely to prevent thrombocytopenia in patients with renal dysfunction receiving LZD. Further investigations of this association are warranted, including whether reductions in the LZD dose will decrease the risk of thrombocytopenia in patients with renal dysfunction.

Declaration of interest The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Zurenko GE, Gibson JK, Shinabarger DL, Aristoff PA, Ford CW, Tarpley WG. Oxazolidinones: a new class of antibacterials. Curr Opin Pharmacol 2001;1:470–6.

[2] Mascini EM, Troelstra A, Beitsma M, Blok HE, Jalink KP, Hopmans TE, et al. Genotyping and preemptive isolation to control an outbreak of vancomycin-resistant Enterococcus faecium. Clin Infect Dis 2006;42:739–46.
[3] Chang S, Sievert DM, Hageman JC, Boulton ML, Tenover FC, Downes FP, et al. Infection with vancomycin-resistant Staphylococcus aureus containing the *vanA* resistance gene. N Engl J Med 2003;348:1342–7.
[4] Pfizer. ZYVOX (linezolid) label information. Pfizer; 2008. Retrieved 20 June 2008.
[5] Pfizer Co. New phase 4 study shows higher rates of clinical and microbiological success for Zyvox versus vancomycin in MRSA nosocomial pneumonia. The 48th Annual Meeting of the Infectious Diseases Society of America, Vancouver, Canada, October 21–24, 2010.
[6] Wu VC, Wang YT, Wang CY, Tsai IJ, Wu KD, Hwang JJ, et al. High frequency of linezolid-associated thrombocytopenia and anemia among patients with end-stage renal disease. Clin Infect Dis 2006;42:66–72.
[7] Minson Q, Gentry CA. Analysis of linezolid-associated hematologic toxicities in a large veterans affairs medical center. Pharmacotherapy 2010;30:895–903.
[8] Gerson SL, Kaplan SL, Bruss JB, Le V, Arellano FM, Hafkin B, et al. Hematologic effects of linezolid: summary of clinical experience. Antimicrob Agents Chemother 2002;46: 2723–6.
[9] Tsuji Y, Hiraki Y, Matsumoto K, Mizoguchi A, Kobayashi T, Sadoh S, et al. Thrombocytopenia and anemia caused by a persistent high linezolid concentration in patients with renal dysfunction. J Infect Chemother 2010;17: 70–5.
[10] Majcher-Peszynska J, Haase G, Sass M, Mundkowski R, Pietsch A, Klammt S, et al. Pharmacokinetics and penetration of linezolid into inflamed soft tissue in diabetic foot infections. Eur J Clin Pharmacol 2008;64:1093–100.
[11] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31–41.
[12] Ihaka R, Gentleman R. R: A language for data analysis and graphics. J Comput Graph Stat 1996;5:299–314.
[13] Sauerbrei W, Schumacher M. A bootstrap resampling procedure for model building: application to the Cox regression model. Stat Med 1992;11:2093–109.
[14] Lin YH, Wu VC, Tsai IJ, Ho YL, Hwang JJ, Tsau YK, et al. High frequency of linezolid-associated thrombocytopenia among patients with renal insufficiency. Int J Antimicrob Agents 2006;28:345–51.
[15] Matsumoto K, Takeshita A, Ikawa K, Shigemi A, Yaji K, Shimodozono Y, et al. Higher linezolid exposure and higher frequency of thrombocytopenia in patients with renal dysfunction. Int J Antimicrob Agents 2010;36: 179–81.

Clinical and Experimental Hypertension, 33(3): 147–152, (2011)

ISSN 1064-1963 print/1525-6006 online
DOI: 10.3109/10641963.2010.531839

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Individual Assessment of Inherent Arterial Stiffness Using Nomogram and Pulse Wave Velocity Index: The Ohasama Study

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Abstract

We measured the brachial-ankle pulse wave velocity (baPWV) in 491 normotensives and determined the "PWV index" (measured baPWV–theoretical baPWV) in 491 normotensives and 83 controlled hypertensives. Linear regression analysis revealed that the theoretical baPWV (cm/sec) was 0.21 × age^2 (years^2)–13.73 × age (years) + 0.05 × mean arterial pressure^2 (mmHg^2) + 3.95 × heart rate (bpm) + 36.49 × gender (1 male; 0 female) + 733 (R^2 = 0.53). The calculated PWV index was significantly higher in 13 smokers than 70 nonsmokers among controlled hypertensives. The calculated PWV index might provide more precise information about inherent arterial stiffness.

Keywords: arterial stiffness, pulse wave velocity, nomogram

INTRODUCTION

The elastic properties of the large arteries are important determinants of circulatory physiology in health and disease. Arterial stiffness plays an important role in the pathogenesis of cerebrovascular and cardiovascular disease (1–3). Arterial stiffening increases ventricular afterload through earlier return of the reflected wave (4), and reduces coronary blood flow by decreasing diastolic pressure (5). This leads to cardiovascular events, such as coronary ischemia and heart failure (5, 6). Arterial stiffness is also associated with microvascular diseases (7). Therefore, evaluation of inherent arterial stiffness for individuals could help not only risk assessment but also risk reduction through effective treatment.

