Table 3. Comparison of Non-MetS and MetS Patients With Symptomatic CHF

	Male			Female		
	Non-MetS (n=1,343)	MetS (n=1,111)	P value	Non-MetS (n=929)	MetS (n=220)	P value
Age (years)	68.9±0.3	66.6±0.3	<0.001	70.8±0.4	72.6±0.7	<0.05
Cigarette smoking, n (%)						
Never	441 (48.6%)	370 (49.3%)	NS	688 (79.4%)	168 (81.6%)	NS
Former	195 (21.4%)	148 (19.7%)	NS	128 (14.8%)	25 (12.1%)	NS
Current	272 (30.0%)	233 (31.0%)	NS	51 (5.8%)	13 (6.3%)	NS
Alcohol intake, n (%)						
Never	402 (37.9%)	320 (35.3%)	NS	606 (81.0%)	154 (80.6%)	NS
Former	131 (12.3%)	89 (9.8%)	NS	31 (4.2%)	4 (2.1%)	NS
Current	529 (49.8%)	498 (54.9%)	NS	111 (14.8%)	33 (17.3%)	NS
BMI (kg/m^2)	21.3±0.2	25.3±0.2	<0.001	21.1±0.2	26.5±0.5	<0.001
Blood pressure (mmHg)						
Systolic	122.6±0.6	129.4±0.5	<0.001	125.1±0.7	131.6±1.4	<0.001
Diastolic	70.2±0.3	74.6±0.4	<0.001	70.4±0.04	72.2±0.9	NS
Heart rate (beats/min)	71.5±0.4	72.2±0.4	NS	74.7±0.6	74.7±1.0	NS
NYHA class						
I	240 (18.0%)	250 (22.6%)	<0.01	114 (12.3%)	19 (8.6%)	NS
II	929 (69.5%)	754 (68.2%)	NS	647 (69.7%)	167 (75.9%)	NS
III	149 (11.1%)	97 (8.8%)	NS	154 (16.6%)	33 (15.0%)	NS
IV	19 (1.4%)	5 (0.4%)	<0.001	13 (1.4%)	1 (0.5%)	NS
Stage C/D	1,296 (96.7%)/ 44 (3.7%)	1,085 (98.3%)/ 19 (1.7%)	<0.05	897 (96.7%)/ 31 (3.3%)	216 (98.2%)/ 4 (1.8%)	NS
LVEF (%)	53.3±0.5	55.8±0.5	<0.001	59±0.5	61.4±1.0	NS
HFREF (EF <50%)	468 (38.7%)	329 (33.2%)	<0.01	232 (27.3%)	37 (18.8%)	<0.05
HFPEF (EF ≥50%)	740 (61.3%)	662 (66.8%)	<0.01	617 (72.7%)	160 (81.2%)	<0.05
SAS	5.6±0.06	5.8±0.06	<0.05	4.6±0.07	4.5±0.1	<0.001
HT	870 (64.8%)	1,006 (90.5%)	<0.001	655 (70.5%)	209 (95.0%)	<0.001
DM or fasting glucose ≥110 mg/dl	506 (37.7%)	747 (67.2%)	<0.001	384 (41.3%)	142 (64.5%)	<0.001
Dyslipidemia	787 (58.6%)	967 (87.0%)	<0.001	615 (66.2%)	201 (87.3%)	<0.001
IHD	632 (47.1%)	632 (56.9%)	<0.001	250 (27.0%)	102 (46.4%)	<0.001
HHD	104 (7.7%)	126 (11.3%)	<0.01	88 (9.5%)	38 (17.3%)	0.001
CM	294 (21.9%)	214 (19.3%)	NS	183 (19.8%)	33 (15.0%)	NS
VHD	332 (24.7%)	162 (14.6%)	<0.001	382 (41.3%)	46 (20.9%)	<0.001
CHD	19 (1.4%)	10 (0.9%)	NS	30 (3.2%)	2 (1%)	NS
Medications						
ACEI/ARB	935 (69.6%)	858 (77.2%)	<0.001	599 (64.5%)	166 (75.5%)	<0.01
β-blocker	666 (49.6%)	571 (51.4%)	NS	392 (42.2%)	115 (52.3%)	<0.01
Statin	364 (27.1%)	512 (46.1%)	<0.001	274 (29.5%)	107 (48.6%)	<0.001

Values are mean±SEM.
Abbreviations see in Tables 1,2.

were characterized by higher prevalence of larger body mass index, glucose intolerance/diabetes mellitus, and IHD, whereas the female patients were in a higher NYHA class, had lower exercise tolerance, and higher prevalence of both preserved LVEF and valvular heart disease.

MetS in CHF

In both male and female patients with CHF, those with MetS were characterized by younger age, higher prevalence of current smoking and drinking, IHD, and hypertensive heart disease, lower NYHA class, better exercise tolerance, and more likelihood of taking medications such as ACEI/ARB, β-blockers or statins (**Tables 2,3, Figure 2**). The prevalence of HFPEF was significantly higher in the MetS group compared with the non-MetS group (**Table 3, Figure 3**).

When compared with the patients with HFREF, those with HFPEF were characterized by higher prevalence of elderly and female patients, obesity, hypertensive and valvular heart disease, and less likelihood of taking medications such as ACEI/ARB, β-blockers or statins (**Table 4**).

Metabolic Components in CHF

In the present study, the contribution of single or combined metabolic components was observed in both the ischemic

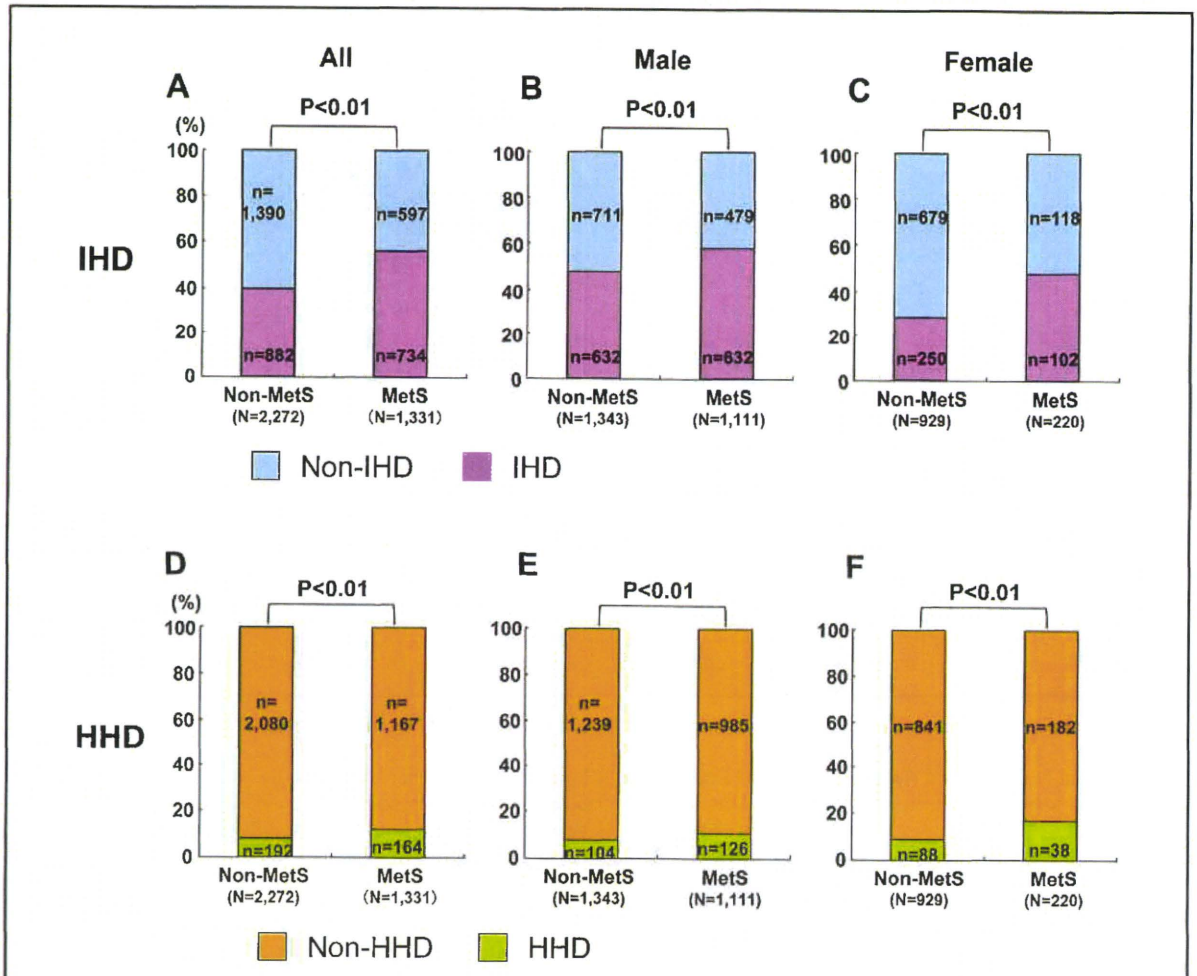

Figure 2. Effect of metabolic syndrome (MetS) on ischemic heart disease (IHD) and hypertensive heart disease (HHD). IHD was significantly more prevalent in CHF patients with MetS than in those without it for all patients (**A**), and in male (**B**) or female patients (**C**). Similarly, for HHD for all patients (**D**), and male (**E**) and female patients (**F**).

and non-ischemic CHF patients (**Figure 4A**). Although the prevalence of ischemic CHF was significantly higher in most of the subgroups with more than 3 metabolic components, the contribution of other single or combined metabolic components was either comparable between the 2 groups or stronger in the non-ischemic CHF group (**Figure 4A**). Although the prevalence of combined metabolic components varied, these components were comparably associated with both HFPEF and HFREF (**Figure 4B**).

Discussion

The novel findings of the present study are that (a) the prevalence of MetS in CHF was more than double that of the general Japanese population, (b) MetS was associated with ischemic or hypertensive heart disease-related heart failure, (c) HFPEF was characterized by a higher prevalence of elderly and female patients with MetS, and (d) the prevalence of the metabolic components was comparable between the ischemic and non-ischemic CHF patients. To the best of our knowledge, this is the first study to provide evidence for a relationship between MetS and CHF.

Prevalence of MetS in CHF

It has been reported that the prevalence of MetS in the general Japanese population is 10–20% in men and 2–8% in women, as defined by the current Japanese criteria.[7,18,19] In contrast, the present study demonstrated a prevalence of MetS (45% in men and 19% in women) that is more than 2-fold that of the general population, suggesting that the presence of MetS is an important therapeutic target of CHF treatment. It is conceivable that the increased prevalence of MetS in CHF patients is both the cause and the result of CHF, as activation of both the sympathetic nervous system and renin–angiotensin system causes the metabolic components.[21] In order to address this important issue, we are now performing a cohort study in which we follow-up MetS patients without CHF to examine the development of CHF in them.

