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The Initial Drug in Combination Therapy —Is ARB superior?

Kazuyuki Shimada*¹

Currently the number of hypertensive patients with 140/90 mmHg or above in Japan is estimated to be approximately 35 million. Recent westernization of life styles is causing a rapid increase in metabolic disorders. The number of diabetic patients is reported to be approximately 7.4 million. Furthermore, half the cases are concomitant diabetes with hypertension. There is no doubt that hypertension and diabetes are central issues of health policy in modern society, because not only is the number of patients large, but also hypertension and diabetes are the greatest risk factors for cerebrovascular, cardiovascular, and renovascular diseases.

As long as the goal of treatment for hypertension and diabetes is the eventual prevention of cerebrovascular, cardiovascular, and renovascular diseases, the cost-benefit analysis needs to be evaluated together with the patients' lifetime benefits and losses. In fact, from the viewpoint of preventive medicine, the effectiveness of antihypertensive therapy has been continuously discussed, because unlike surgical treatment, of which the therapeutic effect is clear for each patient, the effects of antihypertensive therapy can only be seen in one in a couple of dozens and hundreds of patients after a couple of years of treatment. That is why the effectiveness of antihypertensive therapy has been so controversial.

This paper by Saito et al. compares and investigates the cost-benefit analysis of various combination therapies using calcium antagonist and angiotensin II receptor blockers (ARB), which are the most used antihypertensive drugs in antihypertensive therapy in Japan, and diuretics which are the first-line drugs used in the United States. The relation of expected costs and expected survival calculated for patients with and without diabetes was examined in the study. The results showed no difference in expected survival and expected costs in hypertensive patients without diabetes for these antihypertensive therapies. On the other hand, for patients with concomitant diabetes, there were differences between different antihyperten-

sive therapies. In other words, antihypertensive therapy with initial ARB with additional calcium antagonist or diuretic was superior in both expected survival and expected costs to antihypertensive therapy with initial calcium antagonist or diuretic with additional ARB if the therapy was insufficient.

The following are suggested as reasons why there was no difference among the antihypertensive therapies for patients without diabetes: there was no difference in the incidence rate of cerebrovascular and cardiovascular diseases and the frequency of the incidence of new-onset diabetes was low in the initial ARB group, causing less effect. On the other hand, for concomitant diabetes, though there was no difference in the incidence rate of cerebrovascular and cardiovascular diseases, since the progression to end-stage renal disease was inhibited in the initial ARB group, it is reasonable to assume that it affected both the estimated survival and costs.

In this paper, the calculations were based on significantly simplified hypotheses in each stage such as the blood pressure lowering effects of various antihypertensive drugs, the inhibition ratio of incidence of cerebrovascular, cardiovascular, and renovascular diseases with and without concomitant diabetes, the inhibition ratio of the occurrence of new on-set diabetes, and so forth. The legitimacy of each hypothesis is left to be investigated further. With this assumption admitted, this paper is remarkable in the sense that it points out the theoretical probability that the inhibition of occurrence of new on-set diabetes by antihypertensive drugs may not have a great effect overall and that the inhibition of the progression of diabetic nephropathy may be a key factor in determining the superiority of antihypertensive therapy.

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Pharmacoeconomical Evaluation of Combination Therapy for Lifetime Hypertension Treatment in Japan

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Abstract

Background As the observation periods of large-scale clinical studies are relatively short, the pharmacoeconomical advantage of different first-line drugs in lifetime hypertension treatment is unknown.

Methods Based on the results of large-scale clinical studies, phase III clinical trials and epidemiological data, we constructed a Markov model for patients with moderate essential hypertension to study the cost-effectiveness of lifetime hypertension treatment. In 55-year-old patients with moderate hypertension in the absence and presence of concomitant diabetes, four treatment regimens were compared: initial angiotensin II receptor blocker (ARB) with additional calcium antagonist if ARB therapy was insufficient (A + C group); initial calcium antagonist with additional ARB (C + A group); initial ARB with additional diuretic (A + D group); and initial diuretic with additional ARB (D + A group). It was assumed that approximately 20% of patients received combination therapies and there was no difference in the treated blood pressure. Olmesartan medoxomil, amlodipine and furosemide were the ARB, calcium antagonist and diuretic used, respectively.

Results Among patients without diabetes, expected survival and costs were similar in all treatment groups. Among patients with concomitant diabetes, expected survival was longest and expected costs were lowest in the A + C group. Expected survival decreased and expected costs increased in the order of A + D group, C + A group, and D + A group. The presence of concomitant diabetes affected cost-effectiveness.

Conclusion Our study suggests no pharmacoeconomical advantage among any of the treatment regimens in those patients without diabetes. In contrast, treatment with ARB with additional calcium antagonist may be a superior lifetime hypertensive treatment regimen for hypertensive patients with diabetes.

Key words Essential hypertension, Antihypertensives, Cost-effectiveness analysis, Cardiovascular disease, Combination therapy

Introduction

Large-scale clinical studies demonstrated that cardiovascular disease (CVD) in hypertensive patients can be prevented with favorable blood pressure control.^{1–3} Evidence-based guidelines for hypertension management have been pub-

lished or revised in many countries and areas^{4–7}; in 2004, the Japanese Society of Hypertension revised its guideline JSH 2000,⁸ which was first published in 2000.

Most of the guidelines recommend aiming at similar blood pressure targets. However, there are differences among the guidelines as to which first-line drug should be used in uncomplicated

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hypertension. The US 7th Report of the Joint National Committee (JNC7) and revised World Health Organization and International Society of Hypertension (WHO/ISH) statement recommend diuretics as first-line drugs mainly due to price considerations. In contrast, the European Society of Hypertension/European Society of Cardiology guideline recommends diuretics, beta-adrenergic antagonists, calcium antagonists, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARB),

and states that selection of antihypertensives should be based on the pathological condition of patients and that effectiveness and tolerability should not be compromised by drug prices. The recently published guideline of Japanese Society of Hypertension in 2004 has similar recommendations.

The JNC7 and WHO/ISH statement apparently evaluate cost-effectiveness chiefly from the aspect of blood pressure lowering effect and drug prices; however, it seems more prudent to

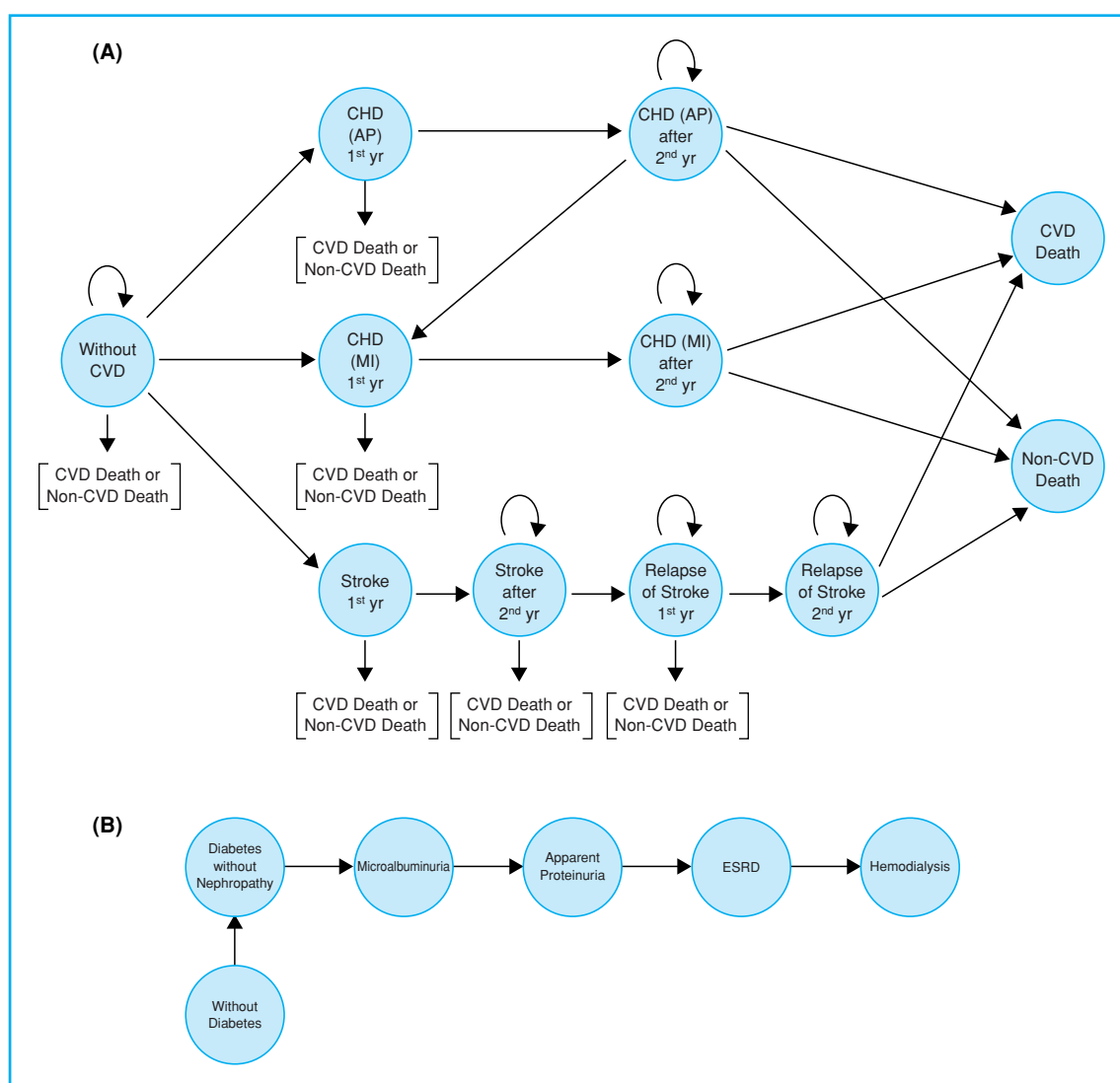


Fig. 1 (A) The Markov model; (B) A diabetes submodel for the progression of diabetic nephropathy
 CVD: Cardiovascular disease; CHD: Coronary heart disease; MI: Myocardial infarction;
 AP: Angina pectoris; ESRD: End stage renal disease.

include other factors such as metabolic changes associated with long-term treatment and patient compliance into such calculations to obtain a more accurate estimate of cost-effectiveness.⁹ Observation periods in large-scale clinical studies typically are 5 years, whereas in many patients medical management of hypertension lasts a lifetime. It is possible that diabetes associated with antihypertensive therapy did not result in CVD because of a relatively short period of observation after the development of diabetes. Hence, evidence obtained in large-scale clinical studies could be inadequate for true assessments of cost-effectiveness of lifetime hypertension management. Pharmacoeconomic analysis can explore the association between extension of survival resulting from antihypertensive treatment and associated costs by mathematical analytic models.

We previously constructed an analytic model (based on the Markov model) for prognosis of patients with essential hypertension and thereby evaluated the cost-effectiveness of single-drug regimens with different ARB.¹⁰ In the present study, in order to assess the cost-effectiveness for general practice, we modified our analytic model to evaluate the pharmacoeconomical effects of four different scenarios of first-line drug selected plus additional drug given as needed in hypertensive patients with and without concomitant diabetes.

Methods

Markov model

Our Markov model was originally comprised of coronary heart disease (CHD) and stroke sub-models, as described in our previous report (Fig. 1A).¹⁰ CHD consisted of myocardial infarction and angina pectoris; stroke consisted of cerebral infarction, cerebral hemorrhage and subarachnoid hemorrhage. Causes of CVD death consisted of myocardial infarction, acute coronary syndrome, and stroke. For the present study, we added two models mapping development of diabetes and progression of diabetic nephropathy. The progression of diabetic nephropathy model included the following five conditions from the report by Ikeda and Kobayashi.¹¹ and was constructed based on the results of the Kumamoto study¹²: no nephropathy, microalbuminuria, apparent proteinuria, chronic renal failure and hemodialysis (Fig. 1B). The mortality rate after hemodialysis was assumed to be 12.9%/year using an exponential distribution based on the 5-year survival rate by Nakai and Shinzato.¹³

Briefly, the CHD submodel included a risk estimation equation formulated based on the findings of the Framingham Study¹⁴ and adjusted for Japanese patients; their incidence was assumed to be 20% of those in the United States based on the WHO mortality database.¹⁵ This equation

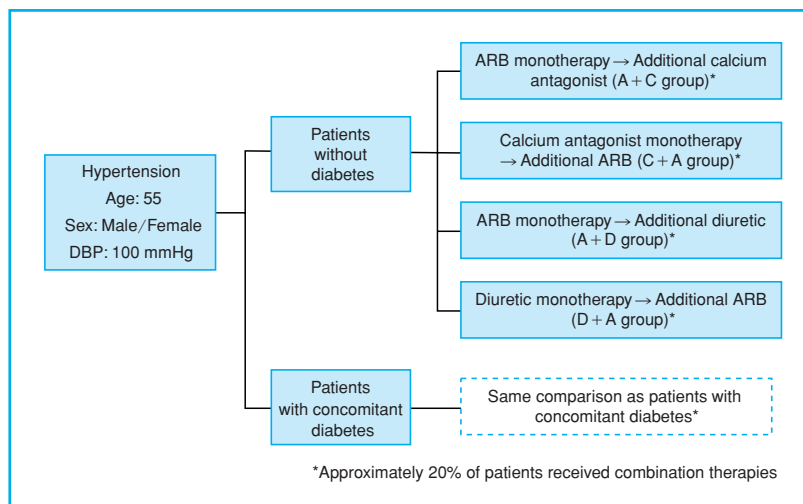


Fig. 2 Subject patient groups and control

incorporated some risk factors: gender; age; diastolic blood pressure (DBP); diabetes; smoking; and ECG-left ventricular hypertrophy. Lowering DBP leads to a decrease in annual CHD risk. The stroke submodel was constructed based on various epidemiological reports on the Japanese population. The model was slightly modified so as to estimate the risk reduction associated with lowering DBP by 10 mmHg at 46.9%, by adopting the Weibull distributions with the shape parameter of 0.5.¹

DATA Professional™ Software (Treeage Software, Inc., Williamstown, MA) was used for the construction and analysis of the Markov model.