Measuring pulse wave velocity (PWV) is one of the most representative methods for assessing arterial stiffness (8, 9). However, normative values of PWV have not yet been fully defined. A single, specific PWV value as a cut-off is inappropriate because various physiological factors, such as age (10, 11), blood pressure (BP) (11, 12), heart rate (HR) (13, 14), and gender (15) affect PWV. Therefore, to evaluate "inherent" arterial stiffness in individuals, the confounding effect of these factors must be considered. Although Blacher et al. (16) have calculated a PWV index that could reflect inherent arterial stiffness in patients with end-stage renal disease, this has not been achieved in an apparently healthy general population. Over the past several years, in addition to conventional carotid-femoral PWV (cfPWV) measurements, the brachial-ankle PWV (baPWV) can provide useful information about arterial stiffness (17, 18), particularly in large populations (19). Therefore, we aimed to construct a nomogram for theoretical baPWV in a general population and to propose an index of inherent arterial stiffness which might be better related to cardiovascular risk for individuals.

METHODS

Study Subjects

The present study was based on a health examination survey performed on residents of Ohasama town, Japan, who were aged 34 years or older. The geographic and demographic characteristics of Ohasama have been reported elsewhere (20). Of 1612 individuals

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Received 2 March 2010; revised 16 April 2010; accepted 29 April 2010.

who underwent a health examination, we selected 491 healthy subjects, according to the following criteria: normotension (systolic BP [SBP] <140 mmHg, diastolic BP [DBP] <90 mmHg, and no medication for hypertension), nondiabetes (fasting blood glucose <126 mg/dl or post-prandial glucose <200 mg/dl, and no medication with insulin or oral hypoglycemic agents), normocholesteloremia (total cholesterol <240 mg/dl and no medication for dyslipidemia), ankle-brachial index (ABI) >0.9, no history of cardiovascular and cerebrovascular diseases, and body mass index (BMI) <30 kg/m^2. These individuals were defined as healthy and normotensive and we constructed a nomogram for calculating theoretical PWV using data from these participants.

We also selected another group of 83 hypertensive patients whose BP was controlled with anti-hypertensive medication. The selection criteria for the hypertensive patients are the same as the one mentioned except treatment by medications for hypertension. They were defined as having controlled hypertension.

Biochemical data were obtained from venous blood samples collected on the same days as the PWV measurements. Information concerning lifestyle, habits, and therapeutic status was collected using a questionnaire.

The study protocol was approved by the Institutional Review Board of Tohoku University School of Medicine, the Department of Health of the Ohasama Town Government, and conducted in accordance with the Declaration of Helsinki and its amendments. All participants gave informed, written consent.

Measurement of PWV

The baPWV was measured in the supine position after at least 5 min of rest using an automatic device (Form PWV/ABI; Colin Co., Ltd., Komaki, Japan), as described (17, 19). Thus, cuffs connected to a plethysmographic sensor are wrapped on both the brachia and ankles for pressure waveforms recordings. The baPWV was determined as length of an arterial segment by the transit time of the pulse wave: baPWV = (La-Lb)/T, where T is the time difference in the foot between the right brachial pulse wave and the right ankle pulse wave, La is the path length from the suprasternal notch to the ankle, and Lb is the path length from the suprasternal notch to the brachium. La and Lb are automatically calculated according to individual height and we used right brachial-to-right ankle baPWV. The validity of the device has been confirmed and the reproducibility of baPWV has been published (13).

We simultaneously measured BP and HR with baPWV in the supine position using the same device. Pulse pressure (PP) and mean arterial pressure (MAP) were calculated according to the formula: PP = SBP − DBP, and MAP = DBP + PP/3, respectively.

Statistical Analysis

The characteristics between healthy normotensives and controlled hypertensives were compared using the student's t-test for continuous variables and the χ^2-test for categorical variables.

For the 491 healthy normotensives, correlations between baPWV and various parameters were calculated. Multivariate stepwise regression analyses were used to evaluate which of linear, quadratic, and exponential functions optimally described the relationships between baPWV and age, and between baPWV and MAP. Covariates entered into the stepwise analysis included the variables that were significantly associated with PWV according to the univariate analysis. To construct a nomogram for theoretical baPWV, a multivariate stepwise regression analysis was performed using terms that described the optimal relationship between baPWV and age, and MAP. The upper limit of the 95% confidence interval (CI) of the theoretical baPWV value was calculated and we constructed a nomogram of the upper limit of baPWV.

To assess the applicability of the nomogram, we calculated the PWV index for each individual (16). Briefly, the PWV index was obtained by subtracting the theoretical baPWV from the measured baPWV. The PWV index was compared between 491 normotensive and 83 controlled hypertensive individuals and between subjects with and without smoking in controlled hypertensives after adjusted for confounding factors. We also examined the optimal cut-off point in the PWV index to distinguish controlled hypertensives from normotensives, using the receiver-operator characteristic (ROC) curve.

All statistical analyses were performed using SPSS software version 11.0 (SPSS Inc., Chicago IL, USA). A value of P <0.05 represented statistical significance.

RESULTS

The clinical characteristics of healthy normotensives and controlled hypertensives are given in Table 1.

Figure 1 shows the relationship between age and baPWV in the 491 healthy normotensives. BaPWV increased with age and in a nonlinear manner, being much more prominent in individuals over 50 years of age. The mean + SD, mean + 2 SDs, and 95th percentile values of baPWV in this population were 1607, 1844, and 1848 cm/sec, respectively.