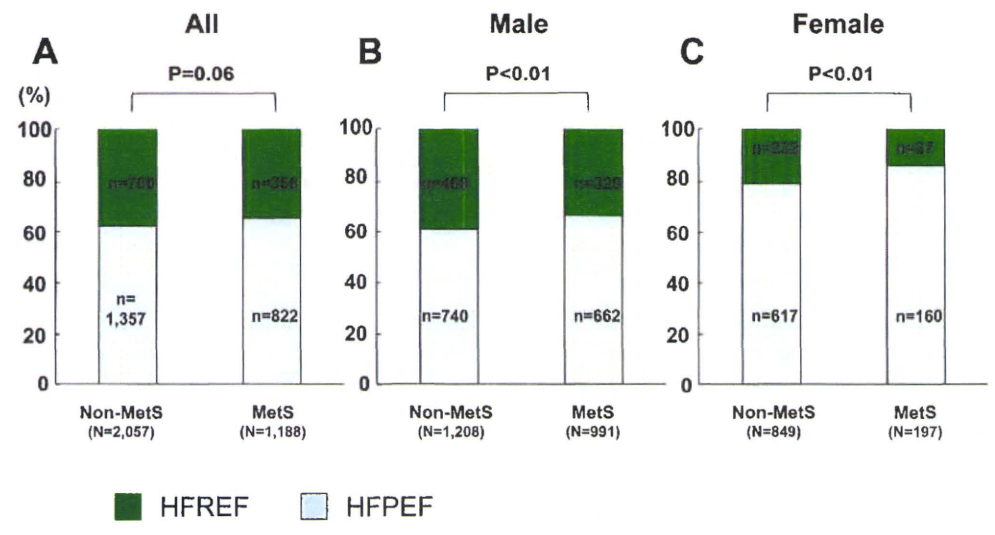

Figure 3. Effect of metabolic syndrome (MetS) on left ventricular ejection fraction. The prevalence of heart failure with preserved ejection fraction (HFPEF) was significantly greater for males (**B**) and females (**C**) with MetS than in those without MetS and tended to be so for all patients with chronic heart failure (**A**). HFREF, heart failure with reduced ejection fraction.

Role of MetS in Ischemic and Hypertensive Heart Disease

MetS has been identified as a risk and prognostic factor for IHD and stroke.[8,22,23] In the present study, MetS was highly associated with IHD in both male and female patients with CHF. Thus, the prevention of IHD is extremely important for preventing the development of CHF, both by life-style modification and the use of anti-atherosclerotic drugs in order to achieve stabilization and regression of systemic atherosclerosis. Furthermore, because hypertension is associated with obesity,[24] it is also important to treat obesity for blood pressure control in order to prevent the development of hypertensive heart disease.

Comparison of HFPEF and HFREF

It has been demonstrated that heart failure can also occur in patients with preserved LVEF, which is often observed in hypertensive heart disease mainly caused by LV diastolic dysfunction.[14] It is now widely accepted that HFPEF is a major cardiovascular disorder with poor prognosis, accounting for approximately 50% of patients with heart failure symptoms,[15,16] and our study demonstrated that 67% of CHF patients had HFPEF (**Table 4**). The present results also indicate the different clinical characteristics of HFPEF and HFREF patients, and the former were characterized by a higher prevalence of elderly and female patients, obesity, and hypertensive and valvular heart disease. Although it has been previously demonstrated that the major determinants of diastolic dysfunction are enhanced myocardial stiffness and impaired relaxation capacity,[25] further studies are needed to clarify the association between these clinical factors and LV dysfunction.

Metabolic Components in Ischemic and Non-Ischemic CHF

In the present study, among the metabolic components in the CHF patients, the prevalence of both hypertension and dyslipidemia was higher, followed by glucose intolerance/diabetes mellitus, probably because of environmental and genetic factors. In order to prevent the development of CHF, all components of MetS should be controlled (ie, blood pressure by anti-hypertensive drugs, lipid-lowering by HMG-CoA reductase inhibitors, and glucose control by diet therapy, exercise and antidiabetic drugs), which is known to ameliorate vascular function and stabilize atheroma.[26–33] In contrast, smoking and alcohol intake may not be highly related to the development of CHF compared with hypertension or dyslipidemia, so smoking cessation and moderate alcohol intake are recommended in the early stage of CHF.[9]

In the present study, MetS was related to the development of HFPEF (LVEF≥50%) in both male and female patients with CHF. Although the precise mechanisms are unknown, coronary microvascular dysfunction with preserved systolic function might be linked to this phenomenon.[34,35]

The present study also demonstrated that there are single or combined metabolic components in both non-ischemic CHF and ischemic CHF patients, a consistent finding with a previous report regarding the lipid levels and heart failure incidence in Caucasians.[36] Therefore, these metabolic components should be regarded as important therapeutic targets for CHF caused by both ischemic and non-IHD.

Study Limitations

First, although we were able to collect the data for a relatively large number of CHF patients, their prognoses need to be elucidated. As we are currently performing a follow-up study for them, we will report the results separately in the future. Second, we used the 2005 definition of the Japanese Committee for the Diagnostic Criteria of MetS, so we were unable to compare the present data with that of non-Japanese studies. We plan to use other diagnostic criteria, such as the National Cholesterol Education Program-Adult Treatment

Table 4. Comparison of HFPEF and HFREF Patients With Symptomatic CHF

	Total		
	HFPEF (n=2,179)	HFREF (n=1,066)	P value
Sex, n (%)			
Male	1,402 (64.3%)	797 (74.8%)	<0.001
Female	777 (35.7%)	269 (25.2%)	<0.001
Age (years)	69.6±0.3	67.7±0.4	<0.001
Non-MetS	1,357 (62.3%)	700 (65.7%)	0.06
MetS	822 (37.7%)	366 (34.3%)	0.06
Cigarette smoking, n (%)			
Never	453 (41.2%)	470 (60.4%)	<0.01
Former	307 (27.9%)	133 (17.1%)	<0.01
Current	339 (30.8%)	175 (22.5%)	<0.01
Alcohol intake, n (%)			
Never	925 (52.2%)	435 (49.7%)	<0.001
Former	149 (8.4%)	85 (9.7%)	<0.001
Current	699 (39.4%)	356 (40.6%)	<0.001
BMI (kg/m^2)	23.1±0.1	22.4±0.2	<0.01
Waist circumference (cm)			
Male	86.9±0.3	85.9±0.3	<0.001
Female	82.1±0.4	80.9±0.8	<0.001
Blood pressure (mmHg)			
Systolic	128.1±0.4	120.7±0.6	<0.001
Diastolic	72.2±0.3	70.5±0.4	<0.001
Heart rate (beats/min)	72.3±0.3	73.8±0.5	<0.05
NYHA class			
I	429 (19.7%)	134 (12.6%)	<0.001
II	1,510 (69.5%)	743 (69.9%)	NS
III	215 (9.9%)	173 (16.3%)	<0.001
IV	19 (0.9%)	13 (1.2%)	NS
Stage C/D	2,125 (97.7%)/49 (2.3%)	1,027 (96.5%)/37 (3.5%)	<0.05
LVEF (%)	65.3±0.2	37.2±0.3	<0.001
SAS	5.4±0.05	5.0±0.06	<0.001
HT	1,725 (53.2%)	729 (68.4%)	<0.001
DM or fasting glucose≥110mg/dl	1,042 (47.8%)	554 (52.0%)	<0.05
Dyslipidemia	1,499 (68.8%)	802 (75.2%)	<0.001
IHD	894 (41.0%)	501 (47.0%)	<0.001
HHD	258 (11.8%)	73 (6.8%)	<0.001
CM	319 (14.6%)	365 (34.2%)	<0.001
VHD	695 (31.9%)	169 (15.9%)	<0.001
CHD	48 (2.2%)	6 (0.6%)	<0.01
Medications			
ACEI/ARB	1,483 (68.1%)	835 (78.3%)	<0.001
β-blocker	880 (40.9%)	711 (66.7%)	<0.001
Statin	702 (32.2%)	403 (37.8%)	<0.01

Values are mean±SEM.
Abbreviations see in Tables 1,2.

Panel III (NCEP/ATPIII),[37] American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI),[38] and International Diabetes Federation (IDF),[39] in future analyses. Third, although MetS is the association and clustering of metabolic components, we were unable to exclude CHF patients complicated by severe hypertension, severe dyslipidemia, or severe diabetes mellitus. This issue also remains to be examined in future studies. Last, the present study lacks an appropriate control group in the same population, which why we used the data from the Kanazawa Study of the Japanese general population in 2007 that demonstrated a prevalence of MetS of 16–21% in 50- to 80-year-old males and in females, prevalence of 3% in the 50s, 5% in the 60s, 8% in the 70s, and 10% in the 80s.[20]