Subjects and treatment strategies

Male and female patients of 55 years of age with moderate hypertension (DBP, 100 mmHg) in both the absence and presence of concomitant diabetes were subjected to this analysis. We assumed the diabetic patients did not have diabetic complications at baseline. Treatment was initiated with the target DBP of 90 mmHg,

and it was assumed that the DBP of patients who achieved the target value after 3 months of treatment could be maintained at 90 mmHg by single-drug therapy throughout the patients' lives. Furthermore, it was assumed that in patients who did not achieve the target value DBP could be controlled by adding another antihypertensive drug to the original treatment regimen. It was also assumed that the calcium antagonist, ARB and diuretic were azelnidipine, olmesartan medoxomil and trichlormethiazide, respectively. Four treatment strategies using these medications were evaluated (Fig. 2): A + C group, initial ARB with additional calcium antagonist if ARB therapy is insufficient (defined as target DBP not reached by initial monotherapy within 3 months); C + A group, initial calcium antagonist with additional ARB if the treatment is insufficient; A + D group, initial ARB with additional diuretic if the treatment is insufficient; and D + A group, initial diuretic with additional ARB if the treatment is insufficient. We assumed that there was no difference in the treated DBP among the four

Table 1 Probability parameters

	ARB	Ca	DIU	Combined Ca + ARB	Combined ARB + DIU	Untreated
Response rate (%)	80.2	83.4	83.4	100.0	100.0	NA
Cumulative dropout (%)						
First year	5.2	8.8	12.5	8.8	12.5	NA
Second year	6.2	10.4	14.7	10.4	14.7	NA
Third year	6.8	11.4	16.1	11.4	16.1	NA
Fourth year	7.3	12.2	17.2	12.2	17.2	NA
Fifth year	7.7	12.9	18.1	12.9	18.1	NA
Cumulative incidence of diabetes (%)						
First year	4.1	5.1	6.0	4.6	5.1	5.1
Fifth year	9.0	11.0	12.9	10.0	11.0	11.0
Tenth year	12.4	15.2	17.8	13.8	15.2	15.2
Fifteenth year	15.0	18.2	21.3	16.6	18.2	18.2
Twentieth year	17.1	20.7	24.2	18.9	20.7	20.7
Percent progression to diabetic nephropathy (%/year)						
Without nephropathy → Microalbuminuria	4.6	6.0	6.0	4.6	6.0	6.0
Microalbuminuria → Apparent proteinuria	4.9	6.5	6.5	4.9	6.5	6.5
Apparent proteinuria → End stage renal disease	14.4	20.0	20.0	14.4	20.0	20.0
End stage renal disease → Hemodialysis	14.4	20.0	20.0	14.4	20.0	20.0
Incidence of hyperuricemia	0.0	0.0	8.0	0.0	8.0	0.0

ARB: Angiotensin II receptor blocker; Ca: Calcium antagonist; DIU: Diuretic; NA: Not Applicable

types of treatment regimens.

Six parameters were considered in the analysis: antihypertensive effect, discontinuation of treatment, incidence of diabetes, inhibition of progression of diabetic nephropathy, incidence of hyperuricemia and drug price (Table 1). The antihypertensive effect of each treatment regimen was evaluated based on the criteria for lowered blood pressure provided in the guideline for clinical evaluation of antihypertensive medications for new drug applications in Japan: percentage of patients that experienced lowering of systolic blood pressure (SBP) by >20 mmHg and of DBP by >10 mmHg. Based on the results of

phase III clinical studies the antihypertensive effect of the calcium antagonist and ARB were assumed to be 83.4%¹⁶ and 80.2%,¹⁷ respectively. The antihypertensive effect of the diuretic was assumed to be the same as that of the calcium antagonist (i.e. 83.4%) based on evidence from a randomized trial conducted in Japan.¹⁸

We hypothesized a Weibull distribution with shape parameter 0.25 for discontinuation of treatment based on the study of 4-year patient compliance conducted by Conlin et al.¹⁹ To adjust for Japanese patients, the scale parameters according to each antihypertensive were estimated based on 1-year continuation of treatment (ARB,

Table 2 Cost parameters

	Item	Cost (Yen)
Costs of coronary heart disease	Acute myocardial infarction	2,785,000
	Myocardial infarction in the chronic stage (per year)	724,000
	Angina pectoris	1,190,000
	Angina pectoris in the chronic stage (per year)	700,000
Costs of stroke	Cerebral infarction	904,000
	Cerebral hemorrhage	1,841,000
	Subarachnoid hemorrhage	3,799,000
	Stroke in the chronic stage (per year)	213,000
Death costs	Death from acute myocardial infarction	2,859,000
	Death from acute coronary syndrome	2,541,000
	Other deaths	1,469,000
Long-term care costs (per year)	ADL1	0
	ADL2	738,000
	ADL3	2,512,000
	ADL4, 5	3,986,000
Outpatient management costs (per year)	Consultation fee + prescription fee + additional fee	53,040
Costs of antihypertensive drugs (daily, on N.H.I. price basis)	Azelidipine (16 mg/day)	87.0
	Olmesartan medoxomil (20 mg/day)	189.1
	Trichlormethiazide (2 mg/day)	9.7
Cost of a uric acid lowering drug	Allopurinol (200 mg/day)	64.5
Costs of laboratory tests (per year)	Diuretic-subscribed patients	
	First year	11,200
	Second and subsequent years	8,400
	Patients without diuretic therapy	5,600
Costs of treating diabetes and diabetic nephropathy (per year)	Diabetes without nephropathy	73,940
	Microalbuminuria	76,340
	Apparent proteinuria	82,510
	End stage renal disease	100,980
	Hemodialysis	5,765,526

94.8%; calcium antagonist, 91.2%; diuretic, 87.5%) as reported by Saito and Saruta.²⁰ Furthermore, it was assumed that compliance would remain steady after 6 years of treatment. DBP was assumed to return to 100 mmHg if treatment was discontinued.

Annual incidence of diabetes was estimated based on the 4-year incidence of diabetes in the ALLHAT²¹ by hypothesizing a Weibull distribution with shape parameter 0.5. The estimate of incidence of diabetes in the ACE inhibitor group was substituted for that among ARB users since the relative difference was similar in the VALUE study.²² The incidence of diabetes among patients taking combination therapies was assumed to be the mean of that associated with each individual drug. The incidence among patients who did not receive treatment was assumed to be the same as that among those patients using the calcium antagonist.

ARB was assumed to decrease the transition probability to microalbuminuria and proteinuria by 24% based on the findings of MICRO-HOPE²³ using ACE inhibitor, since ARB had a similar renoprotective effect as ACE inhibitor in patients with microalbuminuria, as shown in IRMA2.²⁴

After apparent proteinuria, it was assumed that the transition probability to chronic renal failure and hemodialysis would be inhibited by 28% based on the results of RENAAL.²⁵ If calcium antagonist was concomitantly used, the effectiveness was assumed the same as that of ARB therapy alone. If diuretic was concomitantly used, it was assumed that the progression of diabetic nephropathy would not be inhibited.

The incidence of hyperuricemia was assumed to be 8% among patients taking the diuretic.²⁶

Cost items

This analysis was conducted from the perspective of the general practice. Direct medical costs and long-term care costs incurred after stroke were included in the expense items (Table 2).

Drug prices/day were based on the Japanese drug price list as of April 2004.²⁷ In patients prescribed diuretic, it was assumed that frequent monitoring by blood tests would be necessary. Hence blood tests for Na⁺, K⁺, uric acid, fasting plasma glucose and lipid were assumed to be conducted four times in the first year (11,200 yen/year) and three times after the second year (8,400 yen/year). It was assumed that patients

Table 3 Cumulative incidence of CHD, stroke and diabetes every 5 years and during patients' lifetime

Years	CHD (%)				Stroke (%)				Diabetes (%)				
	A+C group	C+A group	A+D group	D+A group	A+C group	C+A group	A+D group	D+A group	A+C group	C+A group	A+D group	D+A group	
Patients without diabetes													
M	5	1.5	1.5	1.5	1.5	1.4	1.5	1.5	1.5	9.1	10.7	9.3	12.3
	10	3.6	3.6	3.6	3.6	3.3	3.4	3.4	3.6	12.4	14.4	12.6	16.5
	15	6.0	6.1	6.0	6.1	5.8	6.0	5.8	6.2	14.6	16.9	14.8	19.3
	Lifetime	15.5	15.5	15.5	15.7	14.5	14.9	14.6	15.4	18.3	21.1	18.6	23.9
F	5	0.7	0.8	0.8	0.8	0.7	0.7	0.7	0.8	9.2	10.8	9.4	12.4
	10	1.9	1.9	1.9	1.9	1.7	1.7	1.7	1.8	12.6	14.7	12.9	16.8
	15	3.2	3.3	3.2	3.3	3.1	3.2	3.2	3.4	15.1	17.5	15.3	19.9
	Lifetime	8.5	8.7	8.5	8.9	13.3	13.6	13.4	14.1	20.6	23.8	21.0	26.8
Patients with concomitant diabetes													
M	5	2.0	2.0	2.0	2.1	1.4	1.5	1.5	1.5	NA	NA	NA	NA
	10	4.7	4.8	4.7	4.8	3.3	3.4	3.4	3.5	NA	NA	NA	NA
	15	7.9	7.9	7.9	7.9	5.7	5.9	5.8	6.1	NA	NA	NA	NA
	Lifetime	18.8	18.4	18.7	18.4	13.8	13.9	13.8	14.3	NA	NA	NA	NA
F	5	1.5	1.5	1.5	1.5	0.7	0.7	0.7	0.8	NA	NA	NA	NA
	10	3.6	3.6	3.6	3.6	1.7	1.7	1.7	1.8	NA	NA	NA	NA
	15	5.9	5.9	5.9	6.0	3.1	3.2	3.1	3.3	NA	NA	NA	NA
	Lifetime	14.3	14.0	14.2	14.0	12.2	12.1	12.2	12.4	NA	NA	NA	NA

NA, not applicable.

CHD consists of myocardial infarction and angina pectoris.

Stroke consists of cerebral infarction, cerebral hemorrhage and subarachnoid hemorrhage.

who did not use the diuretic would undergo biannual blood tests (5,600 yen/year).

Treatment costs of hypertension, CHD and stroke were the same as those used in our previous model.¹⁰ Annual medical costs for treatment of diabetes and diabetic nephropathy were those described by Ikeda et al.¹¹

Cost-effectiveness analysis and sensitivity analysis

We conducted the analyses according to their gender and whether or not the patient had diabetes and estimated the cumulative incidence rate of CHD, stroke and diabetes. We calculated the expected survival and expected costs in each of the four treatment strategies.

As the primary objective of this study was to provide physicians with useful data which could be referred to in their daily practice, some of the general rules of pharmacoeconomic analysis were omitted. For instance, both costs and expected life years were calculated with a 3% discount,²⁸ but both of them without discount were used in the major results analysis. Furthermore, for the analysis of cost-effectiveness, costs and expected life years were based on relative-ness, and incremental cost-effectiveness ratios were not considered.

In the sensitivity analysis, we calculated the changes in expected survival and expected costs after the values of the five parameters in the ARB treatment in the cost-effectiveness analysis (antihypertensive effect, discontinuation of treatment, incidence of diabetes, inhibition of progression of diabetic nephropathy and drug price) were changed in the range $\pm 10\%$. We also conducted a sensitivity analysis of incidence of hyperuricemia, a parameter related to diuretics therapy, in the range 3–13%.

As concomitant diabetes has been demonstrated to be a risk factor for CVD in several studies,^{9,29} the following adjustments of risks for Japanese patients with concomitant diabetes were made in the sensitivity analysis. In the CHD risk estimation, we assumed that the CHD risk associated with concomitant diabetes was 2.02 times higher than that in patients without diabetes based on the results of a Japanese study conducted by Iwashita et al.³⁰ In the estimation of stroke risk, we estimated the risk associated with concomitant diabetes based on Suzuki's report on the relative risk of developing stroke in

patients with or without concomitant diabetes.³¹ Furthermore, sensitivity analysis was conducted on the assumption that combination of ARB and diuretic would successfully inhibit progression of diabetic nephropathy.

Results

Cumulative incidence

Cumulative incidence of CHD, stroke and diabetes every 5 years and throughout the lives of patients based on the Markov model are shown in Table 3. The incidence of CHD and stroke were similar among all groups, but the incidence of diabetes in initial ARB therapy (A + C group and A + D group) was lower than that in the other groups.

Cost-effectiveness analysis

In the analysis of male patients without diabetes, expected survival and expected costs/patient

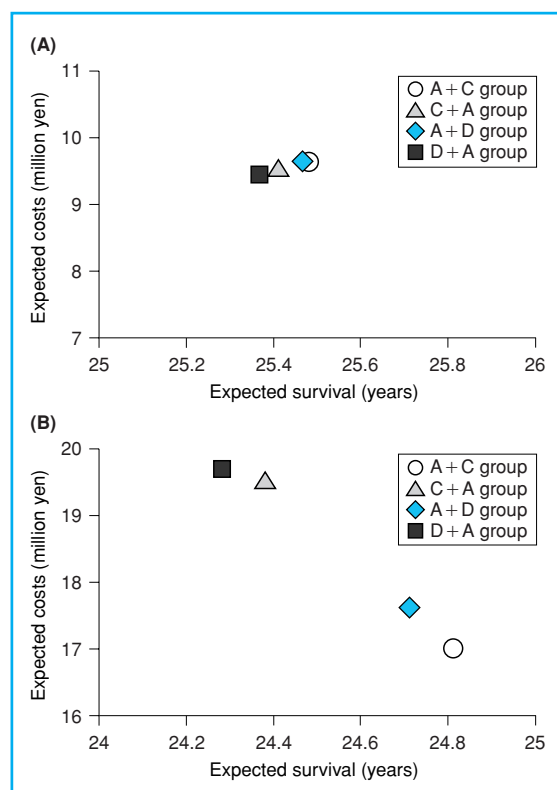


Fig. 3

- (A) Cost-effectiveness of antihypertensive drugs combination therapy (male patients without diabetes);
 (B) Cost-effectiveness of antihypertensive drugs therapy (male patients with concomitant diabetes)

were, respectively, 25.48 years and 9.65 million yen in A + C group, 25.41 years and 9.57 million yen in C + A group, 25.47 years and 9.67 million yen in A + D group, and 25.37 years and 9.44 million yen in D + A group (Fig. 3A). For male patients with concomitant diabetes the estimated values were, respectively, 24.81 years and 17.00 million yen in A + C group, 24.38 years and 19.52 million yen in C + A group, 24.71 years and 17.61 million yen in A + D group, and 24.28 years and 19.68 million yen in D + A group (Fig. 3B).