Table 2 shows correlations between baPWV and various parameters. BaPWV was significantly and positively correlated with age and BP. Among the BP components, the correlation between MAP and baPWV was the closest, with a correlation coefficient of 0.49. The HR was also positively correlated with baPWV. Males had a higher baPWV than females (1,416 ± 232 vs. 1,347 ± 236 cm/sec, P = 0.002). Participants who regularly consumed alcohol had a significantly lower PWV than anyone else (1334 ± 220 vs. 1393 ± 244

Table 1. Clinical characteristics of the study population

	Healthy Normotensives (n = 491)	Controlled Hypertensives (n = 83)
Age (y)	55.9 ± 12.4	68.0 ± 7.9***
Gender (men/women)	169/322	34/49
SBP (mmHg)	123.2 ± 10.0	129.1 ± 7.7***
DBP (mmHg)	75.9 ± 6.8	78.9 ± 5.6***
MAP (mmHg)	92.9 ± 8.3	98.6 ± 5.5***
PP (mmHg)	47.3 ± 7.1	50.2 ± 8.5**
HR (mmHg)	66.2 ± 9.2	66.1 ± 9.6
baPWV (cm/sec)	1371 ± 237	1658 ± 236***
BMI (kg/m^2)	23.2 ± 2.8	24.1 ± 3.0**
Total cholesterol (mg/dl)	191.0 ± 27.5	193.4 ± 28.6
HDL cholesterol (mg/dl)	59.8 ± 15.5	57.7 ± 14.9
HbA_{1c} (%)	4.9 ± 0.4	5.0 ± 0.4*
Smoking habit (%)	17.9	15.7
Alcohol habit (%)	38.3	47.0

Abbreviations: SBP - systolic blood pressure; DBP - diastolic blood pressure; MAP - mean arterial pressure; PP - pulse pressure; HR - heart rate; bpm - beats per minute; baPWV - brachial-ankle pulse wave velocity; BMI - body mass index; HDL - high-density lipoprotein; HbA_{1c} - hemoglobin A_{1c}.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, vs. healthy normotensives. Data are expressed as the mean ± SD.

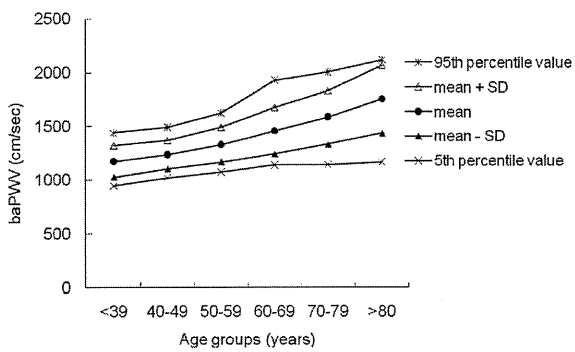

Figure 1. The relations of age to baPWV in 491 healthy normotensives.

cm/sec, P = 0.007). The PWV between those with a smoking habit and others did not significantly differ (1352 ± 198 vs. 1374 ± 244 cm/sec, P = 0.36).

We performed stepwise regression analysis to construct an equation for the theoretical PWV. The variables that were significantly associated with baPWV according to the bi-variate analysis (age, MAP, HR, gender, BMI, total cholesterol, HDL cholesterol, HbA_{1c}, and alcohol habit) were used as potential explanatory variables. Because the relationships between baPWV and age and MAP could be nonlinear, we added a nonlinear term for age and MAP to the equation. We chose a quadratic term rather than an exponential term, since the quadratic functions described the relationship with baPWV better than the exponential function (data not shown).

Table 2. Correlation of baPWV and various parameters in 491 healthy normotensives

	r	P
Age (y)	0.622	<0.001
SBP (mmHg)	0.445	<0.001
DBP (mmHg)	0.396	<0.001
MAP (mmHg)	0.488	<0.001
PP (mmHg)	0.248	<0.001
HR (bpm)	0.161	<0.001
BMI (kg/m^2)	0.095	0.035
Total cholesterol (mg/dl)	0.152	0.001
HDL cholesterol (mg/dl)	−0.134	0.003
HbA_{1c} (%)	0.232	<0.001

Abbreviations: r - Pearson correlation coefficient; baPWV - brachial-ankle pulse wave velocity; SBP - systolic blood pressure; DBP - diastolic blood pressure; MAP - mean arterial pressure; PP - pulse pressure; HR - heart rate; bpm - beats per minute; BMI - body mass index; HDL - high-density lipoprotein; HbA_{1c} - hemoglobin A_{1c}.