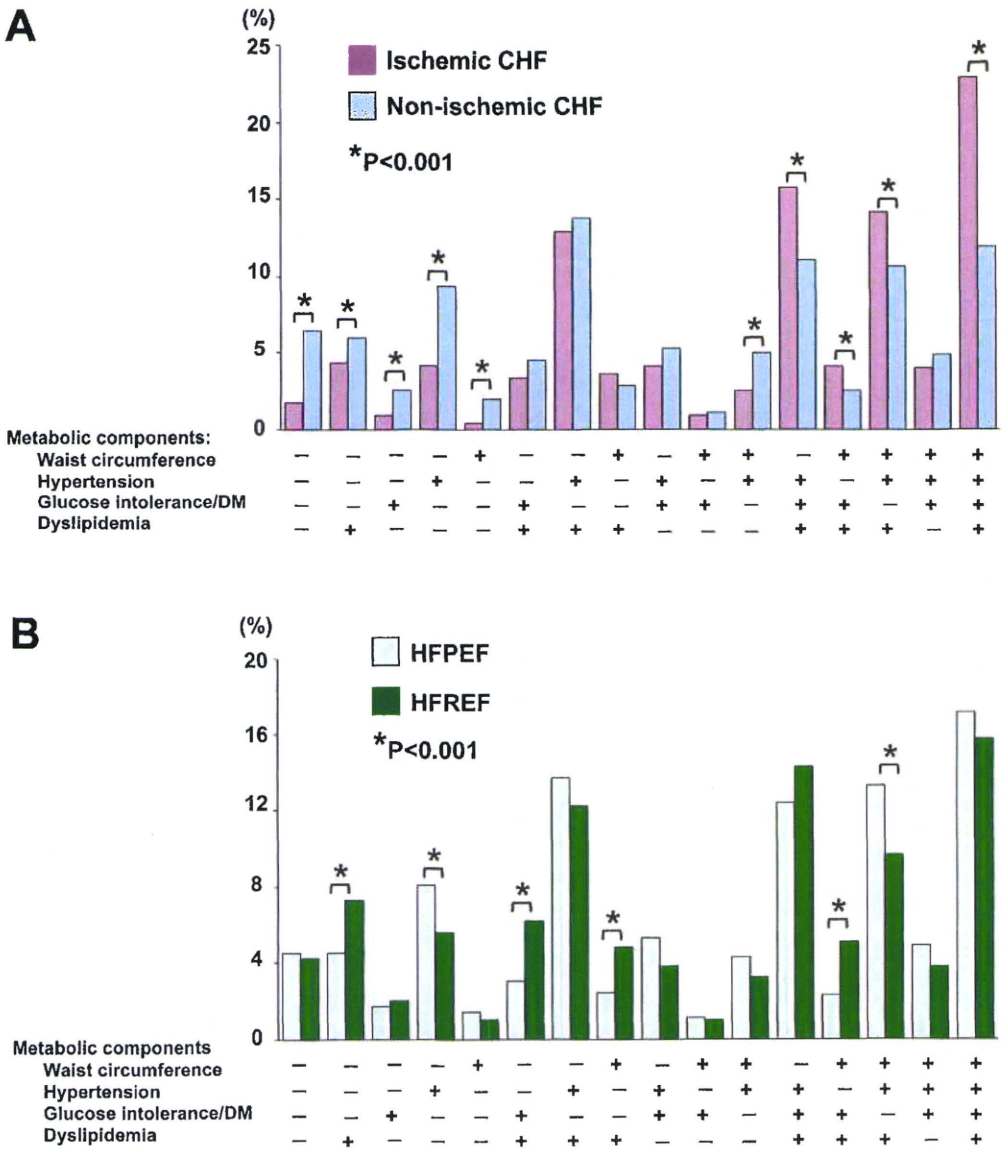

Figure 4. Accumulation of metabolic components in patients with chronic heart failure (CHF). (**A**) When the number of metabolic components was greater than 3, their contribution to ischemic heart failure was greater, whereas the contribution of other single or combined metabolic components was comparable between the 2 groups or stronger in the non-ischemic heart failure group. (**B**) Although the prevalence of combined metabolic components varied, the components were equally associated with both heart failure with preserved ejection fraction (HFPEF) and heart failure with reduced ejection fraction (HFREF). DM, diabetes mellitus.

Conclusion

We found that the prevalence of MetS in CHF patients was more than double compared with the general population in Japan, with a greater involvement of ischemic or hypertensive heart disease and a higher prevalence in elderly and female patients. Because the metabolic components might have a substantial effect on the development of both ischemic and non-ischemic CHF, MetS should be regarded as a new therapeutic target for this disorder.

Acknowledgments

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Disclosures

None.

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話題

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福本義弘** 下川宏明**

Key Words：pulmonary hypertension, Rho-kinase, pulmonary arteriopathy, pulmonary vasoconstriction

はじめに

肺高血圧症は安静時平均肺動脈圧が25mmHg以上と定義され，その成因に器質的肺動脈病変および肺動脈攣縮が大きく関与している．また，進行性の肺血管抵抗上昇および肺動脈圧上昇が特徴で，きわめて予後不良な疾患である．現在，内科的にはワーファリンやカルシウム拮抗薬内服，プロスタグランディン製剤内服もしくは持続点滴投与，エンドセリン受容体拮抗薬内服，PDE5阻害薬内服，外科的には生体もしくは死体肺移植で治療されているが，内科的にはいまだ有効性が不十分であり，また肺移植もドナー不足およびコストの面からも新たな治療法の開発が求められている．

一方，Rhoキナーゼ[1]は，1990年代半ばのほぼ同時期に，2つの日本の研究グループと1つのシンガポールのグループから，低分子量GTP結合蛋白質Rhoの標的蛋白質として同定された細胞内セリンスレオニンリン酸化酵素である．これまでの研究により[2)~4)]，Rhoキナーゼが平滑筋細胞の収縮のみならず，各種細胞の形態制御，遊走，遺伝子発現制御などの生理機能に関与していることが明らかとなっている．さらに，近年の活発な研究により，心血管病の成因にRhoキナーゼが深く関与していることが示されており，Rhoキナーゼ阻害薬の開発と臨床応用が期待されている．

本稿では，Rhoキナーゼが関与する生理機能や病態生理学的意義およびRhoキナーゼ阻害薬の将来展望について概説する．

Rhoキナーゼの機能

1．Rhoキナーゼの構造と発現部位

Rhoキナーゼは分子量約160kDaのセリンスレオニンリン酸化酵素であり，線虫やショウジョウバエなどの下等動物からマウス・ラットなどのげっ歯類，ヒトまで広く保存されている遺伝子である．RhoキナーゼにはRho-kinase α/ROKα/ROCK2とRho-kinase β/ROKβ/ROCK1という2つのアイソフォームがあり，ヒトの場合，それぞれ第2番染色体(2p24)および第18番染色体(18q11.1)に存在する．Rhoキナーゼの構造はN末端側から順に，kinase domain, Rho-binding domain(RBD)を含むcoiled-coil domain, cystein-rich domain(CRD)を含むpleckstrin-homology(PH) domainがあることが示されている．ROCK1とROCK2は高い相同性を有しており，全体として65％の同一性があり，特にkinase domainにおいては92％の同一性がある．

ROCK1およびROCK2は体内に広く発現しているが，ROCK2は特に脳と骨格筋に強く発現している．Rhoキナーゼの発現はアンジオテンシンIIやインターロイキン-1βなどの炎症性刺激により，プロテインキナーゼC-NF-κB依存性経路を介して促進的に制御されている．また，アンジオテ

* Rho-kinase inhibitor.

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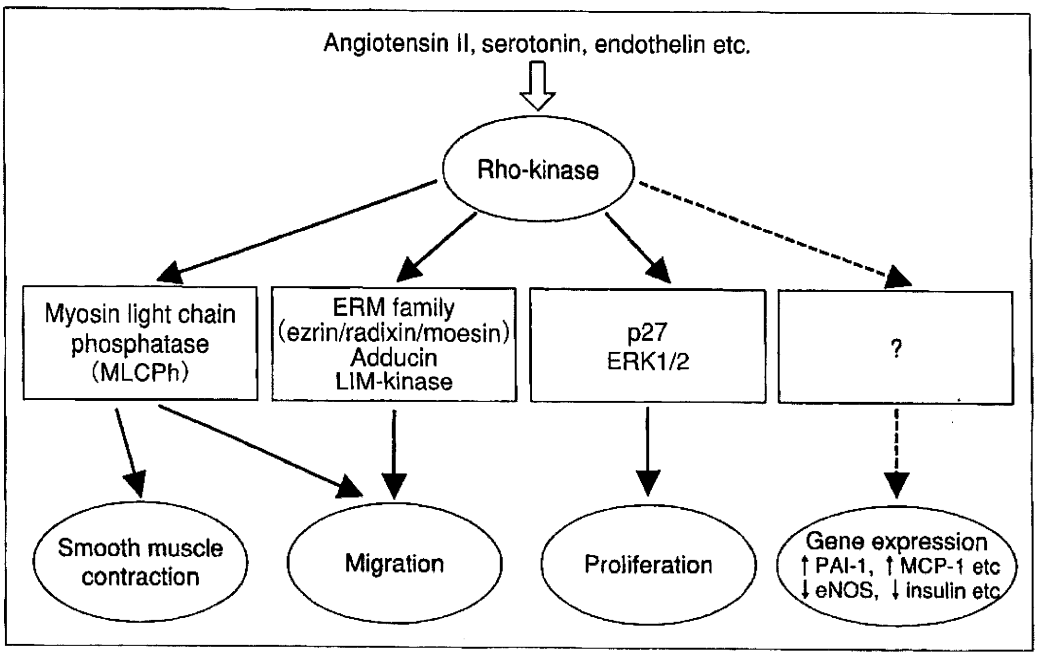

図1　Rhoキナーゼの生理機能
Rhoキナーゼは収縮，増殖，遊走，遺伝子発現誘導など，細胞のさまざまな生理機能に関与している．

ンシンIIによるRhoキナーゼの発現亢進は生理的濃度のエストロゲンにより用量依存的に抑制され，ニコチン投与により約100倍に増加し，かつエストロゲンの抑制作用を消失させた．さらに下川らは，新規動脈硬化促進因子として注目されているレムナントリポ蛋白が，ヒト冠動脈由来血管平滑筋においてRhoキナーゼの発現や活性を著明に上昇させることも見出している．

Rhoキナーゼの細胞内局在に関しては，Rhoキナーゼは主として細胞質に存在し，RhoA活性化に伴って一部が細胞膜に移行することが明らかとなっている．また，ROCK2の一部が核に局在し，p300 acetyltransferaseをリン酸化し，その活性を制御している可能性が示唆されている．しかし，Rhoキナーゼの発現制御や細胞内局在の詳細な分子機構については不明な点が多く残されている．

2．RhoキナーゼC末端側の活性制御への関与

前述のように，Rhoキナーゼのkinase domainはN末端側に存在する．C末端側の一部を欠損したRhoキナーゼはdominant-active formとなり，逆にC末端側の一部を発現させるとdominant-negative formとして働くことから，RhoキナーゼのC末端側が触媒ドメインとの相互作用を介してRhoキナーゼの活性を負に制御していることが示唆されている．また，Rhoキナーゼ同士の多量体形成がATPに対する親和性を制御する機序によってもRhoキナーゼ活性が調整されている可能性も示唆されている．

GTPが結合した活性型RhoAのRBDへの結合は，C末端側の触媒ドメインとの相互作用の乖離を促進し，Rhoキナーゼを活性化する．また，脂質メッセンジャーとして知られているアラキドン酸もRhoAとは独立した機序でRhoキナーゼを活性化することが報告されている．その他にも，Rhoキナーゼ活性を負に制御しているC末端側が切断されることでもRhoキナーゼが活性化することが明らかとなっている．ROCK1はアポトーシスが起こる際，カスパーゼ3によりC末端側が切断される．一方，ROCK2はアポトーシス関連蛋白分解酵素であるgranzyme Bによって C末端側の分解を受ける．興味深いことに，ROCK1はgranzyme Bにより切断される部位を持たず，ROCK2はカスパーゼ3による切断部位を持っていない．これらの正の活性制御機構に加えて，負の活性制御機構も存在する．低分子量G蛋白の一種であるRhoEはROCK1のN末端側触媒ドメインに結合して，その活性を阻害する．この