Among female patients without diabetes, expected survival and expected costs/patient were, respectively, 31.26 years and 10.65 million yen in A + C group, 31.13 years and 10.65 million yen in C + A group, 31.24 years and 10.69 million yen

in A + D group, and 31.06 years and 10.52 million yen in D + A group. For female patients with concomitant diabetes the values were, respectively, 29.91 years and 20.58 million yen in A + C group, 29.09 years and 23.90 million yen in C + A group, 29.73 years and 21.39 million yen in A + D group, and 28.91 years and 24.11 million yen in D + A group.

Expected survival was longer and expected costs were higher in female patients compared with their male counterparts; however, both male and female patients showed similar tendency regarding the analysis results of each combination therapy regardless of concomitant diabetes.

When the 3% discount rate per year for the expected survival and expected costs was applied,

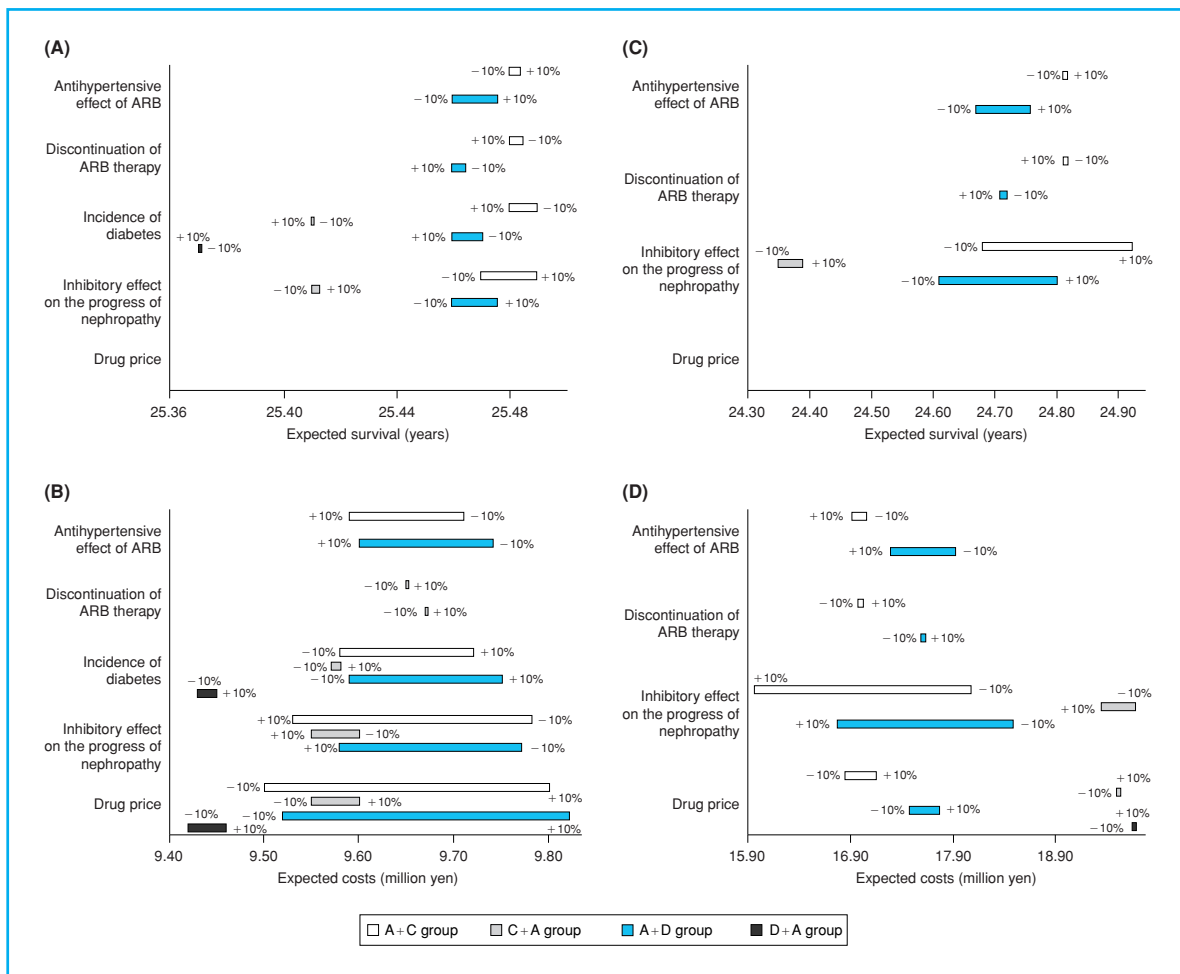


Fig. 4 The results of sensitivity analysis on the ARB-related parameters for male patients without concomitant diabetes (A and B) and with diabetes (C and D)

the results were as follows; the expected survival of patients without diabetes was longest in A + C group (male, 17.38 years; female, 20.06 years) and shortest in D + A group (17.33 years and 19.97 years, respectively) regardless of gender. The expected costs were highest in A + C group (male, 5.59 million yen; female, 5.68 million yen) and lowest in D + A group (5.21 million yen and 5.28 million yen, respectively). Among patients with concomitant diabetes, the life expectancy was longest in A + C group (male, 17.07 years; female, 19.47 years) and shortest in D + A group (16.81 years and 19.03 years, respectively) regardless of gender. The expected costs were highest in C + A group (male, 10.57 million yen; female, 12.01 million yen) and lowest in A + C

group (9.41 million yen and 10.54 million yen, respectively).

Sensitivity analysis

In Figs. 4A–D, the bars show changes in expected survival and expected costs of male patients without diabetes and those with concomitant diabetes when the values of the five parameters of ARB assumed in the cost-effectiveness analysis were altered from -10% to $+10\%$. When the change in the antihypertensive effect of ARB in the range 72.2–88.2% ($80.2\% \times 0.9 - 80.2\% \times 1.1$) was examined, the expected survival and expected cost changed by 0.004 year and 0.12 million yen, respectively. (In this case, the proportion of combination therapy was altered in the range 11.8–27.8%.)

The effect of changes in parameters was smaller in patients without diabetes compared with in those with diabetes, although the effect of changes in parameters related to diabetes was larger than changes of drug price on expected cost (Figs. 4A and B). Inhibition of progression of nephropathy had a large effect on the expected costs and the expected survival with concomitant diabetes: in the A + C group, its effect on expected survival and costs were 0.24 year and 2.11 million yen, respectively (Figs. 4C and D). The longer the length of the bar in the graph, the greater the effect of the parameter on expected survival and cost.

When we adjusted the incidence of CHD and stroke with or without diabetes based on data from Japanese reports,^{30,31} a slight change was observed in the estimated incidence of CVD events. Among patients with concomitant diabetes, cumulative incidence of CHD and stroke in the A + C group were 27.0% and 15.5% in males, and 13.9% and 18.4% in females, respectively. However, this did not influence the superiority of the A + C group (data not shown).

Figures 5A and B show the results of analysis of male patients with concomitant diabetes assuming that the progression of diabetic nephropathy was inhibited by combination therapy with ARB and diuretic (Assumption 1). The expected costs and survival in the A + C group and A + D group became almost equal. The effect of changes in these parameters was also smaller in patients without diabetes (data not shown). Even when the incidence of hyperuricemia, a parameter related to diuretics treatment, was

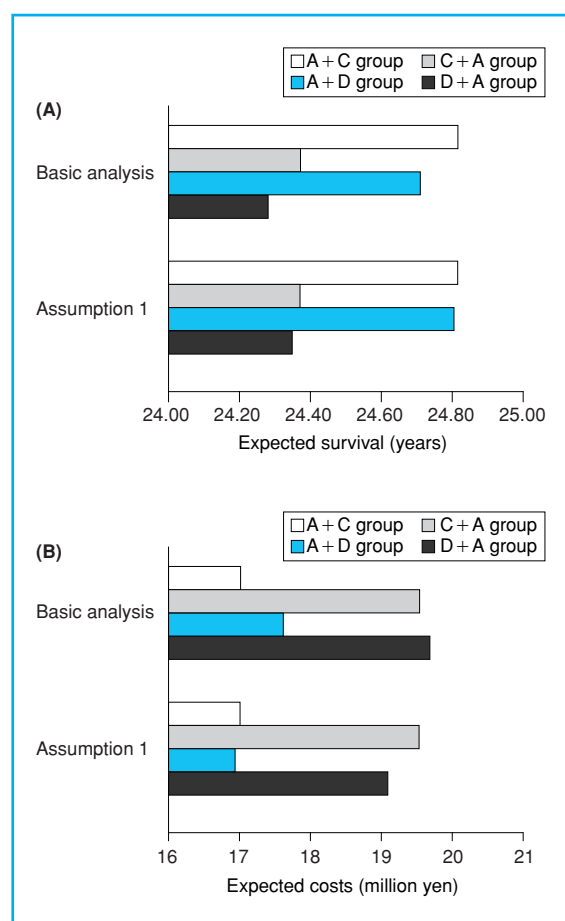


Fig. 5 Sensitivity analysis of male patients with concomitant diabetes
(A) Expected survival; (B) Expected costs

The progression of diabetic nephropathy was inhibited by combination therapy with ARB and diuretic (Assumption 1).

changed by 3–13% in the sensitivity analysis, hardly any change in expected costs was seen (data not shown).

Discussion

According to data published in 2001, heart disease and stroke are ranked the second and third primary causes of death in Japan, respectively³²; prevention of these diseases is therefore an important clinical issue. Annual medical expenses for ischemic heart disease and stroke alone are 2.54 trillion yen (2001), which accounts for about 10.4% of the entire medical expenses for general clinical practice.³³ Prevention of CVD in hypertensive patients by controlling blood pressure is considered pharmacoeconomically important.

Drug cost/day of ARB is highest, followed by calcium antagonists and diuretics. On the other hand, the antihypertensive effect of these three types of drugs does not seem significantly to differ.^{16–18,34} Hence diuretics seem to be the best choice of antihypertensive drug when only drug price and antihypertensive effect are considered; however, unlike calcium antagonists and ARB, diuretics pose certain risks such as abnormal glucose metabolism and hyperuricemia that potentially entail costly treatment. Contrarily, the organ-protective effects of ARB including inhibition of progression of nephropathy have been suggested in large-scale clinical studies, thereby possibly reducing costs in patients taking this therapy. Patient compliance is also an important factor in evaluating the cost-effectiveness of drug treatment. Favorable compliance has been reported among patients using ARB, whereas compliance in patients using diuretics is poor.^{19,35}

In our previous report, olmesartan monotherapy suggested favorable cost-effectiveness compared with other ARB monotherapy and non-antihypertensive treatment.¹⁰ The present analytic model was designed to reflect major factors that clinicians involved in antihypertensive treatment may consider when prescribing drugs and analyzed the cost-effectiveness and safety of four treatment strategies. There was no large difference in cost-effectiveness among the patients without diabetes. Therefore, for those patients, appropriate combination therapy should be chosen case by case based on their conditions as diagnosed by each clinician. However, in patients with concomitant diabetes cost-effectiveness was

noticeably high in the A + C group considering both expected costs and expected survival, suggesting that this treatment regimen is pharmacoeconomically superior to the others tested. Based on our sensitivity analysis of parameters related to ARB therapy, progression of diabetic nephropathy in hypertensive patients with concomitant diabetes had the greatest impact on cost-effectiveness. The incidence of diabetes in initial ARB therapy groups was lower than that in the other groups and the effect of changes in parameters related to diabetes was larger than changes of drug price on expected cost even in non-diabetic patients in the sensitivity analysis. Thus the lack of difference in expected costs for non-diabetic patients is probably due to the comparable medical costs of treatment of new-onset diabetes and diabetic nephropathy in diuretics treatment patients and drug cost of ARB treatment patients. In ARB treatment patients with concomitant diabetes, costs saved by the inhibitory effect of ARB on the progression of diabetic nephropathy exceeded the low price of the diuretic.

It has been reported that the incidence of stroke is higher than that of myocardial infarction in Japan,³⁶ This study included angina pectoris in addition to myocardial infarction in the estimation of the incidence of CHD, thus the incidence of stroke was similar to that of CHD.

Whether the inhibitory effect of combination therapy with ARB and diuretic on the incidence of diabetes and progression of diabetic nephropathy is similar to that of ARB alone and of ARB plus calcium antagonist is unknown. We assumed that the progression of diabetic nephropathy would not be inhibited if a diuretic was concomitantly used. We, however, conducted sensitivity analyses assuming that combination therapy with ARB and diuretic would show inhibitory effects on the progression of diabetic nephropathy. Even when the conditions were altered, cost-effectiveness in A + C group patients with concomitant diabetes was equivalent to the A + D group or superior to D + A group.

There are certain limitations regarding the structure of our model and establishment of parameters. The first limitation lies in the risk estimation equation. Regarding the incidence of CHD, we adjusted the estimation equation formulated based on the Framingham Study to reflect Japanese patients, possibly lending the

results certain bias. It will be necessary to improve the analytic model based on findings in clinical studies now being conducted in Japan^{37,38} in order to obtain more accurate estimations for Japanese patients.

We also may have overestimated the transition probability of progression of diabetic nephropathy, because the model was based on the results of the Kumamoto study in which subjects were treated with insulin.¹²

The second limitation is the fundamental problem that exists in any mathematical model analysis: prognosis models for hypertensive patients do not necessarily include all the factors associated with antihypertensive treatment. Azelnidipine has been reported to cause less changes in heart rates compared with other calcium antagonists³⁹; however, the implications of this was not taken into account in the present study. Moreover, although long-acting calcium antagonists and ACE inhibitors are reported to have similar renoprotective effects in Japan,^{40–42} this was not considered since the renoprotective effect of calcium antagonists is still controversial.⁴³ We also did not consider the indirect costs and utility because estimation was difficult, and this was another limitation of our model. These factors should be considered in future analyses.

The third limitation is the issue of target blood pressure and combination therapy. We assumed that DBP would be controlled at 90 mmHg; however, various guidelines recommend a target DBP of 80 mmHg in patients with concomitant diabetes.^{4,6} If DBP is to be controlled at 80 mmHg, combination therapies with more than two types of antihypertensives are often needed. Pharmacoeconomical analysis of combination therapies with more than two types of antihypertensives is a subject of future investigation. In addition,

there are many other kinds of combination therapies, other than those discussed in this report, e.g. ACE inhibitors and calcium antagonists. Some studies report that ARB show better blood pressure control and compliance than ACE inhibitors^{17,19}; therefore our future analyses should involve comparison with another combination therapy. Furthermore, pharmacoeconomical analysis of control of SBP is needed in future studies.