Table 3. Multiple stepwise regression analysis to construct nomograms

	β	t	P
Nomogram for calculation of a theoretical baPWV value* ($R^2 = 0.53$)			
Age^2 (y^2)	1.26	4.46	<0.001
Age (y)	−0.72	−2.53	0.012
MAP^2 (mmHg^2)	0.32	9.66	<0.001
HR (bpm)	0.15	4.77	<0.001
Gender (1 men; 0 women)	0.07	2.28	0.023
Intercept		4.42	<0.001
Nomogram for calculation of the upper limit of normal baPWV**			
Age^2 (y^2)	1.75	981.47	<0.001
Age (y)	−1.02	−569.45	<0.001
MAP^2 (mmHg^2)	0.48	132.86	<0.001
MAP (mmHg)	−0.04	−10.22	<0.001
HR (bpm)	0.21	1047.94	<0.001
Gender (1 men; 0 women)	0.10	503.20	<0.001
Intercept		324.67	<0.001

Abbreviations: β - standardized partial regression coefficient; baPWV - brachial-ankle pulse wave velocity; MAP - mean arterial pressure; HR - heart rate; bpm - beats per minute.
*Factors excluded from the model: MAP, BMI, total cholesterol, HDL cholesterol, HbA_{1c}, alcohol habit.
**Factors excluded from the model: BMI, total cholesterol, HDL cholesterol, HbA_{1c}, alcohol habit.

When both linear and quadratic terms for age and MAP were thus entered into the models, age^2, age, MAP^2, HR, gender were independently correlated with baPWV, accounting for 53.0% of the variance (Table 3). Other factors were not significant determinants of baPWV. Hence, the theoretical baPWV was calculated from the following equation:

Theoretical baPWV (cm/sec)

$$= 0.21 \times \text{age}^2(\text{years}^2) - 13.73 \times \text{age (years)}$$

$$+ 0.05 \times \text{MAP}^2\ (\text{mmHg}^2)$$

$$+ 3.95 \times \text{HR (bpm)} + 36.49 \times \text{gender}$$

$$(1\ \text{male};\ 0\ \text{female}) + 733.$$

The individual normal upper limit of baPWV was similarly calculated using the following equation:

$$\text{Upper limit of normal baPWV (cm/sec)}$$

$$= 0.22 \times \text{age}^2\ (\text{years}^2) - 14.22 \times \text{age (years)}$$

$$+ 0.05 \times \text{MAP}^2\ (\text{mmHg}^2) - 0.76 \times \text{MAP (mmHg)}$$

$$+ 4.00 \times \text{HR (bpm)}$$

$$+ 37.26 \times \text{gender (1 male; 0 female)} + 1100.$$

Assessment of Nomogram Applicability

The nomogram of a theoretical baPWV was applied to all the participants, and a PWV index was calculated for each individual. The PWV index (measured baPWV – theoretical baPWV) was significantly greater in patients with controlled hypertension than healthy normotensive subjects even after adjusted for confounding factors (95.3 ± 18.1 vs. 1.1 ± 7.4 cm/sec, $P < 0.001$). The PWV index was higher in 13 smokers (148.4 ± 54.6 cm/sec) than 70 nonsmokers (92.6 ± 21.5 cm/sec, $p = 0.36$) after adjusted for confounding factors. According to the ROC curve, cut-off point yielding maximal sensitivity plus specificity to distinguish was 74 cm/sec. Sensitivity and specificity using this cut-off point was 55.4% and 72.0%, respectively.

DISCUSSION

In the present study, we constructed a nomogram for theoretical PWV considering the effects of age, BP, HR, and gender. A definite cut-off value of PWV is not always appropriate for all subjects because PWV depends on various physiological factors (12, 15, 21), although some reports, including the recent ESH-ESC guidelines (22), proposed a cut-off value. Therefore, we chose a nomogram rather than a cut-off value to determine the normalcy of PWV. The PWV index, namely, a difference between measured and theoretical PWV, was significantly greater in patients with controlled hypertension than healthy normotensive subjects.

Blacher et al. (16) and Yamashina et al. (23) have also constructed a nomogram with which to calculate theoretical PWV. Blacher et al. (16) measured PWV between the carotid and femoral arteries. Because their study subjects were nonuremic and included outpatients seen in consultation, members of the paramedical and medical staff, and patients hospitalized in departments other than nephrology, the theoretical PWV calculated on the basis of their nomogram is probably not applicable to the general population. In the present study, we measured PWV between the brachial and ankle regions. Measuring baPWV using pressure cuffs wrapped on the brachial and ankle is a simple and noninvasive method for assessing arterial stiffness. A recent study (24) demonstrated that baPWV were significantly and positively correlated with cfPWV ($r = 0.73$) and baPWV and cfPWV were similarly associated with risk factors for coronary artery disease. It has been reported that baPWV had a predictive power for the mortality of patients with severe renal disease (25, 26). Yamashina et al. (23) represented the relationships between age and baPWV at each SBP level and between SBP and baPWV at each age class as a quadratic curve. This is consistent with the findings of ours and others (12, 15, 21, 23) that the relationships of baPWV with age and BP could be represented by quadratic function. However, despite this similarity, coefficients of each PWV determinant were somewhat different between the studies. One possible explanation for this difference could be the inclusion of HR as a determinant of baPWV in our study but not in the previous study (23). We included HR because a substantial number of previous studies have shown an important effect of HR on PWV (13, 14). Recently, Tomiyama et al. (27) also reported that HR was cross-sectionally and prospectively associated with changes in baPWV in a large sample prospective follow-up study. Therefore, it would be better to take into consideration of HR to construct a nomogram for theoretical baPWV. Alternatively, the difference might be attributable to a difference in study population; our study comprised a relatively older, rural general population with a mean age of 56 years, whereas the previous study (23) comprised a younger urban population with a mean age of 46 years.