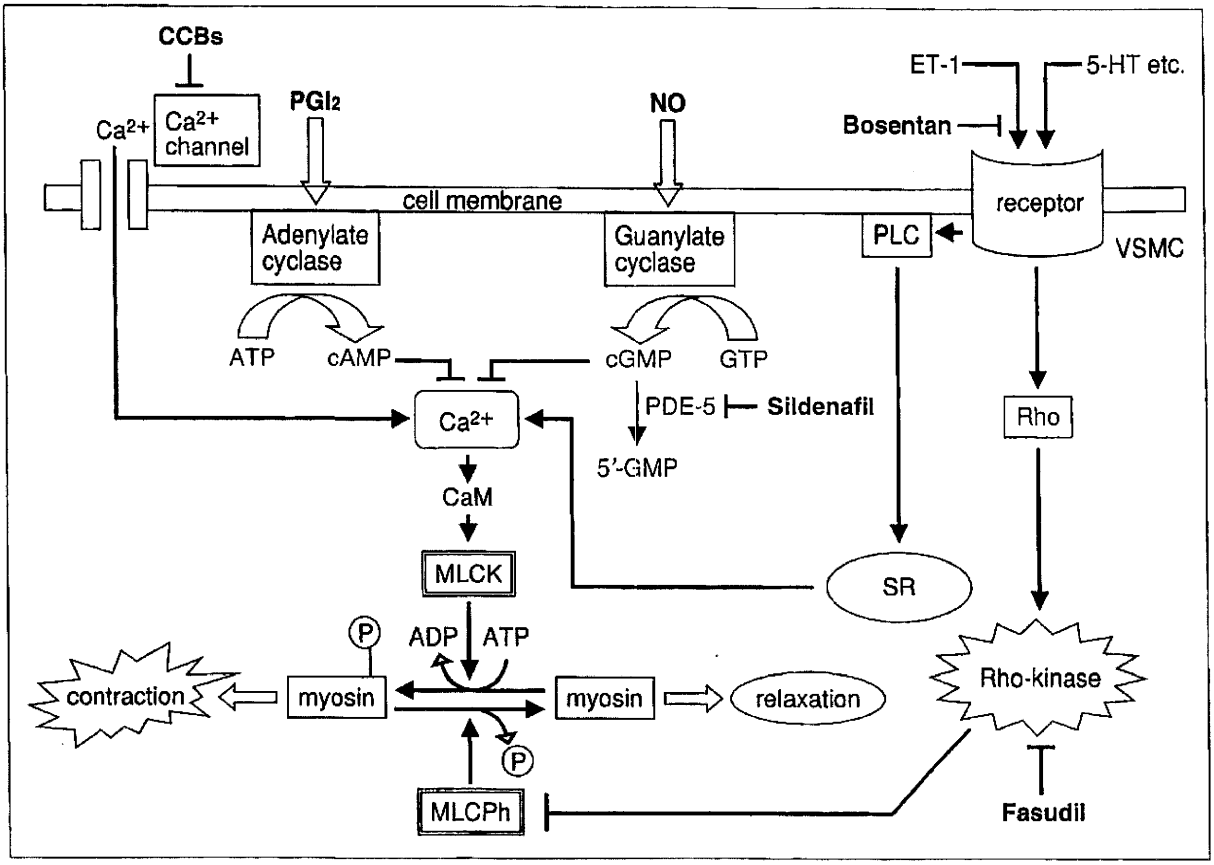

図2　血管平滑筋収縮の細胞内機構

Rhoキナーゼは細胞内Ca^{2+}濃度非依存的に血管平滑筋の収縮弛緩を制御する．すなわち，収縮性血管作動物質の刺激により低分子量G蛋白質であるRhoが活性化され，その標的蛋白の一つであるRhoキナーゼが活性化される．活性化されたRhoキナーゼはMLCPhのミオシン結合サブユニット(MBS)をリン酸化することによりその活性を阻害し，MLCK/MLCPh活性のバランスが崩れ，MLCのリン酸化が上昇することで血管平滑筋は収縮する．

RhoEによる負の制御はROCK2に対しては起こらない．これらのことは，Rhoキナーゼの2つのアイソフォーム間でなんらかの役割の違いが存在する可能性を示唆している．

3．さまざまな細胞生理機能におけるRhoキナーゼの役割

Rhoキナーゼは，収縮，増殖，遊走，遺伝子発現誘導など，細胞の生理機能に深く関与していることが明らかとなっている[2)~4)](図1)．このことは，さまざまなアゴニスト刺激がRhoキナーゼを介してその作用を発現していることを示唆する．今日までの研究により，アンジオテンシンII，セロトニン，トロンビン，エンドセリン，ノルエピネフリン，血小板由来増殖因子(PDGF)，一部のP2Yレセプターを介した細胞外ヌクレオチド，ウロテンシンIIなどがRhoキナーゼを介して作用を発現することが知られている．今後，さまざまなアゴニストによる細胞内シグナル伝達の研究が発展することにより，Rhoキナーゼの関与する新たな生理機能が明らかになる可能性がある．

4．血管平滑筋の収縮弛緩におけるRhoキナーゼ/ミオシン軽鎖の役割

血管平滑筋の主な生理機能である収縮弛緩は交感神経や血管作動物質の刺激に応答して惹起される．この血管平滑筋の機能により，生体は血管径を変化させることで血圧や臓器への血液の分配などの循環調節を行っている．血管平滑筋における収縮弛緩制御は，ミオシン軽鎖キナーゼ(MLCK)活性とミオシン軽鎖フォスファターゼ(MLCPh)活性のバランスにより決定されるミオシン軽鎖(MLC)のリン酸化が中心的役割を果たしている[2)~5)](図2)．

血管平滑筋細胞はアンジオテンシンIIなどの収縮性血管作動物質の刺激に応答して，細胞内のG蛋白に共役したホスホリパーゼC(PLC)の

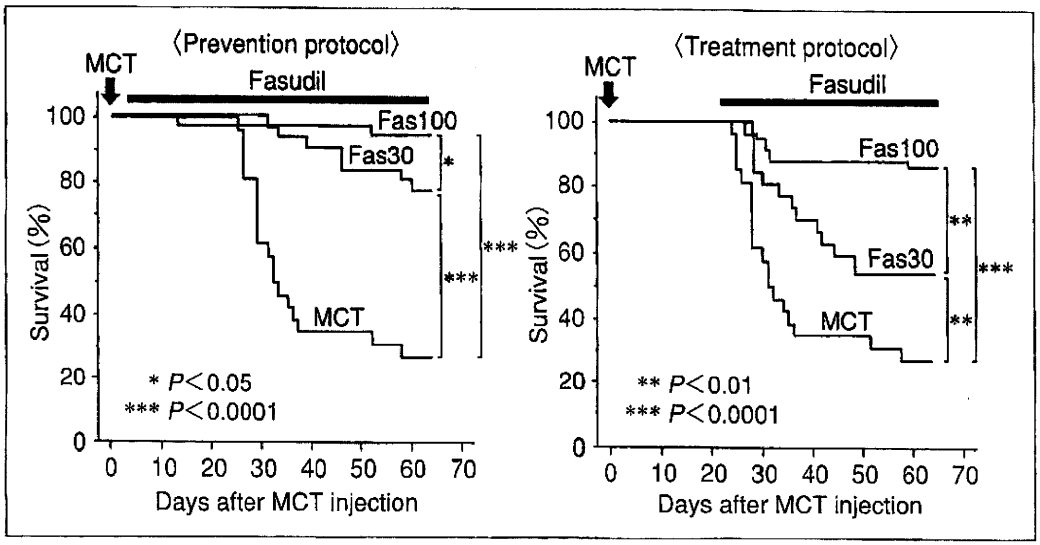

図3 動物モデルにおけるファスジルの効果
モノクロタリン誘発性ラット肺高血圧モデルにおいて，モノクロタリン投与時にファスジルの経口投与を開始する(予防プロトコール)と生存率が著明に改善し，さらに，肺高血圧症を発症させたあとにファスジル投与を開始(治療プロトコール)しても生存率が有意に改善する．
(文献[6]より引用)

作用によりイノシトール3リン酸(IP3)を生成する．IP3は細胞内のCa^{2+}貯蔵部位(筋小胞体)上のCa^{2+}チャネルを開口することによりCa^{2+}放出を惹起し，細胞内のCa^{2+}濃度を上昇させる．また，細胞膜にもCa^{2+}チャネルが存在し，さまざまな刺激に応答してチャネルが開口し，細胞外からのCa^{2+}流入がひき起こされる．筋小胞体からの放出，および細胞外からの流入により上昇した細胞内Ca^{2+}は，カルモジュリンと結合してCa^{2+}/カルモジュリン複合体を形成し，MLCKの触媒サブユニットに結合してMLCKを不活性型から活性型に変換する．活性型MLCKがMLCをリン酸化すると，ミオシン頭部に存在するMg^{2+}-ATPaseのアクチンによる活性化がひき起こされ，血管平滑筋は収縮する．その後，細胞内Ca^{2+}濃度が低下すると，Ca^{2+}はカルモジュリンから解離してMLCKは不活性化される．その結果，MLCPhが優位になり，MLCは脱リン酸化されて血管平滑筋は弛緩する(図2)．

一方，Rhoキナーゼは，細胞内Ca^{2+}濃度非依存的に血管平滑筋の収縮弛緩を制御することが知られている．すなわち，収縮性血管作動物質の刺激により，G蛋白に共役した受容体を介して低分子量G蛋白質であるRhoが活性化され，その標的蛋白の一つであるRhoキナーゼが活性化される．活性化されたRhoキナーゼは，MLCPhのミオシン結合サブユニット(MBS)をリン酸化することによりその活性を阻害する．その結果，MLCK/MLCPh活性のバランスが崩れ，MLCのリン酸化が上昇することで血管平滑筋は収縮する(図2)．

5．血管平滑筋の増殖におけるRhoキナーゼの役割

通常の生体の動脈にみられる平滑筋細胞は前述の収縮弛緩が主な働きであるが，病態や血管障害により増殖因子刺激を受けると平滑筋は形質変換を起こし増殖が誘導される．Rhoキナーゼは，トロンビンやウロテンシンII，PDGF-BB刺激による血管平滑筋細胞の増殖にも重要な役割を果たしている．また，細胞増殖において，サイクリン依存性キナーゼを阻害する働きを持つp27がその制御に重要な役割を果たしていることが知られている[2)~4)]．Rhoキナーゼは活性化するとp27の発現を低下させ，細胞増殖を亢進させる．一方，Rhoキナーゼ阻害はバルーン傷害による血管平滑筋細胞の増殖を抑制しないとの報告もあり，詳細な機序については明らかとなっていない．

6．細胞遊走におけるRhoキナーゼの役割

細胞遊走は白血球や線維芽細胞，平滑筋細胞などの細胞が持つ生理機能であり，さまざまな

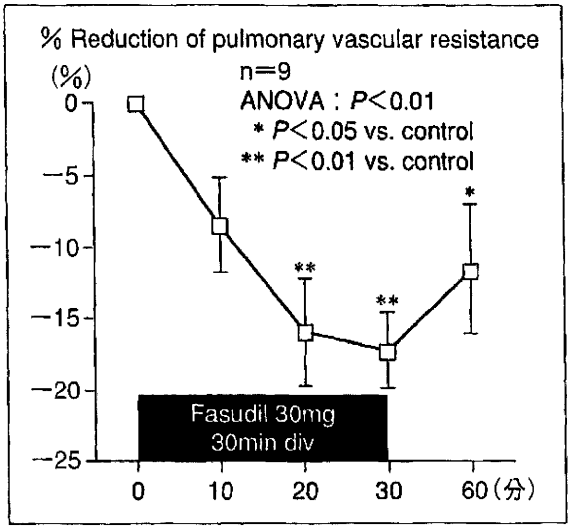

図4　重症肺高血圧症患者におけるファスジルの急性効果
重症肺高血圧症患者において，ファスジルの点滴静脈内投与は肺血管抵抗を有意に低下させる急性効果を有している．　（文献[9]より引用）