Lastly, six parameters were considered in this study (effectiveness, discontinuation of treatment, incidence of diabetes, inhibitory effect of ARB on progression of diabetic nephropathy, incidence of hyperuricemia associated with diuretic, and drug price); there may be others that should also be considered.

Conclusion

Although in hypertensive patients without diabetes the difference in expected survival and costs among the treatment strategies assessed is small, the presence of concomitant diabetes in hypertensive patients dramatically affects cost-effectiveness. Our study suggests that among the combination therapies with ARB, calcium antagonist and diuretic, the most suitable combination for hypertensive patients with concomitant diabetes is ARB plus additional calcium antagonist, from the pharmacoeconomic point of view.

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Ki67 and Tumor Size as Prognostic Factors of Gastrointestinal Stromal Tumors

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Abstract

Background The aim of the study is to determine useful prognostic factors of gastrointestinal stromal tumors (GIST).

Methods 135 patients with GIST in the stomach resected at the Cancer Institute Hospital were retrospectively reviewed.

Results 84% were positive for KIT and/or CD34. All events of death due to GIST, metastasis to lymph nodes, liver and other sites occurred in patients with KIT and CD34 positive GIST. Univariate Cox regression analyses revealed that ulceration, tumor size and Ki67 index were significant predictors of death due to GIST with KIT and/or CD34 positive stain. On the other hand, multivariate Cox regression analysis showed tumor size >50 mm [hazard ratio: 6.9 (95% confidence interval: 1.6–30.1)] and Ki67 index $\geq 40/\text{mm}^2$ [hazard ratio: 8.0 (95% confidence interval: 2.6–25.1)] were the only significant poor prognostic factors. Prognosis of patients with KIT and/or CD34 positive GIST satisfying both conditions of larger than 50 mm in tumor size and Ki67 index $\geq 40/\text{mm}^2$ was significantly poorer than in others (log-rank test: $P < 0.0001$): 5 year-survival rate: 36% vs. 95%.

Conclusion These results suggest that patients with KIT and CD34 positive GIST larger than 50 mm in tumor size and greater than 40 in Ki67 index may have poor prognosis.

Key words Gastrointestinal stromal tumor, Stomach, Clinicopathology, Prognostic factor

Introduction

Until recently, most submucosal tumors of the stomach had been considered to be myogenic or neurogenic and were diagnosed as leiomyoma, leiomyosarcoma, and schwannoma. However, immunohistochemical staining and electron microscopic studies revealed that most of these tumors did not differentiate into smooth muscle or Schwann cells. Mazur termed these tumors gastrointestinal stromal tumors (GIST) in 1983.¹ In 1996, Rosai defined GIST as a general term for mesenchymal tumors of the gastrointestinal

tract and classified these tumors into 1) smooth muscle type, 2) neural type, 3) combined type and 4) uncommitted type according to differentiation to smooth-muscle cells or Schwann cells.² In 1998, Hirota demonstrated mutations of the KIT proto-oncogene in GIST.³ Recently, the term GIST tends to be referred to mesenchymal tumors that are positive for the KIT receptor (CD117, stem cell factor receptor) and/or CD34, and do not differentiate into smooth-muscle cells or Schwann cells. Since both GIST and interstitial cells of Cajal, intestinal pacemaker cells, are positive for KIT protein and/or CD34, the cellular origin of GIST is considered to be mesenchymal

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stem cells or interstitial cells of Cajal. A gain-of-function mutation of the KIT proto-oncogene may play a part in development of GIST.³ Thus, at present, two definitions of GIST are used depending on the pathologists. In a broad sense, GIST is a general term for mesenchymal tumors based on the definition by Rosai.² In a narrow sense, GIST means neural tumors negative for smooth muscle markers, as well as nerve markers, based on the latest WHO classification⁴ and the definition by Miettinen.⁵

Imatinib mesylate, a selective tyrosine kinase inhibitor, has been shown in preclinical models and preliminary clinical studies to have activity against such tumors.⁶ However, no patient had a complete response to the treatment and the median duration of response had not been reached after a median follow-up of 24 weeks after the onset of response.⁷ Moreover, early resistance to imatinib was noted in 14 percent of treated patients.^{7,8} Thus, still delineating prognostic factors for patients with GIST may be important even in post imatinib era.

In this study, we aimed to evaluate significant prognostic factors and determine the subgroup with poor prognosis, who may need intensive treatments.

Patients and Methods

Patients

The study was conducted with the approval of the Institutional Review Board of Jikei University School of Medicine and Cancer Institute Hospital, and patient confidentiality was preserved without going back to obtain individual consent.

From 1946 to 2000, 274 patients diagnosed as having submucosal tumors of the stomach underwent resection at the Cancer Institute Hospital. Among these patients, we examined 135 patients whose postoperative course could be followed and immunohistochemical staining was performed on their tumors. The original diagnoses of these tumors included leiomyoma, leiomyosarcoma and Schwannoma. According to the broad definition of GIST by Rosai,² these tumors were considered to be GIST. Submucosal tumors other than spindle cell tumors, such as carcinoid, accessory pancreas and malignant lymphoma, were not included in this study. In addition, minute tumors of gastric cancer incidentally found in the resected

stomach were also excluded.

Immunohistochemical staining

In addition to hematoxylin and eosin-staining, immunohistochemical staining was performed with KIT (CD117; c-kit proto-oncogene product), CD34 (stem cell factor receptor), SMA (α smooth-muscle actin; smooth-muscle marker), and S100 (nerve marker). Tumors were classified according to the result of immunohistochemical staining. Patients' sex, age, location of the tumor, tumor size, macroscopic growth type, presence or absence of ulceration and metastases were examined by category. Macroscopic growth types of tumors were categorized into the following types according to the classification by Skandalakis: 1) endoluminal type, 2) exoluminal type, 3) intramural type and 4) mixed type.⁹ The location of the tumor was classified into 1) upper, 2) middle and 3) lower stomach. To demonstrate the proliferative activity of tumor cells, the number of Ki67 positive cells per mm² (defined as Ki67 index in this study) was examined.

The presence of metastasis was identified in image findings such as CT and US.

Statistics

Chi-squared test or Student's t-test was used for statistical comparisons of baseline characteristics. Logistic regression model was applied to examine factors that associate with metastasis. The Cox's proportional hazard model was used in univariable and multivariable analyses. Survival rates were calculated by the Kaplan-Meier method, and statistical significance was determined by the log-rank test. A value of $P < 0.05$ was considered statistically significant. All statistical analyses were performed using STATA 8.0 (STATA Corporation, College Station, TX).

Results

Patients' characteristics: Comparison between KIT and/or CD34 positive GIST and others

Patients' characteristics including immunohistochemical staining stratified KIT and/or CD34 positive GIST (N = 114) and others (N = 21) are summarized in Table 1. There were 114 (84%) KIT and/or CD34-positive tumors, 15 (11%) smooth-muscle marker-positive tumors, 7 (5%) neuron marker-positive tumors. Others included

Table 1 Patients' characteristics: Comparison between KIT and/or CD34 positive GIST and others

	KIT and/or CD34 positive GIST N = 114	KIT and CD34 negative GIST N = 21	Total N = 135	P value
CD34 positive	111 (97%)	0 (0%)	111 (82%)	<0.001
KIT positive	109 (96%)	0 (0%)	109 (81%)	<0.001
SMA positive	10 (9%)	13 (62%)	23 (17%)	<0.001
S100 positive	0 (0%)	7 (33%)	7 (5%)	<0.001
Age	59.0 ± 10.6	46.5 ± 14.2	57.1 ± 12.0	<0.0001
Male/Female	56/58	17/4	73/62	0.007
Macroscopic growth type	50/37/13/13/1*	9/5/5/1/0*	59/42/18/14/1*	NS
Ulcer	31 (27%)	2 (9%)	33 (24%)	NS
Location				
Upper/middle/lower	57/35/16	16/2/3	73/37/19	NS
Size (mm)	61.9 ± 47.5	43.3 ± 15.2	59.0 ± 44.4	NS
Size ≥50 mm	60 (53%)	7 (33%)	67 (50%)	NS
Ki67	60.1 ± 125.9	11.8 ± 36.2	52.3 ± 117.4	NS
Ki67 ≥40	36 (33%)	1 (5%)	37 (28%)	0.010
Total death	35 (31%)	2 (10%)	37 (28%)	NS
Tumor death	22 (21%)	0 (0%)	22 (17%)	0.022
Lymph node metastasis	6 (5%)	0 (0%)	6 (4%)	NS
Liver metastasis	18 (16%)	0 (0%)	18 (13%)	NS
Metastasis at any sites	23 (20%)	0 (0%)	23 (17%)	0.024

*: Macroscopic growth type: endoluminal type/exoluminal type/intramural type/mixed type/multiple type

cases with both SMA-positive and -negative lesions. Thus, the tumors were classified into the following three categories: 1) smooth-muscle marker positive, 2) nerve marker positive, and 3) KIT and/or CD34 positive.

Patients with KIT and/or CD34 positive GIST were significantly older in age and more male dominant than others. There were no significant differences in macroscopic growth types, presence of ulcer, location of tumor, size of tumor, score of Ki67 between KIT and/or CD34 positive GIST and others.

The sites of relapse or recurrence in the 23 patients were as follows: Liver: 13.3% (18/135); lung: 0.7% (1/135); bone: 1.4% (2/135); peritoneal: 3.0% (4/135); lymph node: 4.4% (6/135). All events of death due to GIST, metastasis to lymph nodes, liver and metastases at any sites

occurred in patients with KIT and CD34 positive GIST (N = 107). There were 23 cases of metastases and 22 of tumor death, of which 21 overlapped.

Factors affect on metastasis

Using logistic regression model, we examined factors that may associate with liver metastasis (Table 2) and metastases at any sites (Table 3) focusing only on patients with KIT and/or CD34 positive GIST (N = 114). Univariate logistic regression analyses revealed that in men, the presence of ulcerative lesions in the tumor, tumor size ≥50 mm in longer axis and Ki67 index ≥40 significantly increased frequency of liver metastasis. On the other hand, multivariate logistic regression analysis showed that only tumor size ≥50 mm and Ki67 index ≥40/mm² independently increased frequency of liver metastases.

Table 2 Univariate and multivariate logistic regression analyses of the factors associating with liver metastasis (N=18) focusing on patients with KIT and/or CD34 positive GIST (N=114)

	Univariate analysis OR (95%CI)	P value	Multivariate analysis OR (95%CI)	P value
Male	4.50 (1.38–14.68)	0.013	3.47 (0.72–16.71)	NS
Presence of ulcer	3.36 (1.19–9.51)	0.022	4.22 (0.84–21.23)	NS
Size \geq 50 mm	20.56 (2.63–160.79)	0.004	22.17 (2.30–213.54)	0.007
Ki67 \geq 40	29.20 (6.19–137.71)	<0.001	32.12 (5.67–182.03)	<0.001

Table 3 Univariate and multivariate logistic regression analyses of the factor associating with metastasis at any sites (N=23) focusing on patients with KIT and/or CD34 positive GIST (N=114)

	Univariate analysis OR (95%CI)	P value	Multivariate analysis OR (95%CI)	P value
Male	3.78 (1.36–10.47)	0.011	3.06 (0.79–11.83)	NS
Size \geq 50 mm	13.73 (3.04–62.10)	0.001	16.27 (2.93–90.37)	0.001
Ki67 \geq 40	19.84 (5.97–65.94)	<0.001	24.24 (6.08–96.66)	<0.001

Table 4 Univariate and multivariate Cox regression analyses of the death from KIT and/or CD34 positive GIST (N=113^{*1})

	Univariable analysis HR (95%CI)	P value	Multivariable analysis HR (95%CI)	P value
Male	3.82 (1.41–10.38)	0.009	2.01 (0.71–5.72)	NS
Ulcer	2.51 (1.09–5.78)	0.031	1.70 (0.72–4.01)	NS
Size \geq 50 mm	9.76 (2.28–41.77)	0.002	6.90 (1.58–30.07)	0.010
Ki67 \geq 40	12.12 (4.08–36.03)	<0.001	8.02 (2.56–25.09)	<0.001

*1: One patient with KIT positive GIST was loss to follow-up.

Similarly, univariate logistic regression analyses revealed that for men with tumor size \geq 50 mm in longer axis and Ki67 index \geq 40 there was a positive association with the frequency of metastasis at any sites. Multivariate logistic regression analysis showed that only tumor size \geq 50 mm and Ki67 index \geq 40/mm² independently and positively associated with frequency of metastases at any sites.

Survival analyses

Cox hazard regression analyses were applied to determine factors associating with death due to KIT and/or CD34 positive GIST using univariate and multivariate manner (Table 4). Univariate

Cox regression analyses revealed that ulceration, tumor size and Ki67 index were significant predictors of death due to GIST with KIT and/or CD34 positive GIST. On the other hand, multivariate Cox regression analysis showed tumor size \geq 50 mm [hazard ratio: 6.9 (95% confidence interval: 1.6–30.1)] and Ki67 index \geq 40/mm² [hazard ratio: 8.0 (95% confidence interval: 2.6–25.1)] were the only significant poor prognostic factors. The likelihood of a five-year survival of patients with KIT and/or CD34 positive GIST was 81.5%, of which patients satisfying both conditions of larger than 50 mm in tumor size and Ki67 index \geq 40/mm² was significantly poorer than in others (log-rank test: $P < 0.0001$):

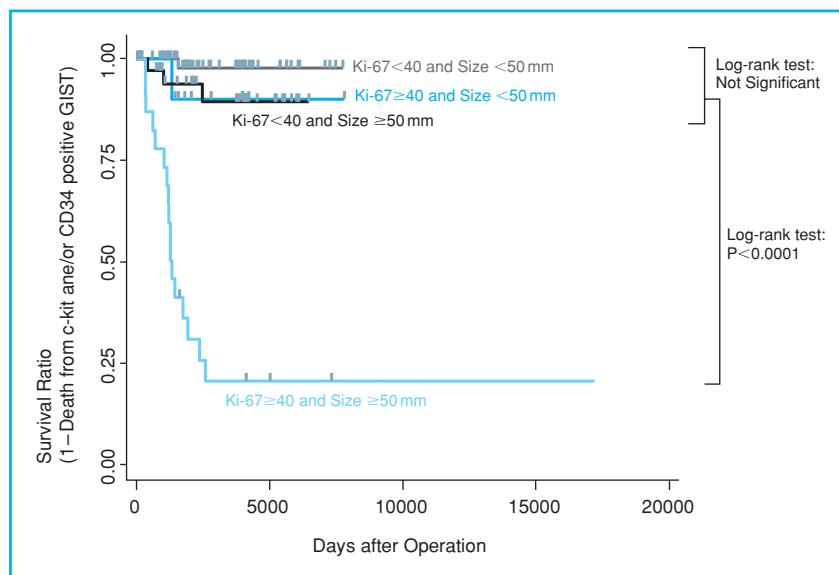


Fig. 1 Kaplan-Meier survival curve without death due to GIST (N=113)

Patients were grouped into 4 categories depending on expressions of KIT and CD34: 1) Ki67 <40 and tumor size <50 mm; 2) Ki67 ≥40 and size <50 mm; 3) Ki-67 <40 and size ≥50 mm; 4) Ki67 ≥40 and size ≥50 mm. Statistical differences were computed with log-rank test.