Blacher et al. (16) originally introduced the PWV index, which was obtained by subtracting a theoretical PWV from a measured PWV, thus reflecting pathologic, rather than physiologic change in arterial stiffness. Consistent with their suggestion, the last part of our results showed that the measured baPWV in patients with controlled hypertension was higher than the theoretical baPWV. This result indicates that the "arterial age" is higher than chronological age in these patients. Moreover, we demonstrated that the PWV index was greater in the controlled hypertensive patients than healthy normotensive subjects. The PWV index was also higher in smokers than nonsmokers. Therefore, the greater PWV index may ensure the existence of severe arterial change. Although we could not evaluate the influence of hypertension history on the PWV index, this finding may suggest that, even if being controlled, long-term history of hypertension increases inherent arterial stiffness. Vergrand et al. (28) previously reported that in spite of the same BP level, treated

hypertensive patients had increased wave reflection and a higher PWV than normotensive subjects. This might be explicable by irreversible structural degeneration of arterial media before the initiation of treatment, or by nonhemodynamic factors relating to arterial stiffening (29). In the present study, we calculated an optimal cut-off point in the PWV index to distinguish controlled hypertensives from normotensives. Blacher et al. (16) previously reported that 1.63 and 0.68 m/sec was the cut-off point in the PWV index for increased risk of cardiovascular and overall death in 242 patients with end-stage renal disease. It is expected that the cut-off point in the PWV index to predict cardiovascular disease mortality and morbidity from the Ohasama study population in the near future.

In conclusion, we constructed a nomogram for theoretical PWV, in which various physiological factors were taken into account. The PWV index based on this nomogram might be applicable not only to general screening but also to clinical practice for evaluating individual inherent arterial stiffness. Future studies are required to clarify whether the PWV index has more potential than conventional markers to assess individual cardiovascular risk and provide effective preventive strategies.

ACKNOWLEDGMENTS

We are grateful to the staff members of the Iwate Prefectural Stroke Registry and to the public health nurses in Ohasama for their valuable support of this project.

The work was supported by Grants for Scientific Research (15790293, 16590433, 17790381, 17790381, 18390192, 18590587, 19590929, and 19790423) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; a Grant-in-Aid (H17-Kenkou-007, H18-Junkankitou [Seishuu]-Ippan-012, and H20-Junkankitou [Seishuu]-Ippan-009, 013) from the Ministry of Health, Labor and Welfare, Health and Labor Sciences Research Grants, Japan; a Grant-in-Aid for Japan Society for the Promotion of Science (JSPS) fellows (16.54041, 18.54042, 19.7152, 20.7198, 20.7477, and 20.54043), Tokyo, Japan; Health Science Research Grants and Medical Technology Evaluation Research Grants from the Ministry of Health, Labor and Welfare, Japan; Japan Atherosclerosis Prevention Fund; the Uehara Memorial Foundation; the Takeda Medical Research Foundation; National Cardiovascular Research Grants; and Biomedical Innovation Grants.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

[1] Laurent S, Katsahian S, Fassot C, Tropeano AI, Gautier I, Laloux B, Boutouyrie P. Aortic stiffness is an independent predictor of fatal stroke in essential hypertension. *Stroke* 2003;34:1203–1206.

[2] Blacher J, Asmar R, Djane S, London GM, Safer ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertension* 1990;33:1111–1117.

[3] Watabe D, Hashimoto J, Hatanaka R, Hanazawa T, Ohba H, Ohkubo T, Kikuya M, Totsune K, Imai Y. Electrocardiographic left ventricular hypertrophy and arterial stiffness: The Ohasama study. *Am J Hypertens* 2006;19:1199–1205.

[4] London GM, Guerin AP. Influence of arterial pulse and reflected waves on blood pressure and cardiac function. *Am Heart J* 1999;138:S220–S224.

[5] O'Rourke MF, Hashimoto J. Mechanical factors in arterial aging: a clinical perspective. *J Am Coll Cardiol* 2007;50:1–13.

[6] Hashimoto J, O'Rourke MF. Is arterial stiffness better than blood pressure in predicting cardiovascular risk?*Curr Cardiovasc Risk Rep* 2008;2:133–140.

[7] O'Rourke MF, Safer ME. Relationship between aortic stiffening and microvascular disease in brain and kidney. *Hypertension* 2005;46:200–204.

[8] Asmar R, Benetos A, Topouchian J, Laurent P, Pannier B, Brisac MA, Target R, Levy BI. Assessment of arterial distensibility by automatic pulse wave velocity measurement. Validation and clinical application studies. *Hypertension* 1995;26:485–490.

[9] Hashimoto J, Chonan K, Aoki Y, Nishimura T, Ohkubo T, Hozawa A, Suzuki M, Matsubara M, Michimata M, Araki T, Imai Y. Pulse wave velocity and the second derivative of the finger photoplethysmogram in treated hypertensive patients: their relationship and associating factors. *J Hypertens* 2002;20:2415–2422.

[10] Najjar SS, Scuteri A, Lakatta EG. Arterial aging: Is it an immutable cardiovascular risk factor? *Hypertension* 2005;46: 454–462.