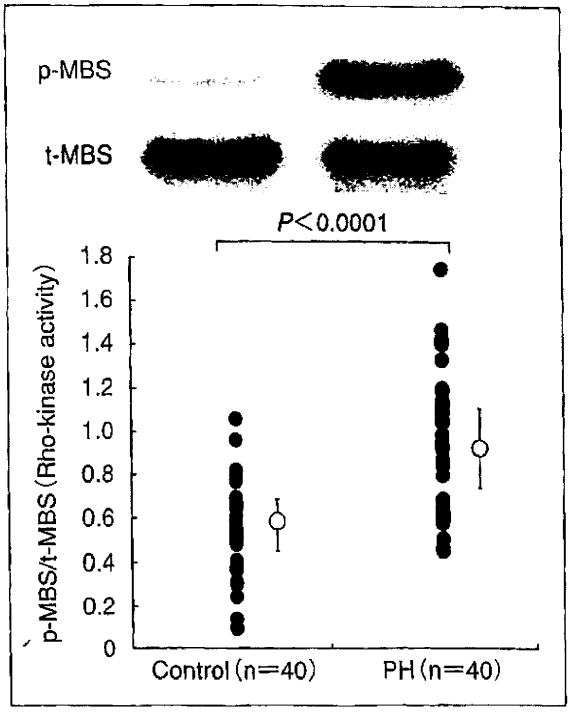

図5　肺高血圧症患者の白血球におけるRhoキナーゼ活性の亢進　（文献[10]より引用）

生理的・病的環境で重要な役割を果たしている．細胞遊走には，遊走刺激によるアクチンフィラメントの重合—脱重合(再構築)，アクチン—ミオシンによる収縮，微小管を介した細胞骨格蛋白質の輸送などが関与している．これら遊走にかかわる細胞機構の制御にRho/Rhoキナーゼが重要な役割を果たしていることが報告されている[2)~4)]．たとえば，血管平滑筋細胞においてRhoキナーゼ阻害薬はPDGFやリソフォスファチジン酸によって誘発される遊走を制御する．その機序として，遊走刺激因子などにより活性化されたRhoがRhoキナーゼを活性化してMLCPhを阻害し，MLCのリン酸化を促進して細胞の収縮性を高め，細胞遊走に関与することが考えられている．また，Rhoキナーゼはアクチンフィラメントの再構築に関与するアデューシン，ERM(ezrin/radixin/moesin)，LIMキナーゼなどの蛋白質をリン酸化することから，これらの蛋白質を介した細胞遊走の制御も考えられている．

肺高血圧症に対する新たな治療ターゲットとしてのRhoキナーゼ

1．基礎研究

下川らはモノクロタリン誘発性ラット肺高血圧モデル(MCTモデル)において[6)]，モノクロタリン投与時にファスジルの経口投与を開始する(予防プロトコール)と生存率が著明に改善することを明らかにした(図3)．次いで，MCTモデルにおいて，肺高血圧症を発症させたあとにファスジル投与を開始(治療プロトコール)しても，生存率が有意に改善することを示した(図3)．MCTモデルの肺動脈ではRhoキナーゼの活性が亢進しており，内皮機能の低下，血管平滑筋の過収縮がみられることを示した．また，組織学的検討ではMCTモデルで認められる肺動脈の中膜肥厚，微小肺動脈の筋性化がいずれもファスジル投与により抑制された．さらに下川らは，低酸素誘発性肺高血圧症マウスにおいてもファスジル経口投与が有効であることを示した[7)]．また，種々の肺高血圧症モデルにおいて，ファスジルまたはY-27632を急性吸入投与することにより肺動脈圧が低下することも報告されている[8)]．

2．臨床研究

一方，臨床的検討として，酸素，一酸化窒素，カルシウム拮抗薬の急性投与に抵抗性を示した重症肺高血圧症患者において，ファスジルの点滴が肺血管抵抗を有意に低下させたことを報告した(図4)[9)]．これらの結果から，肺高血圧症の

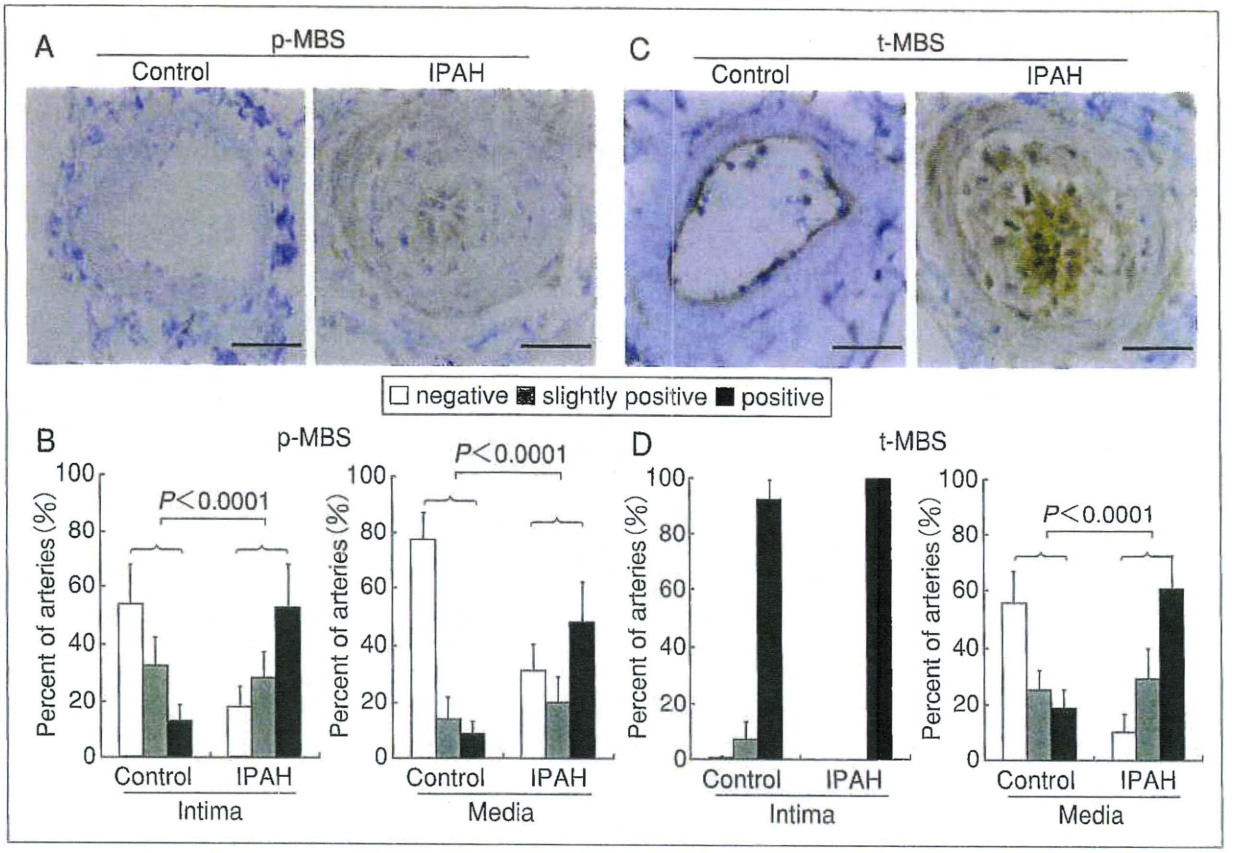

図6　肺高血圧症患者の肺組織におけるRhoキナーゼ活性の亢進（文献[10]より引用）

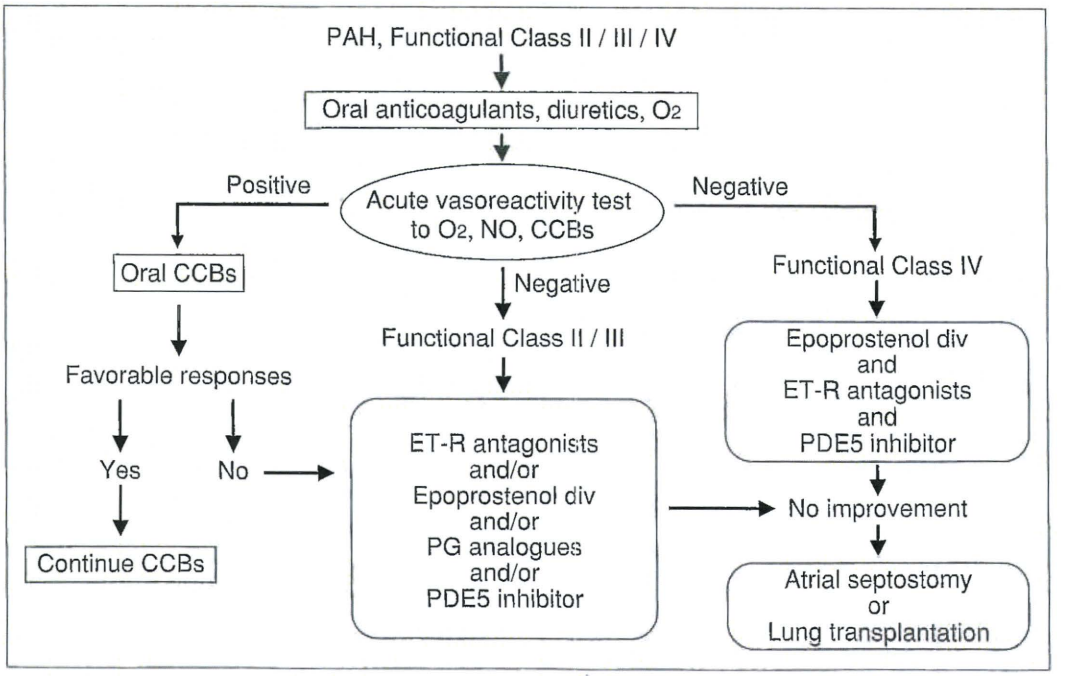

図7　肺高血圧症の治療アルゴリズム
現時点ではベラプロスト，エポプロステノール，ボセンタン，シルデナフィルで加療しているが，Rhoキナーゼ阻害薬の有効性が示されれば重要な治療オプションとなりうる．（文献[5]より引用）

成因（内皮細胞障害，肺動脈病変形成，肺動脈持続的過収縮）にRhoキナーゼが深く関与している可能性が示唆されていたが，最近筆者らは，肺高血圧症患者の肺高血圧症患者における全身性