5 year survival rate: 36% vs. 95% (Fig. 1).

Discussion

In recent reports, GIST has been narrowly interpreted and defined as tumors positive for KIT and CD34. However, tumors negative for a smooth-muscle marker and nerve marker may include tumors 1) positive for both KIT and CD34, 2) positive for only KIT, 3) positive for only CD34 and 4) negative for both KIT and CD34.¹⁰ In this study, KIT and/or CD34-positive tumors accounted for 84% of all spindle cell tumors. This means that 84% of submucosal tumors diagnosed as leiomyoma, leiomyosarcoma or Schwannoma in the past were positive for KIT and/or CD34.

GIST is clinically and pathologically different from myogenic and neurogenic tumors, and their behaviors differ. Thus, myogenic and neurogenic tumors were not included in this study, as recommended by NIH consensus conference. Because myogenic tumors and neurogenic tumors are relatively scarce, we have to accumulate more cases of such tumors, for future research and discussion.

Cases of tumor death almost exactly over-

lapped with cases of metastases. Thus, tumor size ≥50 mm and Ki67 index ≥40/mm² were common independent significant poor prognostic factors in both metastases and tumor death in multiple regression analyses. Prognosis of patients with KIT and/or CD34 positive GIST satisfying both conditions of larger than 50 mm in tumor size and Ki67 index ≥40/mm² was significantly poor than in others (log-rank test: P<0.0001): 5 year survival rate: 36% vs. 95%. Recent studies demonstrated that both tumor size and mitotic activity were significant prognostic factors,¹¹⁻¹⁴ which were consistent with our results.

Tumor size could be a simple and reliable index for operative indication and postoperative follow-up. DeMatteo reported that only tumor size predicted survival in patients with primary lesions who underwent complete gross resection, based on multivariate analysis.¹⁵ Kwon stated that tumor size ≥50 mm was a poor prognostic factor, although this was based on univariate analysis only.¹⁶

The Ki67 index is a cell proliferation marker and could be a good indicator of the risk of metastases and prognosis. In the past, the mitotic index was often used for diagnosis of leiomyoma and leiomyosarcoma.^{17,18} However, the mitotic

index has the following problems: 1) Authors use different criteria for determination of malignancy, 2) assessment of mitosis using HE-stained samples tends to be subjective and 3) there is a possibility that broken nuclei could be mistaken for mitoses.¹⁹ The advantages of the Ki67 index include: 1) Mitosis is clearly observed as brown, 2) concentrated nuclei and karyolysis are negative and 3) information on mitosis can be obtained only by observing the nuclear configuration in Ki67-positive cells.²⁰ Carrillo concluded that MIB-1 (a monoclonal antibody to Ki67 antigen) index was the most powerful predictor of clinical behavior of GIST and should be used to support histological grading, based on multivariate analysis.²¹ Sedial stated that the expression of Ki67 in the nuclei of the tumor cells was the most important prognostic factor.²² Fujimoto reported the correlation with Ki67 and mitotic index, and each of them became prognostic factors in GISTs of the stomach.¹⁴ This was not examined in our study, but we were able to pick up mitosis certainly by calculating Ki67 positive cells.

From the Table 2 and 3 which analyzed factors related to metastasis, we can ascertain that the tumor size were small and Ki67 were low in cases

without metastasis.

It is clear that the tumor size and Ki67 index are reliable prognostic factors. In future research it would be useful to determine whether preoperative diagnosis and estimation of prognosis are possible. GIST is usually diagnosed by immunohistochemical staining and histopathological examination of the resected specimen. To diagnose GIST before surgery is generally difficult. In our study, variation of Ki67 index was frequently detected depending on the location in a single tumor. There are reports on biopsy after artificial ulceration and EUS-guided fine needle aspiration.^{23,24} However, because sufficient tissue for pathological examination cannot be obtained in many cases, these methods have not become widespread. Preoperative percutaneous biopsy theoretically has the risk of peritoneal seeding or tumor rupture.¹⁵

In conclusion, 1) 84% of GIST was positive for KIT and/or CD34, 2) all cases who developed to metastases and all cases who died of tumor were observed in patients with GIST positive for both KIT and CD34, 3) combination of tumor size and Ki67 index were further prognostic factors in GIST positive for both KIT and CD34.

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Immunological Homeostasis for Understanding Inflammatory Bowel Diseases

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Abstract

Inflammatory bowel diseases are thought to be caused by a complex interaction of genetic, immunological, and environmental factors. The involvement of immunological factors in the etiology of inflammatory bowel diseases is suggested by various facts, such as 1) the effectiveness of immunosuppressive agents, steroids, and anti-TNF- α antibody; 2) the presence of autoantibodies, and 3) the spontaneous development of chronic colitis in mice lacking a single immunity-related gene, e.g., IL-2 or TGF- β . In particular, the treatment of Crohn's disease using anti-TNF- α antibody has been applied to clinical practice with satisfactory clinical results all over the world. This article describes the immunological pathology of inflammatory bowel diseases as autoimmune diseases, and outlines prospective new treatment methods based on the nature of the disease.

Key words Regulatory T cells, Inflammatory bowel disease, Colitis, Leukocytapheresis

Inflammatory Bowel Diseases as Autoimmune Diseases

In understanding inflammatory bowel diseases (IBDs) as autoimmune diseases, an important point is the presence of autoantibodies. Unlike systemic lupus erythematosus and myasthenia gravis, autoantibodies involved in ulcerative colitis and Crohn's disease have not been characterized sufficiently to reveal the nature of these diseases. Autoantibodies in this case refer to the molecules that are specific to epithelial cells and interstitial cells in the intestines. However, these must be proved through various approaches, such as:

- 1) Identification of autoantibody molecules and epitopes (8 to 10 amino acid residues);
- 2) Presence of autoantibodies against antigens;
- 3) Development of animal models for conditions resembling human autoimmune diseases associated with hyperimmunization using purified antigens;

- 4) Isolation of T-cell receptors (TCR) reacting to autoimmune epitopes; and
- 5) Experimental development of conditions resembling human IBDs in transgenic mice with TCR genes.

In addition, the pathology of IBDs assuming an autoimmune mechanism is further complicated by the presence of intestinal bacteria.

- (1) Several models for chronic colitis (mice and rats) were established in the 1990s, particularly using gene-manipulated mice. These mice models require the presence of indigenous bacterial flora, and do not develop morbidity in a germ-free environment.¹
- (2) Antibiotics are effective in some patients with Crohn's disease.²

These facts suggest the possibility that the autoantigens involved in the autoimmune mechanism for IBD may be the antigens derived from symbiotic intestinal bacteria, rather than the antigens inherent to the human body. In view of the history of symbiosis starting before the evolution of anthropoid apes, intestinal bacterial

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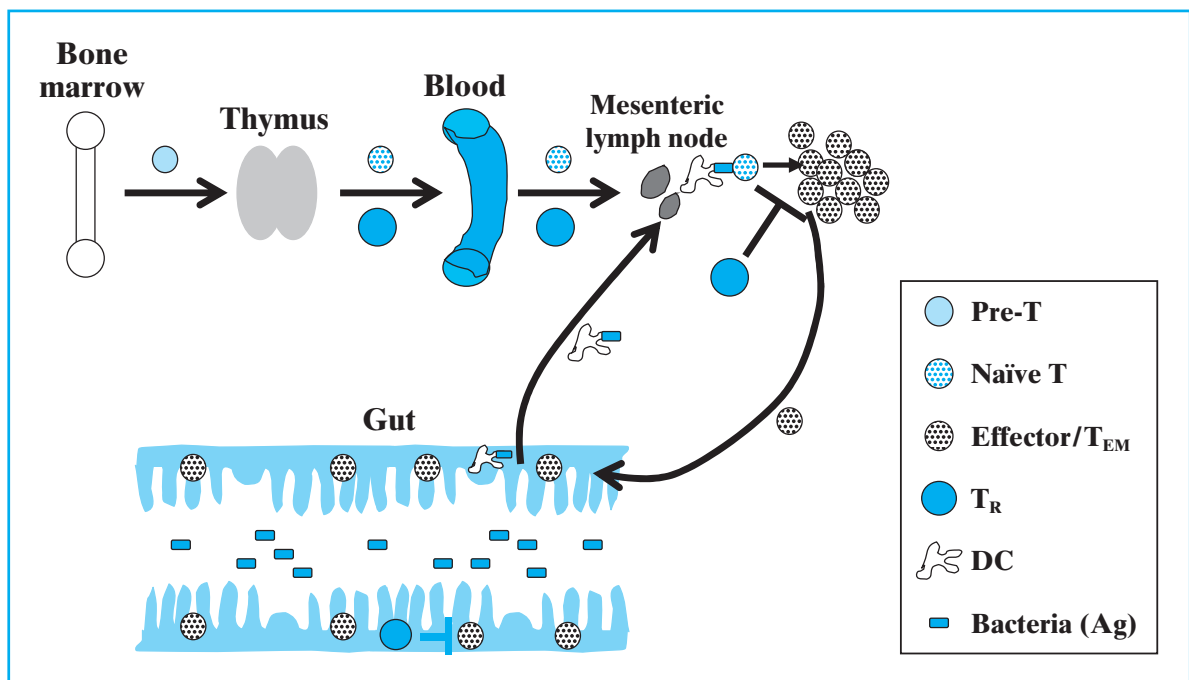


Fig. 1 Life-span of lymphocytes

The lamina propria T cells occurring in the intestines originate as naïve T cells in the thymus, move to the periphery, and are activated in mesenteric lymph nodes by dendritic cells, which are antigen-presenting cells. After activation, these cells express the homing receptor needed for the movement into the intestines, and then the resultant effector cells migrate into the intestines. While the effector cells are constantly kept under growth inhibition by regulatory T cells, some are thought to deviate from this regulation and develop colitis.

flora seems to be a part of self with respect to the immune response of the human body.

A Hypothesis for Autoimmune Mechanism—Central Tolerance and Peripheral Tolerance

Aberrant reactivity (abnormal activation and proliferation) of CD4⁺ helper T cells to self (or intestinal bacteria) is important in the autoimmune mechanism. The immune response in the actual living body consists of the following processes (Fig. 1):

- (1) Precursor T cells move from bone marrow into the thymus.
- (2) After entering the thymus, precursor T cells undergo selective removal of the cells (clones) that are reactive to self (negative selection), achieving elimination of autoreactivity and induction of self-tolerance (central tolerance). However, negative selection is not perfect. A small number of autoreactive clones move out of the thymus, mixed in

the large majority of clones that recognize non-self.

- (3) The clones that recognize non-self extensively proliferate in the thymus (positive selection), and move out of the thymus in the form of naïve T cells.
- (4) There are special clones called regulatory T cells (T_R, Treg), which probably recognize autoantigens in the thymus without being eliminated and differentiate to memory T cells within the thymus. These cells develop following a completely different pathway than that of the clones (naïve T cells) that can recognize self and non-self. Regulatory T cells occur at the rate of 5 to 10% among CD3⁺CD4⁺CD8⁻ cells in the thymus and among peripheral CD4⁺ cells. They normally exert suppressive control over autoreactive clones in the periphery and monitor the aberrant activity of autoreactive clones at the peripheral level (peripheral tolerance).³
- (5) Once moved into the periphery, the naïve T cells that recognize non-self continuously

monitor the invasion of non-self antigens (foreign antigens), patrolling the blood and dropping in mesenteric lymph nodes.

- (6) On the other hand, foreign antigens are taken up by intestinal lymphoid tissues, Peyer's patches, and isolated lymph follicles, and are processed by the dendritic cells occurring there. In this process, dendritic cells themselves are also activated and migrate to regional mesenteric lymph nodes.
- (7) When the naïve T cells recognizing non-self, described in (5) above, incidentally encounter the activated dendritic cells undergoing antigen presentation (6), the naïve T cells are activated quickly, proliferate, and differentiate to effector cells. In this process, the effector cells acquire the expression of homing receptors (integrin $\alpha 4\beta 7$) needed for the movement into the intestinal mucosa. As a result, the effector cells activated in mesenteric lymph nodes re-enter the blood flow via the thoracic duct and eventually enter the intestinal mucosa, where MAdCAM-1, the ligand for integrin $\alpha 4\beta 7$, is expressed.
- (8) The lymphocytes in the intestinal mucosa have a special characteristic. Unlike the cells in peripheral blood and lymph nodes, these cells consist of an overwhelmingly large number of memory T cells, express characteristic homing receptors, and are poorly proliferative. Strangely, most lymphocytes in the mucosa do not return to the blood flow and are believed to undergo apoptosis in the mucosa. However, the possibility remains that a small minority of intramucosal lymphocytes may return to the blood flow via an unknown mechanism and contribute to an autoimmune mechanism.

In the light of the development of lymphocytes as outlined above, there are several possibilities regarding the mechanism for autoimmune diseases including IBDs:

- 1) Abnormal development of regulatory T cells in the thymus;
- 2) Abnormal maintenance of regulatory T cells in the periphery;
- 3) Abnormal activation of dendritic cells;
- 4) Arrival of excessive antigens derived from intestinal bacteria (probably due to failure of epithelial cells and other barriers); and
- 5) Excessive viability of intramucosal lymphocytes (resistance to apoptosis).

To consider these possibilities, we need to understand the immunological basis for IBDs, focusing on regulatory T cells and the innate immunity system, which is closely related to the abnormal activation of dendritic cells and the abnormality of barrier elements such as epithelial cells. Regulatory T cells and the innate immunity system are hot topics in immunology at the present. These new concepts are expected to find application in the development of new therapies for IBDs.