[11] Avolio AP, Deng FQ, Li WQ, Luo YF, Huang ZD, Xing LF, O'Rourke MF. Effects of aging on arterial distensibility in populations with high and low prevalence of hypertension: comparison between urban and rural communities in China. *Circulation* 1985;71:202–210.

[12] Mitchell GF, Parise H, Benjamin EJ, Larson MG, Keyes MJ, Vita JA, Vasan RS, Levy D. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: The Framingham Heart Study. *Hypertension* 2004;43: 1239–1245.

[13] Munakata M, Ito N, Nunokawa T, Yoshinaga K. Utility of automated brachial ankle pulse wave velocity measurements in hypertensive patients. *Am J Hypertens* 2003;16:653–657.

[14] Haesler E, Lyon X, Pruvot E, Kappenberger L, Hayoz D. Confounding effects of heart rate on pulse wave velocity in paced patients with a low degree of atherosclerosis. *J Hypertens* 2004;22:1317–1322.

[15] Tomiyama H, Yamashina A, Arai T, Hirose K, Koji Y, Chikamori T, Hori S, Yamamoto Y, Doba N, Hinohara S. Influences of age and gender on results of noninvasive brachial-ankle pulse wave velocity measurement-a survey of 12517 subjects. *Atherosclerosis* 2003;166:303–309.

[16] Blacher J, Safar ME, Guerin AP, Pannier B, Marchais SJ, London GM. Aortic pulse wave velocity index and mortality in end-stage renal disease. *Kidney Int* 2003;63:1852–1860.

[17] Hashimoto J, Watabe D, Kimura A, Takahashi H, Ohkubo T, Totsune K, Imai Y. Determinants of the second derivative of the finger photoplethysmogram and brachial-ankle pulse-wave velocity: The Ohasama study. *Am J Hypertens* 2005;18: 477–485.

[18] Sugawara J, Hayashi K, Yokoi T, Cortez-Cooper MY, DeVan AE, Anton MA, Tanaka H. Brachial-ankle pulse wave velocity: an index of central arterial stiffness? *J Hum Hypertens* 2005;19:401–406.

[19] Yamashina A, Tomiyama H, Takeda K, Tsuda H, Arai T, Hirose K, Koji Y, Hori S, Yamamoto Y. Validity, reproducibility, and clinical significance of noninvasive brachial-ankle pulse wave velocity measurement. *Hypertens Res* 2002;25: 359–364.

[20] Imai Y, Nagai K, Sakuma M, Sakuma H, Nakatsuka H, Satoh H, Minami N, Munakata M, Hashimoto J, Yamagishi T. Ambulatory blood pressure of adults in Ohasama, Japan. *Hypertension* 1993;22:900–912.

[21] McEniery CM, Yasmin, Hall IR, Qasem A, Wilkinson IB, Cockcroft JR. Normal vascular aging: differential effects on wave reflection and aortic pulse wave velocity The Anglo-Cardiff Collaborative Trial (ACCT). *J Am Coll Cardiol* 2005;46:1753–1760.

[22] European Society of Hypertension-European Society of Cardiology Guidelines Committee. 2007 Guiidelines for the Management of Arterial Hypertension. *Eur Heart J* 2007;28: 1462–1536.

[23] Yamashina A, Tomiyama H, Arai T, Koji Y, Yambe M, Motobe H, Glunizia Z, Yamamoto Y, Hori S. Nomogram of the relation of brachial-ankle pulse wave velocity with blood pressure. *Hypertens Res* 2003;26:801–806.

[24] Tanaka H, Munakata M, Kawano Y, Ohishi M, Shoji T, Sugawara J, Tomiyama H, Yamashina A, Yasuda H, Sawayama T, Ozawa T. Comparison between carotid- femoral and brachial-ankle pulse wave velocity as measures of arterial stiffness. *J Hypertens* 2009;27:2022–2027.

[25] Kitahara T, Ono K, Tsuchida A, Kawai H, Shinohara M, Ishii Y, Koyanagi H, Noguchi T, Matsumoto T, Sekihara T, Watanabe Y, Kanai H, Ishida H, Nojima Y. Impact of brachial-ankle pulse wave velocity and ankle-brachial blood pressure index on mortality in hemodialysis patients. *Am J Kidney Dis* 2005;46:688–696.

[26] Morimoto S, Yurugi T, Aota Y, Sakuma T, Jo F, Nishikawa M, Iwasaka T, Maki K. Prognostic significance of ankle-brachial index, brachial-ankle pulse wave velocity, flow-mediated dilation, and nitroglycerin-mediated dilation in end-stage renal disease. *Am J Nephrol* 2009;30: 55–63.

[27] Tomiyama H, Hashimoto H, Tanaka H, Matsumoto C, Odaira M, Yamada J, Yoshida M, Shiina K, Nagata M, Yamashina A; baPWV/cfPWV Collaboration Group. Synergistic relationship between changes in the pulse wave velocity and changes in the heart rate in middle-aged Japanese adults: a prospective study. *J Hypertens.* 2010;28:687–694.

[28] Vergnaud AC, Protogerou AD, Blacher J, Safar ME. From "optimal" to "borderline" blood pressure in subjects under chronic antihypertensive therapy. *J Hypertens* 2008;26: 138–144.