もしくは局所的なRhoキナーゼ活性を検討した[10]．まず肺高血圧症患者および健常者の末梢血由来多型核白血球におけるRhoキナーゼ活性の検討を行った．Western blot法によりRhoキナーゼの基質であるミオシン結合サブユニット(MBS)のリン酸化体および総MBSの比(Rhoキナーゼ活性)が，肺高血圧症患者の末梢血由来多型核白血球では健常者に比べ有意に亢進していることを認めた(図5)．次に，肺移植を受ける肺高血圧患者の肺組織およびコントロール(肺がん患者)の正常肺組織に対し，Rhoキナーゼ発現および活性を免疫組織染色法および摘出血管で検討を行ったところ，免疫組織学的にRhoキナーゼの発現および活性が，肺高血圧症患者ではコントロールと比べ有意に亢進していることが認められた(図6)．さらに肺高血圧患者の摘出肺動脈において，内皮依存的弛緩反応低下およびセロトニンに対する過収縮を認め，この平滑筋過収縮はRhoキナーゼ阻害薬によって抑制された．これらのデータから，はじめて肺高血圧症患者におけるRhoキナーゼ活性の亢進が証明され，肺高血圧症の発症，進展におけるRhoキナーゼ経路の関与が示された[10]．

おわりに

本稿では，Rhoキナーゼの基礎的知見と肺高血圧症におけるRhoキナーゼ薬の将来性について，これまでに得られてきた知見を中心に解説した．しかしながら，肺高血圧症の発症メカニズムは非常に複雑であり，Rhoキナーゼ経路の役割に関しても，まだ明らかにすべき点は多い．現在われわれは，図7に示すようなアルゴリズムで肺高血圧症の治療を行っているが[55]，今後Rhoキナーゼ阻害薬を含めた新しい薬剤の有効性が示されれば，非常に重要な併用療法オプションになると期待される．

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ORIGINAL ARTICLE
Pulmonary Circulation

Identification of New Prognostic Factors of Pulmonary Hypertension

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Background: Pulmonary hypertension (PH) still remains a serious disease, for which the plasma level of brain natriuretic peptide (BNP) and hemodynamic variables (eg, cardiac index: CI) are established prognostic factors. The aim of the present study was to identify new additional prognostic factors of the disorder to improve the management of PH.

Methods and Results: The study cohort comprised 136 consecutive PH patients admitted to hospital from 1974 to 2008, all of whom were closely followed every 6–12 months. During the follow-up period of 53.5±4.5 [SEM] months, 47 patients died of cardiopulmonary causes. The patients who were initially treated with monotherapy showed improved pulmonary hemodynamics when subsequently treated with combination therapy. Multivariate analysis showed that BNP and CI were significant and independent prognostic factors in all PH patients. However, in PH patients with low CI at diagnosis, only CI improvement by PH therapy was a significant and independent prognostic factor. Indeed, the patients with low CI at diagnosis (CI <2.5) followed by subsequent normalization in response to therapy (CI ≥2.5) showed a significantly better survival compared with those without such normalization.

Conclusions: CI normalization in response to treatment is an independent new prognostic factor of PH in patients with low CI at diagnosis, suggesting the importance of intensive therapy to achieve CI normalization. (*Circ J* 2010; **74:** 1965–1971)

Key Words: Cardiac index; Prognosis; Pulmonary hypertension; Thyroid dysfunction

Pulmonary hypertension (PH), defined as a mean pulmonary arterial pressure (PAP) greater than 25 mmHg at rest,[1,2] is a fatal disease caused by small pulmonary artery obstruction related to vascular proliferation and remodeling.[3] PH is characterized by markedly elevated PAP and increased pulmonary vascular resistance (PVR), frequently leading to right-sided heart failure and death.[3] The pathological changes of the pulmonary arteries in PH include endothelial injury, proliferation and hypercontraction of vascular smooth muscle cells and migration of inflammatory cells.[4,5] Although several vasodilators, anticoagulant agents and lung transplantation are currently used for the treatment of PH, more effective treatment needs to be developed.[6]

Identification of prognostic factors is useful for better management of PH. Possibilities include functional class, exercise capacity (eg, 6-min walking distance, cardiopulmonary exercise testing), pulmonary hemodynamics (eg, mean right atrial pressure (RAP), PVR, right ventricular end-diastolic pressure (RVEDP)), right ventricular function (eg, cardiac output (CO), clinical evidence of right ventricular failure), and plasma levels of brain natriuretic peptide (BNP), endothelin-1, uric acid, and troponin.[1,2,7–10] Among these prognostic factors, plasma BNP levels and CO are considered to be the most influential.[11] We closely follow our PH patients every 6–12 months, which includes cardiac catheterization, with the aim of identify new prognostic factors of PH. Here we report that we were able to identify that normalization of CO in response to the therapy is a significant prognostic factor of PH and probably has more significance than BNP.

Methods

The Ethical Committees of Tohoku University Hospital approved the study protocol and all patients provided written informed consent. Our study cohort consisted of 136 con-

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Table 1. Clinical Characteristics of Patients With PH

Total, n	136
Age (years)	45.6±1.6
Sex	
Male, n (%)	33 (24%)
Female, n (%)	103 (76%)
Survivor, n (%)	89 (65%)
Follow-up period (months)	53.5±4.5
Type of PH	
Idiopathic PAH, n (%)	43 (32%)
Heritable PAH, n (%)	3 (2%)
PAH associated with connective tissue diseases, n (%)	21 (15%)
PAH associated with congenital heart disease, n (%)	22 (16%)
PAH associated with portal hypertension, n (%)	10 (7%)
PVOD	4 (3%)
CTEPH	33 (24%)
Treatment	
Oral prostacyclin, n (%)	71 (52%)
Intravenous prostacyclin, n (%)	51 (38%)
Bosentan, n (%)	45 (33%)
Sildenafil, n (%)	8 (6%)

Values are mean±SEM.
PH, pulmonary hypertension; PAH, pulmonary arterial hypertension; PVOD, pulmonary veno-occlusive disease; CTEPH, chronic thromboembolic pulmonary hypertension.

secutive PH patients admitted to hospital between 1974 and 2008.

Study Population

The 136 patients had PH of various etiologies, including idiopathic pulmonary arterial hypertension (IPAH, n=43), heritable PAH (n=3), PAH associated with connective tissue diseases (n=21), congenital heart diseases (n=22), or portal hypertension (n=10), pulmonary veno-occlusive disease (n=4), and PH because of chronic thromboembolism (CTEPH, n=33) (Table 1). Besides their regular follow-up, we hospitalize patients if their symptoms or right-heart failure worsen.

Diagnosis of PH

We performed right-heart catheterization in all 136 patients, among whom we were able to perform follow-up catheterization in 88. PH was defined as a mean PAP greater than 25 mmHg at rest.[1,2] Connective tissue disease and liver disease were diagnosed clinically and by blood tests. Congenital heart disease was diagnosed by echocardiography, and CTEPH was diagnosed by ventilation–perfusion RI scans and computed tomography (CT). Pulmonary function tests, arterial blood gases, chest X-ray and CT scan were used to diagnose lung disease and hypoxia. If the aforementioned abnormalities were ruled out, the patients were diagnosed as having IPAH.[2,5] Heritable PAH was diagnosed as IPAH with a family history of PAH.[2,5,12]

Data Collection

Baseline demographic information (including age, sex, height and body weight), clinical diagnosis, comorbidities (connective tissue diseases, liver diseases, congenital heart diseases, and thyroid dysfunction) and hemodynamic data obtained during catheterization were recorded for each patient. Hyperthyroidism was defined as either an elevated level of free triiodothyronine (T3 ≥4.12 pg/ml) and/or thyroxin (T4 ≥1.5ng/dl) or reduced level of thyroid-stimulating hormone (TSH ≤0.46 μIU/ml). Hypothyroidism was defined as either elevated TSH (>3.73 μIU/ml) or a reduced level of free T3 (<2.51 pg/ml) and/or T4 (<0.88 ng/dl). Hemodynamic parameters examined included pulmonary capillary wedge pressure, pulmonary artery pressure (PAP), RVEDP, RAP, CO, cardiac index (CI), systolic and diastolic blood pressures, mean blood pressure, PVR, systolic vascular resistance, and mixed venous oxygen saturation ($S\dot{V}O_2$). Lung transplantation and death were defined as cardiopulmonary death. The CI response was evaluated at diagnosis and 1 year after treatment.

Current Medical Treatment of PH

We usually begin oral prostacyclin at a starting dose of 60 mg/day and increase it in a stepwise manner up to 240 mg/day, if tolerable. In daily practice, we usually start intravenous prostacyclin (epoprostenol) therapy during hospitalization at 0.5–1 $ng \cdot kg^{-1} \cdot min^{-1}$, which is carefully increased in a step-wise manner on the basis of symptoms and side-effects of the drug (Figure S1). Similarly, we usually start bosentan at 31.25 mg/day combined with dobutamine and/or milrinone in PH patients with heart failure, and at 62.5 mg/day in those without heart failure, and then carefully increase the dose in a stepwise manner every week (Figure S2). Regarding sildenafil, we usually start at 30 mg/day and increase the dose up to 60 mg/day if patients can tolerate it. Currently, we treat patients with PH using prostacyclin, bosentan, sildenafil as monotherapies or in combination.

Statistical Analysis

Results are expressed as mean±SEM. Unpaired t-test was used for comparison of continuous variables and chi-test for categorical variables. Survival from all-cause death and from lung transplantation was estimated by Kaplan-Meier method and differences between the curves were examined for significance using the log-rank test. Univariate and multivariate Cox proportional hazard models were used to estimate the hazard ratios and 95% confidence intervals. Statistical analyses were performed using GraphPad Prism 5.0E (GraphPad Software Inc, La Jolla, CA, USA) or SPSS (SPSS Inc, Chicago, IL, USA). P values less than 0.05 were considered to be statistically significant.

Results

Clinical Characteristics of Patients With PH

Clinical characteristics of the 136 patients are shown in Table 1. During the follow-up period of 53.5±4.5 months, 47 patients died of cardiovascular causes.

Current Therapies for PH

In the 136 patients with PH, 122 (90%) were treated with oral (n=71) or intravenous prostacyclin (n=51), 45 (33%) with bosentan, and 8 (6%) with sildenafil (Table 1). Although it has already been reported that monotherapy with intravenous epoprostenol or oral bosentan or sildenafil improves symptoms, 6-min walk distance, pulmonary hemodynamics, and survival in patients with PAH,[13–16] some of the present patients needed additional treatment. In particular, among the patients with PAH who did not respond to any monotherapy, combination therapy with oral or intravenous prostacyclin,

Table 2. Prevalence of Thyroid Dysfunction in Patients With PH

N	127
No thyroid dysfunction, n (%)	69 (54%)
Thyroid dysfunction, n (%)	58 (46%)
Hyperthyroidism, n (% of thyroid dysfunction)	11 (19%)
Hypothyroidism, n (% of thyroid dysfunction)	47 (81%)
Treatment	
Antithyroid drugs, n (% of hyperthyroidism)	5 (45%)
Thyroid hormone replacement, n (% of hypothyroidism)	3 (6%)

Abbreviation see in Table 1.