Regulatory T cells

In the 1970s, when the study of immunology was promoted energetically focusing on T cells, the group led by Tada and Okumura proposed the presence of suppressor T cells, which are a type of T cell performing negative control over the immune system. While their proposal was based on experimental evidence, it was not possible to identify a gene expression marker using the techniques in molecular biology and immunology at the time, and the enthusiasm dwindled in the early 1980s. However, the group of Sakaguchi in 1995 demonstrated that the functions ascribed to suppressor T cells were found to occur specifically in CD4⁺CD25 (IL-2R α)⁺ cells, which exist at the rate of 5 to 10% among CD4⁺ T cells in healthy organisms.⁴ Following this discovery, there has been a new boom in the study of suppressor T cells, which are now called regulatory T cells. Many types of regulatory T cells have been proposed so far. This article focuses on the relationship between CD4⁺CD25⁺ regulatory T cells and IBDs.

CD4⁺CD25⁺ Regulatory T Cells

CD4⁺CD25⁺ cells occur at the rate of 5 to 10% among the CD4⁺ cells in peripheral blood (spleen) of normal mice, rats, and humans.^{2,4,10} As mentioned above, CD4⁺CD25⁺ cells are thought to recognize autoantigens in the thymus and undergo selection in a different way from other naïve T cells before they are supplied to the periphery.³ CD4⁺CD25⁺ regulatory T cells suppresses the *in vitro* proliferation of other CD4⁺CD25⁻ responder cells in a way depending on cell-cell contact.¹⁰ Experimental data concerning the ability of CD4⁺CD25⁺ cells to produce cytokines are inconsistent. Some reports

support and others disprove the production of inhibitory cytokines such as IL-10 and TGF- β in *in vitro* stimulation systems.⁴ However, CD4⁺CD25⁺ regulatory T cells characteristically do not produce IL-2, although they express high-affinity IL-2R receptors.⁴ CD4⁺CD25⁺ regulatory T cells are the only cells in the body that constitutively express CTLA-4, a type of co-stimulatory molecule, in cytoplasm.^{5,6} In fact, CD4⁺CD25⁺ regulatory T cells differ from other naïve T cells in that they are CD45RB^{low}CD44^{high} memory-type cells, and some of them are CD69 positive and have the phenotype of activated T cells. Recently, a number of groups identified Foxp3 molecules as a specific transcription factor in CD4⁺CD25⁺ regulatory T cells.⁷⁻⁹ A mouse strain with Foxp3 gene mutation is known as the scurfy mouse. This mouse has a phenotype (symptoms) closely resembling that of CTLA-4 deficient mice manifested in CD4⁺CD25⁺ regulatory T cells, and develops severe autoimmune disorders including IBDs.¹⁶ Importantly, this mouse shows a decrease in the number of CD4⁺CD25⁺ regulatory T cells. In addition, forced expression of the Foxp3 gene in naïve T cells causes them to act as regulatory T cells and inhibit the development of chronic colitis mediated by transfer of CD4⁺CD45RB^{high} T cells in mice.¹³

CD25 is the alpha chain of IL-2 receptor, which combines with beta and gamma chains to form the high-affinity IL-2 receptor complex. In fact, CD4⁺CD25⁺ cells express high affinity IL-2 receptors and show high affinity to IL-2. Interestingly, IL-2 deficient mice and IL-2 receptor alpha chain deficient mice also develop autoimmune diseases in various parts of the body including chronic colitis.^{17,18} Before the concept of CD4⁺CD25⁺ regulatory T cells was introduced, IL-2 was thought to be a T-cell growth factor inducing autoimmunity. However, IL-2 is now believed to be a factor that is essential to the differentiation, growth, and maintenance of CD4⁺CD25⁺ regulatory T cells. More importantly, CD4⁺CD25⁺ regulatory T cells suppress the development of morbidity in various animal models for autoimmune diseases including the models for IBDs.⁵ Although one group has proposed that the CD45RB^{low} cell population consists of regulatory T cells, current theory identifies it with a cell population representing about 30% of the CD4⁺ T cells

including CD4⁺CD25⁺ regulatory T cells. Recent studies have suggested the presence of subpopulations having regulatory function within the CD4⁺CD25⁻ T-cell population. It may be the case that CD45RB^{low} cells consist of many cells other than CD4⁺CD25⁺ regulatory T cells in addition to all regulatory T cells. When CD4⁺CD45RB^{high} cells are isolated from normal mouse spleen cells and transferred to syngeneic immunodeficient SCID mice or Rag deficient mice, chronic colitis develops. The fact that co-transfer of spleen or lymph node CD4⁺CD25⁺ regulatory T cells suppresses the development of chronic colitis indicates that CD4⁺CD25⁺ regulatory T cells possess the suppressive function to exert *in vivo* inhibition of chronic colitis.¹¹

Regulatory T Cells in the Human Mucosa and Inflammatory Bowel Diseases in Humans

Despite the large body of knowledge being accumulated concerning the roles of regulatory T cells in the mouse models of chronic colitis, little is known regarding the involvement of regulatory T cells in IBDs in humans. We examined the presence and function of CD4⁺CD25⁺ regulatory T cells localized in various parts of the intestines. In the past, T cells occurring in the intestines were thought to be memory cells, and CD25⁺ cells occurring at low percentages among CD4⁺ and CD8⁺ T cells were regarded as activation markers indicating IL-2 receptor alpha chain. These cells were understood to be pathological cells, particularly in the context of IBDs. After the recent drastic changes in our understanding, we conducted this study as an attempt to answer the fundamental question of whether human CD4⁺CD25⁺ T cells in the mucosa are regulatory T cells or pathogenic T cells in the current perspective.

We confirmed that CD4⁺CD25⁺ cells in normal human intestinal mucosa occurred at a rate of 4 to 8% of CD4⁺ cells. Regulatory T cells in human peripheral blood, unlike those in mice, have been reported to occur predominantly in the CD25^{high} cell population, which is a subclass of CD25⁺ cells characterized by considerably high fluorescence intensity.¹⁰ We, therefore, examined CD4⁺CD25^{high} cells, and found that CD4⁺CD25^{high} T cells in normal human intestinal mucosa occurred at the rate 1 to 3% of CD4⁺ T cells.¹¹

The CD4⁺CD25^{high} cells in the mucosa actually expressed CTLA-4, GITR, and Foxp3 genes. In addition, we clarified that CD4⁺CD25^{high} T cells in the mucosa are poorly proliferative (anergic) and suppress the proliferation of CD4⁺CD25⁻ responder T cells dependently on cell-cell contact. These results provide the first demonstration that CD4⁺CD25^{high} T cells in normal human intestinal mucosa possess the regulatory T cell function. This finding will be important in maintaining immunological tolerance in the intestines despite the presence of various antigenic stimuli in the intestines.

The study using the mouse models of chronic colitis suggested that the quantitative decrease in the number and the qualitative decline of the function of CD4⁺CD25⁺ regulatory T cells are the key to developing chronic colitis. However, our results surprisingly show increases in both CD4⁺CD25⁺ cells and CD4⁺CD25^{high} cells in patients with ulcerative colitis and Crohn's disease as compared with the mucosa of healthy subjects. The increase in CD4⁺CD25⁺ cells may be explained if we consider that they are pathogenic T cells in the inflammatory mucosa in IBDs, similarly to activation markers such as the transferrin receptor (CD71) and CD69 described in past reports. However, the CD4⁺CD25^{high} cells in the inflammatory mucosa in IBDs expressed CTLA-4, GITR, Foxp3, etc. in the same manner as those in healthy subjects and at the same time they were anergic, suppressed the proliferation of CD4⁺CD25⁻ cells, and had the same regulatory T cell function as in healthy subjects. Thus, our results show that T_R cells in the intestinal mucosa of patients with IBDs increased in number without qualitative decline of function. Although these result seem contradictory, they suggest a possible explanation that the enhancement of cytokine production, including the secretion of IL-6 and IL-15 from activated dendritic cells and the production of IL-2 from pathologic effector cells localized to the site of inflammation, may promote the proliferation and activation of effector cells at the site of mucosal inflammation in IBDs to an extent that cannot be controlled by the increased regulatory T cells. In fact, we confirmed that CD4⁺CD25^{high} T cells lost their regulatory function after the addition of IL-2. Alternatively, it is also interesting to consider IBDs as a pathology resembling chronic infection like intestinal tuberculosis. The

presence of regulatory T cells can be detrimental to the body for the purpose of eliminating particular microorganisms.¹² As the elimination of microorganisms depends on the presence of potent effector cells, the action of regulatory T cells to suppress the effector function may result in the inability to achieve complete elimination of microorganisms, leading to chronic inflammation. In this respect, interesting clinical studies conducted recently by two groups reported that the administration of anti-human CD25 antibodies improved active ulcerative colitis.^{13,14} While the authors of these reports also developed their study protocols on the assumption that IL-2 was a factor aggravating autoimmune diseases, the possibility remains that regulatory T cells may also be the target in ulcerative colitis. Future development of clinical and basic studies in this direction is awaited.

After revealing that regulatory T cells occur locally in the human intestines, we next considered the origin of these regulatory T cells. There are several possibilities:

- 1) The cells that have moved directly from the thymus to the intestines in the form of regulatory T cells;
- 2) The cells that have moved from the thymus to the regional lymph nodes (mesenteric lymph nodes in this case), educated there to gain gut-homing receptors, and then moved to the intestines; and
- 3) The cells that have developed and differentiated locally in the intestines.

Further examination is needed for better understanding of the regulation of intestinal inflammation.

Leukocytapheresis-assisted Retransfusion of Regulatory T Cells

In developing a new treatment method for IBDs using regulatory T cells, we considered the use of leukocytapheresis. In Japan, leukocytapheresis is indicated for severe, fulminant, and refractory cases of ulcerative colitis. Three methods are available at present: granulocyte apheresis (GCAP), lymphocyte apheresis (LCAP), and centrifugal leukocytapheresis. Considering the fact that large quantities of lymphocytes are removed in the process of treatment, we devised a new treatment method in which the above-mentioned regulatory CD4⁺CD25⁺ T cells are

isolated from the removed lymphocytes and retransferred. However, in the mainstream of clinical immunology, development of this kind of treatment is focused on collecting a small quantity of peripheral blood from the patient, separating CD4⁺CD25⁺ regulatory T cells, culturing them in vitro, and retransferring them to the patient. Such methodology has already entered the stage of clinical application in the field of tumor immunology. However, it cannot be applied to IBDs unless we solve significant problems, including: the applicability to benign diseases in younger patients; the safety of the use of various factors and cells needed for culturing (IL-2, anti-CD3 antibodies, and allogeneic cells); the possibility of carryover of these factors and cells during retransfer; and biohazard management against serious infections associated with long-term culturing.

We consider that the best approach at present is to develop a method that does not require a cell culture system, like the one using leukocytapheresis being developed in Japan. Leukocytapheresis is performed in a closed system and its safety has already been acknowledged. Through a joint study with Dynal, we developed Basiliximab-ClinExVivo, which is a preparation of anti-human CD25 chimeric antibodies (Basiliximab; Simulect, Novartis, Switzerland), approved for clinical use, bound to clinically applicable ClinExVivo (Dynal, Norway) separation beads. In addition, we are using the separation system of Dynal to ensure execution of the recovery process in a complete closed circuit. In this system, the recovered leukocytes are directly reacted with Basiliximab-ClinExVivo and subjected to magnetism while they were kept in the leukocyte bag, and the closedness during separation can be ensured easily. At present, the development of this revolutionary therapy (Leukocytapheresis-assisted Retransfusion of CD4⁺CD25⁺ T_R cells, LART-25) is promoted under the support of Center for Cell Therapy, Tokyo Medical and Dental University. The purpose of this therapy is to correct the decrease in peripheral regulatory T cells occurring in patients with active ulcerative colitis, aiming at the final result of increasing the supply of regulatory T cells to the site of inflammation and the lymphoid tissues responsible for inflammation.

Evidence concerning the use of various

immunosuppressive agents for the treatment of IBDs is being accumulated, and these agents are used widely. However, they are also causing serious side effects such as damage to the kidneys and pancreas. Regulatory T cells are the living body's own immunosuppressive agent, and the use of them is not expected to cause such side effects. On the other hand, retransfer of large quantities of regulatory T cells may cause excessive suppression of immunity, potentially causing problems of carcinogenesis and infection. The new therapy must be performed only after sufficient informed consent, absolute confirmation of safety based on in vitro analysis, animal experiments, etc., and the establishment of consensus between immunologists and the public.

Innate Immunity

Innate immunity is an immune mechanism that is contrasted with acquired immunity.¹⁵ It has long been known that lipopolysaccharide (LPS) acts as an endotoxin and induces dramatic immune reaction, sometimes causing shock and other responses. It was found in 1997 that the true nature of this response is the reaction mediated by toll-like receptors (TLR). Innate immunity is the immune mechanism working in the important early stage before the characteristic establishment of acquired immunity. It is a defense system against infection consisting of non-lymphatic immune cells such as neutrophils, macrophages, and dendritic cells. TLR is the receptor for pathogen-associated molecular patterns (PAMP) occurring in pathogens such as bacteria, fungi, and viruses. The subtypes from TLR1 to TLR10 have been identified in humans. For example, TLR2 recognizes the peptidoglycan (PGN) of Gram-positive bacteria, TLR2 recognizes viral dsRNA, TLR4 recognizes lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria, TLR5 recognizes a structural protein of bacterial flagella called flagellin, TLR7 recognizes imidazoquinolines, which are synthetic compounds with antiviral activity (no ligands of biological origin have been identified), and TLR9 recognizes CpG DNA of microorganisms.

On the other hand, acquired immunity is an immune mechanism that exists only in vertebrate animals. It depends on antigen-specific reactions

of T cells via TCR and B cells via B cell receptors and antibodies (immunoglobulin). The ability of acquired immunity to recognize a great variety of antigens derives from the use of receptors with randomly rearranged VDJ sequences. Acquired immunity is thought to be mainly involved in antigen recognition at the time of re-infection and the elimination of pathogens in the later stages of infection. In mammals, innate immunity and acquired immunity cooperate to constitute a secure and economically effective defense system.