[29] Nichols WW, O'Rourke MF. *McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles.* London: A Hodder Arnold Publication, 2005.

blood

2011 117: 2113-2120
Prepublished online November 24, 2010;
doi:10.1182/blood-2010-05-285502

A Markov decision analysis of allogeneic hematopoietic cell transplantation versus chemotherapy in patients with acute myeloid leukemia in first remission

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Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.

A Markov decision analysis of allogeneic hematopoietic cell transplantation versus chemotherapy in patients with acute myeloid leukemia in first remission

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Various prospective trials have been performed to assess the roles of allogeneic hematopoietic cell transplantation (allo-HCT) and chemotherapy in patients with acute myeloid leukemia (AML) in first complete remission (CR1). However, the results have not always been consistent, and there has been a limited evaluation of quality of life (QOL) in these postremission strategies. We performed a Markov decision analysis that enabled us to compare survival outcomes with a QOL evaluation using a database of 2029 adult AML patients who achieved CR1. The Markov decision model compared 2 strategies: allo-HCT or chemotherapy in CR1. Patients who had intermediate- or unfavorable-risk AML had a longer life expectancy when they received allo-HCT in CR1 than patients treated with chemotherapy alone. Likewise, patients who had a suitable related donor who received allo-HCT in CR1 had a longer life expectancy. The life expectancy was shortened to a greater degree by adjustment for QOL in the allo-HCT group. Nevertheless, QOL-adjusted life expectancies in most of the subgroups remained longer in the allo-HCT group than in the chemotherapy group. Our results showed that older patients with a related donor and younger patients with unfavorable cytogenetics benefited the most from allo-HCT in CR1. (*Blood.* 2011;117(7):2113-2120)

Introduction

Although 60%-80% of patients with acute myeloid leukemia (AML) achieve first hematologic complete remission (CR1) with chemotherapy, a substantial number of patients have an individualized risk of relapse.[1] Allogeneic hematopoietic cell transplantation (allo-HCT) has been established as a powerful treatment method to reduce the risk of relapse in patients with AML. However, this approach still leaves concerns associated with a certain probability of nonrelapse mortality. Although several prospective trials that used genetic allocation have been performed to clarify the roles of postremission strategies, the results have not always been consistent.[2-9] The role of allo-HCT in patients with AML in certain subgroups, including patients with intermediate-risk AML and elderly patients who have remained in CR1, remains unclear. A large meta-analysis that considered many of these prospective studies reported that allo-HCT in CR1 provided survival advantages not only in an unfavorable-risk group but also in an intermediate-risk group.[10] Even with these numerous studies performed in a prospective setting, it is still controversial to simply define allo-HCT as a better decision because of concerns about various late effects such as graft-versus-host disease (GVHD) that might lower the quality of life (QOL) after cure of the disease.

A decision analysis is a statistical technique that is used to help decision making under uncertain conditions with the assumption of a QOL evaluation.[11] When it is combined with a Markov process, it gives a flexible analytical method that makes it possible to track clinical events that occur after a certain decision with different probabilities and desirability over time.[12] This technique can offer valuable information about what clinical decision should be taken by quantitatively integrating the risks and benefits of a certain decision, and, hence, has been widely applied in making decisions in various fields. For example, in the field of hematology, on the basis of the results of a Markov decision analysis, Lee et al[13] reported the indications of allo-HCT for chronic myeloid leukemia in the era before imatinib, and Cutler et al[14] elucidated the recommended timing of allo-HCT for younger patients with myelodysplastic syndrome. Regarding AML, Sung et al[15] reported the results of a decision analysis with a conventional decision tree concerning consolidation strategies for patients in CR1. However, a Markov decision analysis has not yet been reported for postremission strategies in AML in CR1. To address this point, we performed a Markov decision analysis with the use of clinical information collected from 2029 patients.

Submitted May 14, 2010; accepted November 10, 2010. Prepublished online as *Blood* First Edition paper, November 24, 2010; DOI 10.1182/blood-2010-05-285502.

An Inside *Blood* analysis of this article appears at the front of this issue.

The online version of this article contains a data supplement.

Presented at the 51st annual meeting of the American Society of Hematology in New Orleans, LA, December 6, 2009.

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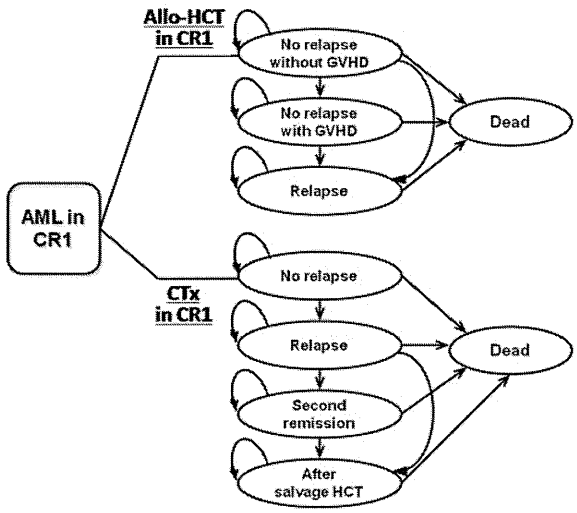

Figure 1. Markov decision model. Markov model that compares allo-HCT in CR1 and chemotherapy in CR1 is shown. Possible health states for each of the 2 groups are indicated in circles. Arrows indicate possible transitions between states. CR1 indicates first complete remission; allo-HCT, allogeneic hematopoietic cell transplantation; CTx, chemotherapy; and GVHD, graft-versus-host disease.