Table 3. Comparison of Thyroid Dysfunction Between PAH and CTEPH

	PAH	CTEPH	P value
N	98	29	
No thyroid dysfunction, n (%)	47 (48%)	22 (76%)	<0.05
Thyroid dysfunction, n (%)	51 (52%)	7 (24%)	
Hyperthyroidism, n (% of thyroid dysfunction)	9 (18%)	2 (29%)	NS
Hypothyroidism, n (% of thyroid dysfunction)	42 (82%)	5 (71%)	
Treatment			
Antithyroid drugs, n (% of hyperthyroidism)	3 (27%)	2 (18%)	NS
Thyroid hormone replacement, n (% of hypothyroidism)	3 (6%)	0 (0%)	

Abbreviations see in Table 1.

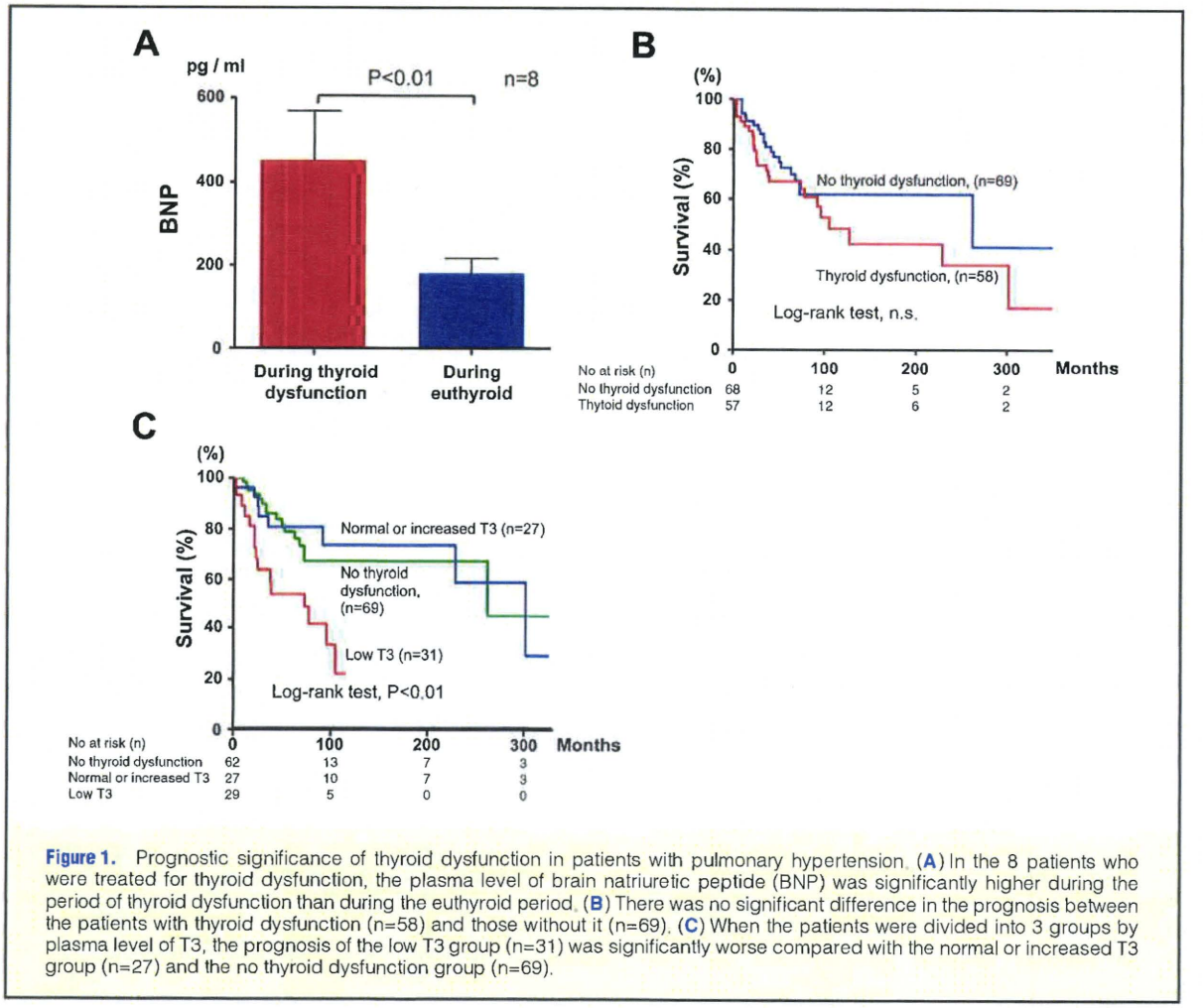

Figure 1. Prognostic significance of thyroid dysfunction in patients with pulmonary hypertension. (**A**) In the 8 patients who were treated for thyroid dysfunction, the plasma level of brain natriuretic peptide (BNP) was significantly higher during the period of thyroid dysfunction than during the euthyroid period. (**B**) There was no significant difference in the prognosis between the patients with thyroid dysfunction (n=58) and those without it (n=69). (**C**) When the patients were divided into 3 groups by plasma level of T3, the prognosis of the low T3 group (n=31) was significantly worse compared with the normal or increased T3 group (n=27) and the no thyroid dysfunction group (n=69).

bosentan, and sildenafil significantly improved pulmonary hemodynamics (Figure S3A–D).

Biochemical Prognostic Factors

Of the complications in this group of PH patients, thyroid dysfunction was most the common (n=58, 46%), comprising hyperthyroidism in 11 (19%) and hypothyroidism in 47 (81%); 5 of the 11 patients with hyperthyroidism were treated with antithyroid drugs, and 3 of the 47 patients with hypothyroidism were treated with thyroid hormone replace-

Table 4. Univariate and Multivariate Analyses of Prognostic Factors of PH

	Survivors	Deaths	P value	
			Univariate analysis	Multivariate analysis
All PH patients				
N	89	47	–	–
Follow-up (months)	61.9±5.3	37.5±7.7	–	–
Age (years)	47.3±1.9	42.3±2.7	NS	NS
Sex				
Male, n (%)	67 (75%)	36 (77%)	NS	NS
Female, n (%)	22 (25%)	11 (23%)		
mPAP (mmHg)	51.6±1.8	58.9±2.5	NS	NS
RAP (mmHg)	6.1±0.4	8.7±0.9	<0.05	NS
$S\dot{V}O_2$ (%)	67.8±1.1	62.7±1.8	<0.05	NS
Free T3 (pg/ml)	3.31±0.25	3.17±0.37	<0.05	NS
BNP (pg/ml)	260.4±36.4	517.5±65.5	<0.001	<0.05
CI at diagnosis	2.68±0.08	2.29±0.11	<0.01	<0.05
Low CI patients				
N	27	18	–	–
Follow-up (months)	60.6±8.0	30.1±4.4	–	–
Age (years)	44.0±3.2	43.5±3.9	NS	NS
Sex				
Male, n (%)	22 (82%)	13 (72%)	NS	NS
Female, n (%)	5 (19%)	5 (28%)		
mPAP (mmHg)	56.6±2.6	63±4.1	NS	NS
RAP (mmHg)	9.0±1.1	6.4±0.9	NS	NS
$S\dot{V}O_2$ (%)	61.2±2.8	62.0±1.3	NS	NS
Free T3 (pg/ml)	3.53±0.78	3.98±0.86	NS	NS
BNP response at low CI improvement (pg/ml)	368.5±82.6 to 188.5±60.7	538.1±106.5 to 305.3±85.0	0.053	NS
CI response in low CI at diagnosis ($L \cdot min^{-1} \cdot m^{-2}$)	1.98±0.06 to 2.80±0.11	2.00±0.08 to 2.55±0.13	<0.0001	<0.05

NS, not significant; mPAP, mean pulmonary artery pressure; RAP, right atrial pressure; $S\dot{V}O_2$, mixed venous oxygen saturation; T3, triiodothyronine; BNP, plasma level of brain natriuretic peptide; CI, cardiac index. Other abbreviation see in Table 1.

ment therapy (Table 2). In the present study, 90% of the patients were treated with oral or intravenous prostacyclin (Table 1), and there was no significant difference in the prevalence of thyroid dysfunction between those treated with prostacyclin (55 of 116 patients, 47%) and those without it (3 of 11 patients, 27%).

Interestingly, the prevalence of thyroid dysfunction was significantly higher in the patients with PAH than in those with CTEPH (Table 3). Although the patients with thyroid dysfunction had higher plasma levels of BNP than those without it (450±120 vs 177±39 pg/ml, P<0.05) (Figure 1A), thyroid dysfunction itself did not have a significant prognostic impact (Figure 1B). However, the patients with low T3 levels had a significantly poor prognosis by univariate analysis (P<0.01, hazard ratio: 2.368, 95% confidence interval: 1.221–4.593) (Figure 1C).

An elevated plasma level of BNP, which indicates right-heart failure, was also a significant prognostic factor in the univariate analysis (P<0.001, hazard ratio: 3.609, 95% confidence interval: 1.856–7.016), whereas multivariate analysis demonstrated that BNP was a significant independent prognostic predictor in all PH patients (Table 4).

Hemodynamic Prognostic Factors

Hemodynamic parameters, such as CI at diagnosis, RAP and $S\dot{V}O_2$, which are established prognostic factors, were also significant prognostic factors in our univariate analysis, whereas mean PAP was not (Table 4).

Low CO at diagnosis was a poor prognostic factor for death within 1 year (Figure 2A, Table 4). Importantly, in the univariate analysis patients with low CO at diagnosis (CI <2.5, n=45) followed by subsequent CI normalization in response to treatment (CI ≥2.5, n=34) survived significantly longer than those who did not respond to the treatment (CI <2.5, n=11) (Figure 2B, Table 4). However, among 43 patients with normal CI at diagnosis (CI ≥2.5), there were 37 with a subsequent normal CI (CI ≥2.5), of whom 29 survived and 8 died. Of the remaining 6 patients whose CI was reduced during the follow-up (CI <2.5), 5 survived and 1 died, indicating the insignificant difference in the CI response among patients with normal CI at the diagnosis.

Importantly, multivariate analysis demonstrated that among the significant prognostic factors in the univariate analysis, only CI improvement in response to treatment was an independent prognostic factor of PH in patients with low CI at diagnosis (Table 4).

Furthermore, there were no significant differences in PH treatment between survivors and those who died, except for monotherapy with intravenous prostacyclin in all PH patients (Table 5).