As mentioned above, intestinal bacteria are essential to the development of chronic colitis. However, it has not been determined whether the antigen protein of intestinal bacteria, TLR signal, or both is important. Recently, Takeda and colleagues found that the development of chronic colitis is inhibited in the double knockout mouse produced from the STAT3 knockout mouse, a model for chronic colitis, and the MyD88 knockout mouse lacking the innate immunity system.¹⁵ On the other hand, we found that the MyD88 knockout mouse surprisingly shows aggravation of DSS-induced colitis in comparison with normal control mice.¹⁷ We hope to see further developments in study related to the role of innate immunity in colitis, including the hot topic of the therapeutic application of probiotics.

Therapy for Inflammatory Bowel Diseases Using Probiotics

As discussed above, the presence of intestinal bacteria is an indispensable condition for the development of colitis. However, not all bacteria aggravate chronic colitis. The species in the category of probiotics (e.g., *Lactobacilli*, *Bifidobacteria*, *Streptococci*, and non-pathogenic *Escherichia coli*) may act beneficially to the body. In fact, the effectiveness of the administration of probiotics has been demonstrated in several mouse models for IBDs.^{18,19} In addition, studies

have shown the effectiveness of probiotics in preventing pouchitis after surgical treatment of ulcerative colitis,^{20,21} as well as the effectiveness in inducing and maintaining remission in mild to moderate ulcerative colitis.²² On the other hand, the value of the use of probiotics in Crohn's disease has not been established. The proposed mechanisms for the action of probiotics include the antagonism against pathogenic bacteria through competitive binding to epithelial cells and the promotion of the production of secretory IgA and suppressive cytokines. Expectations are running high for probiotic therapy, as it poses little risk of side effects and it is closely related to intestinal bacteria, which are suggested to be involved in the early stages of disease. Even unexpected developments are anticipated, such as a suppressive TLR signal.

Conclusion

Due to advances in immunology, the treatment of IBDs is making tremendous progress both in Japan and in the rest of the world. The development of unexpected new therapies may be realized within years. To this end, we hope that the readers gain a better grasp of IBDs based on an accurate understanding of immunology in general and immunology of the mucosa, including the recent remarkable breakthroughs in immunological studies.

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Clinical Management of Pulmonary Aspergillosis

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Abstract

Pulmonary aspergillosis is deep seated mycosis that occurs as an opportunistic infection, and is known as the disease whose diagnosis and treatment are particularly difficult. Japan's first Guidelines for the Diagnosis and Management of Deep Seated Mycosis were published recently, and it is expected that the guidelines may encourage the standardization of the management of deep seated mycosis. The algorithm of the guidelines is composed of three categories of diagnosis: "Proven infection", "Clinically documented infection or Probable infection" and "Possible infection"; and 2 categories of therapy: "empiric", and "targeting" therapy. Treatment using amphotericin B (AMPH-B) is a standard practice even now. However, a more effective voriconazole (VRCZ) has become available and several other combination therapies are being performed. A new treatment standard should be established by collecting more knowledge and clinical experience.

Key words Pulmonary aspergillosis, Guidelines, Immunocompromised host, Amphotericin B, Voriconazole, Combination therapy

Introduction

Recently the number of immunocompromised hosts is increasing because of the application of chemotherapy, organ transplantation and the long-term administration of immunosuppressants. Deep seated mycosis as an opportunistic infection in immunocompromised hosts has become one of the clinically significant disorders. The introduction of azole antifungal drugs and the development of various serum diagnostic methods have resulted in the cure of some types of deep seated mycosis, thereby reducing the share of deep seated mycoses in the total number of pathological autopsy cases every year. However, the frequency of aspergillosis is rather increasing.¹ In particular, invasive pulmonary aspergillosis is a disease whose definitive diagnosis is difficult to establish and often has poor prognosis, with progression to an acute form. Non-invasive pulmonary aspergillosis may often progress to an acute

form and lead to poor prognosis. Even aspergillosis as non-invasive aspergillosis after repeating of hemoptysis, and chronic necrotizing pulmonary aspergillosis (CNPA) after following a chronic progression, often become resistant to therapy.² The early diagnosis of, and the establishment of treatment methods for, pulmonary aspergillosis, neither of which are adequate presently, are a pressing need. However, the recent development of several serodiagnostic methods and the release of new antifungal drugs are changing the diagnostic and management strategy for pulmonary aspergillosis. In every case, sufficient understanding of the morbidity of pulmonary aspergillosis is considered to be most important.

In Japan, the first 'Guidelines for the Diagnosis and Management of Deep Seated Mycosis' was published in February 2003.³ This paper discusses recent trends of clinical practice and management of pulmonary aspergillosis, including the Japanese Guidelines.

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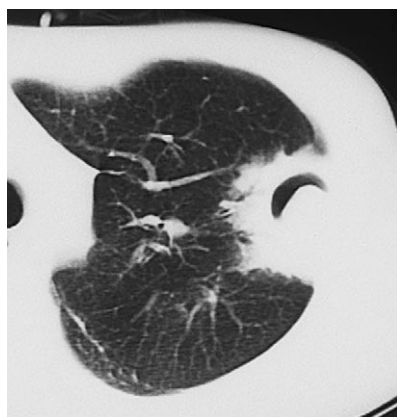


Fig. 1 CT scan of IPA patient with acute myeloid leukemia, showing an air-crescent sign

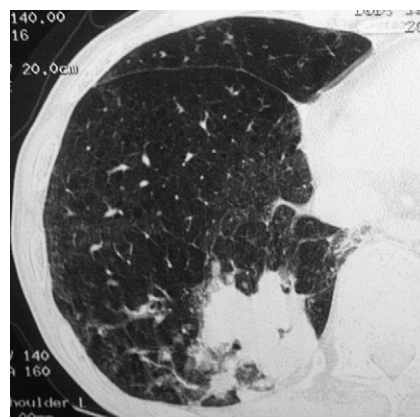


Fig. 2 CT scan of IPA patient with adult T-cell lymphoma, reveals consolidation and irregular infiltration

Classification

Pulmonary aspergillosis is mostly contracted by a fungus (aspergillus) which enters the body by inhaling conidia and colonizes and grows in the alveolus, bronchiole or existing cavities. Pulmonary aspergillosis presents various morbidities depending on the interaction between the pathogenicity of aspergillus, the immune status of the host (decreased immunity and allergic predisposition), and the pulmonary structure (existing cavitory lesions and cysts). Usually, pulmonary aspergillosis is divided into three categories: invasive pulmonary aspergillosis (IPA); non-invasive pulmonary aspergillosis mainly of fungus ball pulmonary aspergillosis (aspergilloma); and allergic bronchopulmonary aspergillosis (ABPA).⁴ Some researchers advocate that chronic necrotizing pulmonary aspergillosis (CNPA), considered to be clinically positioned between invasive and non-invasive types, is defined as aspergillosis but this has not undergone histopathological examination.⁵ Recently a report proposed to categorize chronic pulmonary aspergillosis into various types.⁶

Clinical Feature and Diagnosis

Invasive pulmonary aspergillosis: (IPA)

IPA is a fungus infection (mycosis) with poor prognosis, which occurs in compromised hosts who have undergone chemotherapy and take immu-

nosuppressant, including steroids, for malignant hematopoietic diseases, hematopoietic stem cell transplantation and malignant tumors. It is known that if the patients who have undergone bone marrow transplantation contract IPA, then 90% of them die.⁷ IPA occurs at a higher frequency in the patients with neutropenia, particularly with neutrophils of 500/ μ l or less.⁸ Histopathologically, discrete nodular lesions, accompanied by hemorrhage around the lesions, are observed with strong fungal invasion into blood vessels and the lesions in these cases.⁹ As radiological findings single or multiple nodules and infiltrations are often observed in plain chest X-rays. Increased density of groundglass opacity around the nodule in the CT, is a characteristic finding in the relatively early stage. It is called the 'CT-halo sign' and is a very useful finding for early diagnosis.¹⁰ This is known to histologically correspond to the hemorrhage around the nodular lesion, but it is not specific to IPA. The recover of the leukocyte count to normal in the progress of the disease would suggest that necrotic tissues are absorbed and removed from the periphery of the nodular lesion to fill air in the periphery, which is known as an 'air-crescent sign' in the imaging findings.¹¹ It sometime reveals radiological findings which look like the fungus ball of aspergilloma at a glance (Fig. 1). However, histopathologically, the nodule in the cavity is the pulmonary tissue coagulated and necrotized by fungal invasion and growth. On the other hand, IPA which occurs in those without neutropenia, shows non-specific

infiltration like bronchopneumonia (Fig. 2).¹² Japanese Guidelines divide diagnostically IPA into the following three categories: “Proven infection”; “Clinically documented infection or Probable infection”; and “Possible infection”.³ For the proven infection, the presence of aspergillus hyphae in infectious lesions must be mycologically and/or histopathologically proven. Transcutaneous and transbronchial lung biopsies, and invasive examinations such as thoracoscopic biopsy with video assisted thoracoscopic surgery (VATS), may be rarely performed and it is difficult to apply them, in view of the systemic conditions of the patients. The Guidelines include the detection of aspergillus from sputum and bronchoalveolar lavage (BAL) specimen as requirements for diagnosing proven infection. However, the aspergillus culture positive rate is low in many patients with IPA, and the number of patients who can undergo bronchoscopic examination is limited. “Clinically documented infection or probable infection” is diagnosed when the case shows typical imaging findings, such as the CT-halo sign or air-crescent sign, and when the galactomannan antigen and β -D glucan in serum or gene diagnosis using the PCR method is positive.^{13–15} This “Clinically documented infection” corresponds to the “Probable infection” used by the European Organization for Research and Treatment of Cancer (EORTC). Many cases diagnosed in routine medical practice are considered to meet these criteria. “Possible infection” is diagnosed when either imaging findings or serum/genetic diagnosis is positive. However, in the field of respiratory medicine, the cases with risk factors and clinical conditions as well as typical imaging findings, are diagnosed as “Clinically documented infection”. In the clinical setting the early detection of clinical symptoms or risk factors, or imaging findings from which IPA is suspected in the patients with a clinical background such as immunosuppression or risk factors, is of critical importance. It should be followed by serum or genetic diagnosis, and then by prompt administration of antifungal drugs with the performance of invasive examination, if possible, in mind.

Aspergilloma

Aspergilloma is contracted by the secondary colonization of aspergillus conidia through the airway into existing cavitory lesion, bronchiectasis or

cavitory lesion produced by old lung tuberculosis, and its proliferation to form fungus balls. Many patients remain asymptomatic, whereas blood sputum, fever, systemic weariness, weight loss, and massive hemoptysis are observed in some patients. According to a report, blood sputum is seen more than once in over 75% of the patients, and 5% of the patients die from mass bleeding.¹⁶ Chest X-ray showed cavity wall, thickened pleura, round fungal balls in the cavity and air layers surrounding the cavity. These findings are named as “air-crescent sign” or “meniscus sign”. Partially thickened pleura is considered as an initial finding of aspergilloma.¹⁷ Diagnosis is relatively easy. Proven infection is diagnosed by detecting aspergillus in the culture of sputum and BAL, or proving the presence of aspergillus in the tissue of transcutaneous and transbronchial lung biopsies. Anti aspergillus precipitation antibody, a parameter in a serological test, is positive in about 90–100% of the aspergilloma patients, and is a useful parameter in adjuvant diagnosis.¹⁸ Aspergilloma demonstrates various radiological findings and clinical progressions depending on the immunosuppression and changes in the existing lung structure, ranging from asymptomatic with little change in radiological findings in the progress of disease, to those with the factors of CNPA and IPA. A recent report defines chronic pulmonary aspergillosis, including CNPA, as consisting of chronic cavitory pulmonary aspergillosis (CCPA), chronic fibrosing pulmonary aspergillosis (CFPA), and simple aspergilloma. The entity of disease will be the subject of future discussion.⁶

CNPA, Semi-invasive PA

The disease type of CNPA is defined as positioned between aspergilloma and invasive pulmonary aspergillus. It is interpreted as a disease categorized within aspergilloma. But as its clinical symptoms may progress to a severe form, utmost care is necessary. CNPA is often observed in the relatively mild immunocompromised hosts due to malnutrition, diabetes and malignant tumor, or in those with the lung whose immunity is locally reduced due to lung surgery, radiation therapy, chronic obstructive pulmonary disease and pneumoconiosis.² It is reported that approximately 25% of the patients underwent low-dose steroid therapy.¹⁹ CNPA follows a chronic progress



Fig. 3 CT scan of patient with CNPA, showing a fungus ball and consolidation

but often shows a poor prognosis. Chest X-rays demonstrate the thickened pleura and infiltration in the initial stage, to be followed later by cavity lesion. Fungus balls and the air-fluid level may be observed in the cavity. In addition to this, infiltration of consolidation or ground-glass appearance surrounding the area may be also progressed (Fig. 3). Although no detailed histopathological examination has been performed, various findings such as necrotizing granulomatous pneumonia, bronchocentric granulomatosis (BCG), eosinophilic pneumonia and organizing pneumonia have been reported, and there is no histopathological definition for diagnosis.²⁰ CNPA is diagnosed on the basis of clinical course, consisting of clinical background, symptoms and imaging findings, and the presence of aspergillus in the culture of sputum. In the serum diagnosis aspergillus precipitation antibody shows the highest positive rate, and galactomannan and β -D glucan are also useful in the diagnosis.

Treatment

Successful treatment depends on the early administration of a sufficient dose of effective drugs as well as whether or not the recovery from neutropenia or immunocompromised status caused by the administration of immunosuppressive drugs. So far, only four drugs were effective for lung aspergillus, namely AMPH-B, Flucytosine (5-FC), Itraconazole (ITCZ) and Micafungin (MCFG). In addition, the use of VRCZ in Japan has been authorized since July 2005. The treatment methods for pulmonary aspergillus are explained below in

accordance with the Japanese Guidelines. IPA cases which were diagnosed as proven and clinically documented infection are subjected to, as a targeted therapy, intravenous drip injection of 1.0–1.5 mg/kg/day of AMPH-B. If this produces an insufficient effect, it is combined with the oral administration of 100–150 mg/kg/day of 5-FC or 200–400 mg/day of ITCZ. Or, the intravenous drip injection of 150–300 mg/day of MCFG is chosen. For the patients who continue chemotherapy, or who are scheduled to undergo bone marrow transplantation, surgical removal of remaining lesion should be considered. The patients with suspected IPA should be subjected to the oral administration of 200–400 mg/day of ITCZ as an empiric therapy, or a drip injection of 0.75–1.5 mg/kg/day of AMPH-B, or 150 mg/day of MCFG will be chosen.