Methods

Data source

The study protocol was approved by the Institutional Review Board at National Cancer Center Hospital. We constructed a new database that included the clinical data of adult patients (age 16-70 years) whose conditions were diagnosed as AML by the World Health Organization classification between 1999 and 2006 and who had achieved CR1 after 1 or 2 courses of induction chemotherapy. Clinical information on > 2600 patients was collected from 70 institutions across the country. Patients with biphenotypic leukemia who were treated with chemotherapy for acute lymphocytic leukemia; patients who had extramedullar AML without marrow invasion, an extramedullary lesion that did not totally disappear after remission induction chemotherapy, or acute promyelocytic leukemia; and patients who received autologous HCT in CR1 were excluded from the analysis. Consequently, a total of 2029 patients were considered for this analysis.

Decision strategy

The primary decision examined in this study was whether to perform allo-HCT in patients with AML who remained in CR1. Statistical analyses were performed as of January 2010 with the use of the software package TreeAge Pro 2009 (TreeAge Software Inc) and the SPSS software package (SPSS Inc).

Markov model. We constructed a Markov decision model to compare 2 strategies: performing allo-HCT in CR1 (HCT group) and continuing chemotherapy without allo-HCT in CR1 (CTx group; Figure 1). The possible health states that were considered to occur after each decision/strategy included, for the HCT group, (1) no relapse without GVHD, (2) no relapse with GVHD, (3) relapse, and (4) dead, and for the CTx group, (1) no relapse, (2) relapse, (3) second remission, (4) after salvage allo-HCT, and (5) dead. The "GVHD" state included chronic extensive GVHD. The "dead" state included death from any cause. A schematic of the tree file is shown in supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article.

State transition probabilities. Transition probabilities between the states were calculated from the information in the database collected for this analysis as described in "Data source." The probabilities of state transition were allowed to vary over time. As a result, patients were distributed in various health states with different proportions along with cycle advances, that is, as time advanced from CR1, as shown in Figure 2. To take into account patients who were unable to receive allo-HCT in CR1 even though they had made a decision to receive allo-HCT, patients who died or relapsed within 3 months from CR1 were excluded from the database when we calculated the probabilities. The cycle length between state transitions has previously been set at the time considered to represent the clinical features and decision-making process for the target disease. In a Markov decision analysis that targeted myelodysplastic syndrome,[14] the cycle length was set at 6 months. In this analysis that targets patients with AML, we chose a shorter cycle length (3 months), and the analysis was performed for 40 cycles (10 years). The results are presented as life expectancy (LE), which is the average duration of life when patients are followed up for 10 years.

QOL utilities. We also assessed QOL-adjusted life expectancy (QALE) for the HCT and CTx groups. The time spent in each health state was adjusted for the estimated QOL that patients experienced while they remained in that state, which was represented by a utility value. In this study, utility values were derived from a questionnaire (supplemental Figure 2) that used a visual analog scale and was presented to 35 physicians who were familiar with the treatment of AML. Among them, 25 were physicians who were mainly involved in transplantation, and 10 were physicians mostly involved in chemotherapy with knowledge of transplantation. The utility values were expressed as numerical values between 0 (a

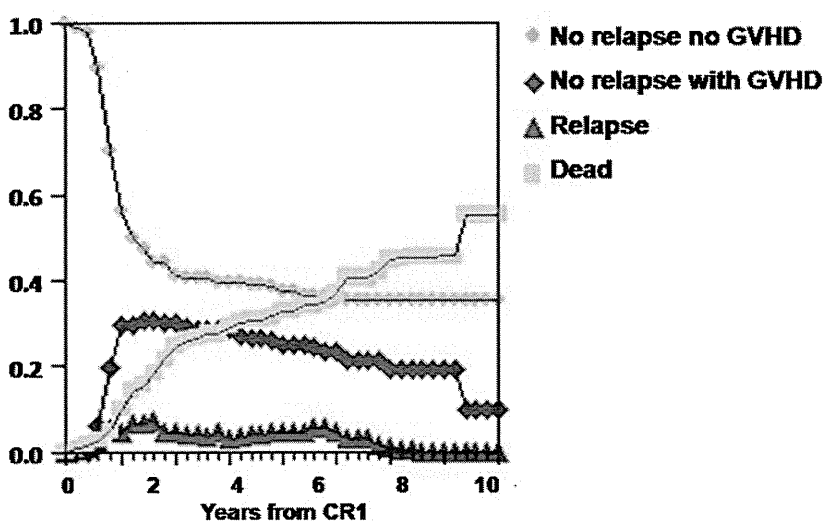

Figure 2. Distribution of patients in each health state. Distribution of patients with intermediate-risk AML in each health state is shown. Transition probabilities between the states were calculated for each subgroup with the use of the database. The probabilities of state transition were allowed to vary along with the cycle (1 cycle = 3 months) advances, depending on the states that the cohorts move from and to. As a result, the patients were distributed in each health state in changing proportions at different times from CR1. GVHD indicates graft-versus-host disease; and CR1, first complete remission.