Discussion

In the present study, we aimed to identify new prognostic factors of PH in a relatively large cohort of patients with a long-term close follow-up. The results suggest that CI

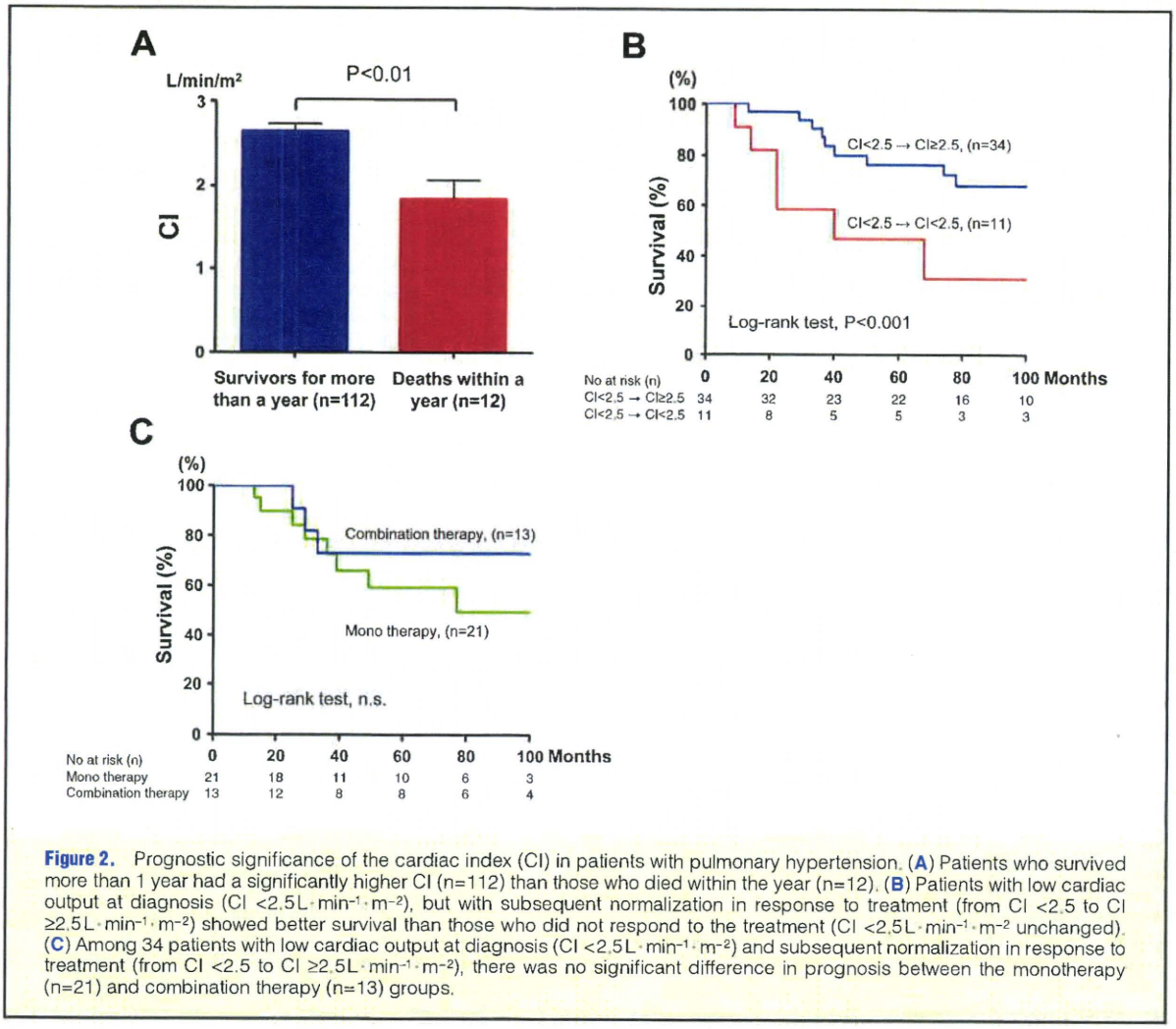

Figure 2. Prognostic significance of the cardiac index (CI) in patients with pulmonary hypertension. (**A**) Patients who survived more than 1 year had a significantly higher CI (n=112) than those who died within the year (n=12). (**B**) Patients with low cardiac output at diagnosis (CI <2.5L·min^{-1}·m^{-2}), but with subsequent normalization in response to treatment (from CI <2.5 to CI ≥2.5L·min^{-1}·m^{-2}) showed better survival than those who did not respond to the treatment (CI <2.5L·min^{-1}·m^{-2} unchanged). (**C**) Among 34 patients with low cardiac output at diagnosis (CI <2.5L·min^{-1}·m^{-2}) and subsequent normalization in response to treatment (from CI <2.5 to CI ≥2.5L·min^{-1}·m^{-2}), there was no significant difference in prognosis between the monotherapy (n=21) and combination therapy (n=13) groups.

Table 5. Use of PH Drugs in Mono and Combination Therapies

	All patients			Patients with CI normalization		
	Survivors	Deaths	P value	Survivors	Deaths	P value
Monotherapy						
N	60	35		14	7	
PGI_2 oral, n (%)	36 (60.0%)	12 (34.3%)	NS	4 (28.6%)	2 (28.6%)	NS
PGI_2 intravenous, n (%)	14 (23.3%)	19 (54.3%)	<0.05	9 (64.3%)	4 (57.1%)	NS
Bosentan, n (%)	3 (5.0%)	3 (8.6%)	NS	1 (7.1%)	1 (14.3%)	NS
Sildenafil, n (%)	2 (3.3%)	0 (0%)	NS	0 (0%)	0 (0%)	–
CCB, n (%)	2 (3.3%)	1 (2.9%)	NS	0 (0%)	0 (0%)	–
No anti-PH drugs, n (%)	3 (5.0%)	0 (0%)	NS	0 (0%)	0 (0%)	–
Combination therapy						
N	33	8		11	2	
PGI_2 oral+bosentan, n (%)	16 (48.5%)	5 (62.5%)	NS	3 (27.3%)	0 (0%)	NS
PGI_2 intravenous+bosentan, n (%)	13 (39.4%)	1 (12.5%)	NS	8 (72.7%)	1 (50.0%)	NS
PGI_2 oral+sildenafil, n (%)	1 (3.0%)	0 (0%)	NS	0 (0%)	0 (0%)	–
PGI_2 intravenous+sildenafil, n (%)	0 (0%)	1 (12.5%)	NS	0 (0%)	1 (50.0%)	NS
PGI_2 oral+bosentan+sildenafil, n (%)	1 (3.0%)	0 (0%)	NS	0 (0%)	0 (0%)	–
PGI_2 intravenous+bosentan+sildenafil, n (%)	2 (6.1%)	1 (12.5%)	NS	0 (0%)	0 (0%)	–

CCB, calcium-channel blocker; PGI_2, prostaglandin I_2. Other abbreviation see in Table 1.

normalization in response to treatment is a new prognostic factor of PH and probably has more prognostic significance than BNP and other previously established prognostic factors.

Cohort of PH Patients

Our cohort of PH had more female patients with a more prevalence of PH, and most of the patients were treated with prostacyclin alone or in combination with other vasodilators, a finding consistent with previous reports.[1,17–19] Our cohort is unique because all patients were closely followed by right-heart catheterization performed every 6–12 months in order to monitor their clinical course and to adjust the doses of drugs, including epoprostenol, bosentan and/or sildenafil. All of the drugs, especially the combination with intravenous prostacyclin and other vasodilators, have already been reported to improve symptoms, 6-min walk distance, pulmonary hemodynamics, and survival.[9,20–24] Indeed, in the present study, combination therapy with intravenous prostacyclin and other vasodilators significantly improved pulmonary hemodynamics in patients with PH who were not improved by any monotherapy. However, if combination therapy is unavailable, monotherapy with intravenous prostacyclin might not be enough in patients with severe PH, as shown in **Table 5**.

Our current strategy for advanced PH is to improve quality of life and pulmonary hemodynamics (especially CI and RAP) and to prevent the development of right-heart failure, although more therapeutic progress needs to be made.[25–29]

BNP Levels in PH

Elevated BNP level is a significant prognostic factor,[10] but in the present study this was the case in the univariate analysis only, not in the multivariate analysis when we included the CI response to treatment. This result suggests that BNP and CI closely influence each other through right ventricular dysfunction and that the CI response has more prognostic significance than BNP. However, BNP should remain in use as a prognostic factor because it is easily and non-invasively measured.

Thyroid Dysfunction in PH

The present study demonstrated that 46% of the PH patients had thyroid dysfunction (hyperthyroidism in 19%; hypothyroidism in 81%). Indeed, 5 of 11 patients with hyperthyroidism were treated with antithyroid drugs, and 3 of 47 patients with hypothyroidism were treated with thyroid hormone replacement therapy, which is consistent with previous studies in which 22–49% of patients with PAH were complicated with thyroid dysfunction.[30,31]

The precise mechanisms of thyroid dysfunction in PH remain unclear; however, involvement of autoimmune mechanisms and the side-effects of prostacyclin therapy may be involved.[30] Indeed, it was reported that the treatment of thyroid dysfunction ameliorated PAP in PH patients complicated with thyroid diseases.[32–34] It also was reported that prostacyclin plays an important role in the modulation of thyroid function in patients with Graves disease, because prostacyclin therapy may cause thyrotoxicosis as a result of downregulation of prostaglandin I_2 receptors, with subsequent reduced production of cAMP.[32,33] However, in the present study, no significant influence of prostacyclin therapy was noted on the occurrence of thyroid dysfunction. Furthermore, a low plasma T3 level was a significant prognostic factor by univariate analysis but not by multivariate analysis. In contrast, a low plasma T3 level has been reported as associated with poor prognosis in patients with chronic heart failure.[35–37]

Hemodynamic Variables in PH

Although echocardiography is a non-invasive and useful tool for the follow-up of PH patients, because it can estimate systolic PAP, it alone is not enough to evaluate pulmonary hemodynamics, because RAP, $S\bar{V}O_2$ and CI are more important prognostic factors that cannot be obtained by echocardiography.[38–40] Therefore, right-heart catheterization is essential for both accurately evaluating pulmonary hemodynamics and decision making in each PH patient.[38–40] Indeed, low CO at diagnosis is a prognostic factor of PAH[41] and further, the present study demonstrated that CO normalization in response to treatment is a new prognostic factor.

During the course of the development of PAH, pulmonary arteriopathies first occur, followed by RV pressure-overload. Thus, the final goal of PH should be regression of pulmonary arteriopathies.[6] At present, because none of the available therapies is able to normalize pulmonary hemodynamics (especially during exercise), RV function (eg, CO) may determine the prognosis of PH patients.[41]

Study Limitations

Several limitations should be mentioned. First, this was an observational cohort study from a single center, so the present finding regarding the prognostic impact of CI normalization remain to be confirmed in a large, multicenter clinical study. Second, the PH patients were treated individually, but not in a randomized manner, based on their pulmonary hemodynamics and comorbidities, so some bias could be involved. However, considering the severity of the disease, such a randomized clinical study would not be appropriate from the ethical point of view.

Conclusions

In the present study, we were able to identify that CI normalization in response to treatment is a new independent prognostic factor of PH in patients with low CI at diagnosis, suggesting the importance of intensive therapy to normalize CO in PH patients.

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Disclosures

None.

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Supplementary files

Figure S1. Administration protocol for epoprostenol.

Figure S2. Administration protocol for bosentan.

Figure S3. Effects of combination therapy on pulmonary hemodynamic variables in patients with pulmonary hypertension.

Please find supplementary file(s);
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