The treatment of aspergilloma, including CNPA, is explained below. Asymptomatic or slowly-grows aspergilloma may be placed under observation without treatment. The patients who show an aggravating trend of symptoms of fever, bloody sputum and hemoptysis require active treatment in which surgical removal is the most reliable method. The patients diagnosed as having proven infection should be subjected to surgery as soon as possible. Those who cannot undergo surgical removal because of advanced age or declined lung functions should be subjected to the following medical treatment: oral administration of 200–400 mg of ITCZ once per day, or intravenous drip of 0.75–1.5 mg/kg/day of AMPH-B, or 150–300 mg/day of MCFG. Inhalation or injection of AMPH-B may be performed, though it is not described in the Guidelines. However, when it is directly administered through the air respiratory tract, safeguards should be taken against the symptoms caused by direct stimulation such as fever, coughing, airway contraction and shock.

The administration of AMPH-B has been the standard therapy for aspergillosis for some time. However, it has often been experienced that renal dysfunction and various adverse effects have forced the administration to be discontinued. In Western countries the use of the liposomal preparation of AMPH-B is known to have identical efficacy with, and less adverse effect than, AMPH-B, and ITCZ injections have produced good results. Liposomal preparation of AMPH-B has good transfer into the infected lesions, and

the less adverse effect of AMPH-B has enabled the administration of a sufficient dose of the drug for the patients with poor systemic conditions.²¹ Also VRCZ is the latest azole antifungal which shows a much better outcome in terms of efficacy, survival rate and toxicity than AMPH in IPA, and is thought to be the standard drug of first choice for the treatment.²² However, as it causes vision impairment, hepatic disorder and drug interaction in many cases and 23% of Oriental people are poor metabolizers, utmost care should be taken in these cases. In 2002 the clinical use of MCFG, a candin antifungal drug developed in Japan become possible. In the USA caspofungin (CAP), a candin antifungal drug, was approved in 2001. It is an antifungal drug with a new action mechanism to inhibit the synthesis of 1,3- β -D glucan, one of the main components forming the antifungal cell wall. This drug is very safe and has no drug interaction, though it is only in the injection dosage form as yet.²³

VRCZ has been newly approved after the publication of the Japanese Guidelines, and liposomal AMPH-B and ITCZ injections are scheduled to be approved. Conventionally only AMPH-B has been an effective drug for aspergillosis, whereas there are several options of antifungal drugs with anti-aspergillus activities. It is considered that the choice and optimal use of effective drugs are keys to the success of treatment. However, there is no antifungal drug which may easily cure aspergillosis. Therefore, the combination therapy, the effect of which is improved by the coadministration of an antifungal drug with different action mechanisms, has attracted increasing attention in recent years.²⁴ In Western countries combination therapies such as VRCZ+CAP and AMPH+CAP are being tried.²⁵ The therapy with the coadministration of AMPH-B+MCFG is being performed.²⁶ To

establish an evidence base for the combination therapy, it is desirable to collect many cases and perform controlled clinical trials.

Conclusion

In the field of fungal infection the treatment of pulmonary aspergillosis has become the most important clinical challenge. There has been great advancement in the prevention of onset and early diagnosis of the disease, and less adverse effects but not yet to a satisfactory level. The publication of the 'Guidelines on the Diagnosis and Management of Deep Seated Mycosis' in Japan has improved and standardized the clinical practice for the disease.

In every case it is of prime importance to adequately understand the morbidity of pulmonary aspergillosis. Moreover, it is considered that the full understanding of the characteristics of all diagnostic methods, including serological diagnosis, and the most effective usages of them would lead to early accurate diagnosis of the disease. In the treatment, it seems necessary to understand characteristics of each antifungal drug, and to choose the optimal treatment method taking into account any underlying diseases and immune conditions. Also, the effectiveness of the combination therapy is examined in an increasingly number of cases, and the release of several new antifungal drugs is now scheduled. Previously we had to treat the aspergillosis cases with a limited number of drugs, but we are now entering the era when we ourselves should choose the effective and appropriate drugs and the methods of administration, and accumulate experiences in the use of the drugs. A new standard for the management strategy of aspergillosis infection is awaited.

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Policy Address

JMAJ 48(12): 607–608, 2005

Haruo Uematsu*¹

Health Cost Control

The Liberal Democratic Party won a landslide victory in the elections that were held in September, 2005 following the dissolution of the Lower House in August of this year. The pros and cons regarding the privatization of the postal system were the major issues that were addressed and it is regrettable that the controversial issues related to health care reforms were not disputed. As a result, the pace of health care reforms is expected to gain momentum. In fact, statements by the Council on Economic and Fiscal Policy and Council for the Promotion of Regulatory Reform have increased in severity.

Meanwhile, the Ministry of Finance publicly announced a two to five percent reduction in medical fees in order to control medical costs, reviewed the scope of health insurance benefits, part of hospital fees shouldered by users, the increased burden of the elderly, reductions in hospitalization periods, and other issues.

The rationale for these cost controls are based on the statistics issued by the Ministry of Health, Labor, and Welfare, which claims that medical costs will rise to 69 trillion yen (US\$577 billion) by 2025. However, in looking back on the ministry's past statements, their projected statistics in 1995 were 50 trillion yen (US\$419 billion) for 2004, but in actuality they were 32 trillion yen (US\$268 billion). Additionally, their projected health cost estimates for 2025 were 141 trillion yen (US\$1.18 trillion). As mentioned earlier, their projected estimate for this year was 69 trillion yen. Their estimates have been drastically

halved within only ten years.

Furthermore, the ministry estimated that the rise in per capita medical costs was 2.1% for the elderly under 75 years and 3.2% for the elderly over 75 years of age. However, according to an analysis of the Japan Medical Association Research Institute (JMARI) based on actual data from 1998 to the present, increased per capita medical costs was much lower and on average, were 1.1% for the elderly under 75 and 0.6% for the elderly over 75 years of age.

As can be seen, the data by which medical cost control policies are based on are very nebulous at best. Therefore, through the use of a variety of data, we will continue to refute the ministry's arguments that are based on an undependable rationale of medical cost controls.

The Shortage of Physicians and Hospital-based Doctors

Difficulties in providing medical care due to the shortage of physicians and their uneven distribution have begun to surface, and complaints by overworked hospital-based doctors have begun to be heard. Physician shortage and their uneven distribution is an extremely important issue especially in the field of pediatrics and obstetrics, where it has reached critical levels. But, this is not an issue that can be resolved by the JMA's executive board alone. Changes in the awareness of JMA members as well as those of medical students are required and we ask for your cooperation in addressing this issue.

In pursuing the issue of overworked hospital-based doctors, we must return to the fundamental

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This is a main part of the policy address delivered by Haruo Uematsu, president of the JMA at the 113th Extraordinary General Assembly of the JMA House of Delegates held in Tokyo on October 2, 2005

concepts surrounding the health care delivery system. Although hospitals should focus on inpatient care, in actuality, their efforts have been concentrated on providing outpatient services. The discussion should focus on going back to basic fundamental concepts. An approach that includes revising medical fees in tandem with proposals about how to realize the ideas that come out of these discussions should be taken.

In addition, a review of the medical plan is also expected to be included in health reforms.

Protecting the Quality of Health Care in Cooperation with the Public

According to the mass media recently, top officers in charge of the policies of the ruling party allegedly made a comment that “the excessive number of physicians is affecting the rise in health care costs”. The control measures of health costs should not be discussed with this kind of incorrect understanding. If the public’s burden is increased and the universal health insurance system is threatened, we should start a campaign to protect the quality of health care and the uni-

versal health insurance system together with the public as was done in 2004 to prevent the introduction of mixed medical care, which means the combined use of public insurance and private insurance for treatment. Therefore, when this campaign is started, we would like to request the similar participation of each area as in the previous movement.

As for the issue on medical fees, it will be impossible to maintain the quality of medical care services according to the revision of a two to five percent reduction advocated by the Ministry of Health, Labour, and Welfare, the Ministry of Finance, and others. Therefore, we will strive to change this to a plus revision. Presently, we are reviewing the basic data together with JMARI and will be submitting JMA’s views and demands to the Central Social Insurance Medical Council, other government bodies, and the ruling party.

As was reported recently by the mass media, the health hazards of asbestos have become a very important issue. However, we must review not only measures to address this specific issue, but other environmental and industrial health related policies from a broader perspective.

Overcoming the Crisis in Obstetric and Gynecologic Practice

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Key words Shortage of obstetrician-gynecologists, Postgraduate educational system, Female doctors, Medical care by reduced personnel, Obstetrician-gynecologist-intensive hospital, Open system

Introduction

Obstetrician-gynecologists (ob-gyns) are well known for irregular working hours and frequently having lawsuits filed against them, factors that cause medical students to avoid choosing the field as a specialty. In Japan, two-year postgraduate education has been obligatory since April 2004, a stipulation that will result in no new ob-gyns entering the field between 2004 and 2006. In contrast to the usual expectation of about 400 new ob-gyns per year, no new ob-gyn physicians are entering practice. When compounded by the usual attrition from physician retirement, the decrease in the number of ob-gyns is substantial. The number of hospitals no longer able to provide ob-gyns increased rapidly in some areas as a result of these factors. A survey of university-affiliated hospitals throughout the country as of September 2004 revealed that 119 hospitals already were unable to provide obstetric and gynecologic care. This figure likely has risen even further by now. It is possible that the nation will suffer if the current situation prevails. Indeed, a survey in the Hokkaido area revealed that the lack of ob-gyns is closely associated with neonatal mortality. In other words, a crisis is occurring in the field of obstetrics and gynecology, and measures need to be taken.

Number of Ob-Gyns in Japan

Members of the Japan Society of Obstetrics and Gynecology number approximately 15,000, among whom those aged 50 years old or older and those aged 70 years old or older account for 52% and 20%, respectively. The total number of members has not changed significantly since 1985, i.e., in the past 20 years. This is attributed to the fact that the members tend to be long-lived. In fact, the number of members in their 20s, 30s and 40s is decreasing. Among new members, female doctors now account for more than 60%, outnumbering male doctors.

Current Situation of Ob-Gyns Working in Hospitals

According to a survey on the current situation of ob-gyns working in hospitals, doctors usually have night duty 6–8 times per month. In order to cope with emergencies, such as caesarean sections, an additional doctor needs to be on call besides the one on night duty. Including on-call duty, each ob-gyn is obliged to be available at least 12 times per month. This frequency of obligation may be possible to endure for a few years, but may become burdensome after a longer period. Since the supply of new doctors has been halted for 2 years, since the beginning of 2004, existing hospital ob-gyns have to put up with

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a difficult situation. Doctors in senior positions tend to leave the department of obstetrics and gynecology after gradually experiencing burn-out, whereas mid-level doctors are headhunted by private hospitals with better conditions, a situation that is becoming increasingly common throughout Japan. Universities that have been providing doctors to affiliated hospitals now are desperate to secure enough doctors for their own institutions. Further, it has become very difficult to find doctors who are ready to work as part-time attending staff on a limited salary.

Changes in Obstetric and Gynecologic Care

After 1970, Japan's economy underwent rapid growth, and the level of affluence increased. As more women entered the workforce, the number of births decreased from 2 million in 1974 to 1.1 million in 2004, a notable decline. This decline in the number of births and recent trends toward later marriage and childbearing have caused delivery to take on much greater significance. This in turn has tended to make obstetric and gynecologic care more fraught and stressful than previously. On the other hand, progress in assisted reproductive medicine has made in-vitro fertilization and embryo transfer more common. Consequently, we have seen a higher incidence of multiple births and high-risk delivery due to increased premature deliveries and low-birth-weight infants. This has further increased the responsibility of hospital doctors.

The Challenging Nature of Obstetric and Gynecologic Care

The main part of obstetric care is assisting nature in achieving a natural, spontaneous delivery. However, if problems in delivery occur, they may lead to death or abnormality of the infant, or serious impairment or death of the mother. At this point, obstetric care requires high-level medical expertise. Some lives can be saved only when high-level care is given by a multidisciplinary team of doctors, midwives, and nurses who can provide on-the-spot decision-making. This is a challenge in obstetrics. Therefore, it is necessary to produce as many ob-gyns as possible who can rise to this challenge. Such ob-gyns are the very ones most needed for reliable medical care.

Strengths and Weaknesses of Japan's Obstetric and Gynecologic Care System

Since the natural form of delivery has been preferred in Japan, facilities that deal with childbirth through the work of a single care provider or a small number of care providers have become widespread. As a result, Japan has a very large number of childbirth facilities (about 6,000), a situation that is unusual among leading industrialized countries. When a childbirth facility managed by one person or a small staff is located in her neighborhood, it is convenient for the pregnant woman because gestational management and institutional delivery are available nearby. While pregnant women tend not to consider childbirth risky or dangerous, they also may be unaware that small practices can be ill equipped to handle emergencies. If a sudden adverse event occurs, the patient will be transferred to a secondary care hospital. However, even in public secondary care hospitals, there are usually only 3–5 ob-gyns, and thus the true strength of multidisciplinary care cannot be realized.

Medical practice by a small staff has inherent limitations in fostering the ability to handle sudden changes in a patient's condition. The dispersal of human resources to small local facilities could lead to deterioration in the quality of care, educational functions, and research potential in the field of obstetrics and gynecology in Japan.

Need for Structural Reform

Given these circumstances, it is necessary for the current system of obstetric and gynecologic practice to change in structure from the dispersal of human resources in small local clinics to facilities that are more comprehensive and have more personnel. In other words, there is a need to produce hospitals in which ob-gyns are more centralized. Small, local obstetrics and gynecology clinics should be abolished or merged into more doctor-intensive hospitals, and advanced care and education should be provided in these settings. If such change were to take place, improved quality of life could be expected for ob-gyns working in hospitals. Doctors in private practice could provide safer services

and enhance their own quality of life by making contracts that provide limited or unlimited collaboration with the more comprehensive hospitals (semi-open or open system). It is also important to create working conditions that encourage the participation of more female ob-gyns and to foster an environment in which

they can exert their expertise.

To provide safe obstetric and gynecologic care through the sharing of human resources, the implementation of structural reform and the establishment of a better working environment for female doctors are needed.

Erratum

In the article titled, “Increase of ‘Health and Human Rights’ Research Articles in Japan” by Jimba M, Nomura Y, Poudel KC, et al. published in the JMAJ 2005; 48(11): 545–549, the fourth line of the abstract on page 545 should read ‘substantially from 1995’ instead of ‘substantially from 1994’.

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