Mutation Assay for T790M in Exon 20

The restriction enzyme *Nla*III was used to digest the CATG sequence in the amplicon of the mutant type (T790M) allele because of the base substitution of C to T at the third base of CACG. In contrast, wild-type allele was not digested. The PCR products after digestion were run on 2% agarose gel and the existence of the mutation was assessed (Figure 1).

EGFR Gene Sequencing

EGFR gene mutations in the cDNA samples were examined using PCR-based direct sequencing for exons 18, 19, and 21 to confirm the results of RFLP analysis. Sequencing was performed using the Applied Biosystems PRISM dye terminator cycle sequencing method with an ABI PRISM 3100 Genetic Analyzer (Perkin-Elmer Corp., Foster City, CA) at the Central Research Center of Keio University Hospital.

Patients and Clinical Samples

Tumor samples from 109 patients diagnosed as having primary NSCLC by histopathological examination were obtained from Keio University (82 samples) and Kawasaki Municipal Hospital (27 samples). Ninety-one frozen tumor specimens were obtained either by surgery ($n = 48$), computed tomography-guided needle lung biopsy ($n = 32$) or ultrasonography-guided needle lung biopsy ($n = 5$), or TBLB ($n = 6$). Fourteen samples from pleural effusion, three samples from pericardial effusion, and one sputum sample were also obtained. Malignant effusion collected by pleurocentesis or cardiocentesis was centrifuged and the cell pellet was collected after removal of the supernatant. All samples were stored at −80°C until the DNA and RNA extraction procedures described above were performed.

All patient samples were collected or tested with informed consent, as approved by our respective institutional review boards. Clinical parameters for the patients were obtained from their medical records.

Clinical information was available for all 109 patients and Table 1 summarizes the demographic and clinical data of the study cohorts.

TABLE 1. Clinicopathological Features of All Patients

Variables	Subset	No.	(%)
No. patients		109	
Age (yr)	Mean	64.9	
	Range	30–85	
Sex	Male	59	(54.1)
	Female	50	(45.9)
Stage	I	18	(16.5)
	II	7	(6.4)
	III	33	(30.3)
	IV	51	(46.8)
Smoking history	Never smoker	37	(34.0)
	Ever-smoker	72	(66.0)
Tumor type	Adenocarcinoma	79	(72.6)
	AWBF	1	(0.9)
	BAC	5	(4.6)
	Squamous cell carcinoma	13	(11.9)
	Large cell carcinoma	1	(0.9)
	LCNEC	1	(0.9)
	Adenosquamous carcinoma	1	(0.9)
	Pleomorphic	2	(1.8)
	Others	5	(5.5)
Tumor samples	Resected tumor	48	(44.0)
	CT-guided lung biopsy	32	(29.4)
	US-guided lung biopsy	5	(4.6)
	TBLB	6	(5.5)
	Pleural fluid	14	(12.8)
	Pericardial fluid	3	(2.8)
	Sputum	1	(0.9)
EGFR mutations		37	(33.9)
	Exon 18		1/37, 2.7%
	Exon 19	22	22/37, 59.5%
	Exon 21	14	14/37, 37.8%

AWBF, adenocarcinoma with bronchiolo-alveolar carcinoma features; BAC, bronchiolo-alveolar carcinoma; EGFR, epidermal growth factor receptor; LCNEC, large cell neuroendocrine carcinoma; TBLB, transbronchial lung biopsy.

Statistical Analyses

The association between EGFR mutational status and tumor response to gefitinib was assessed using the χ^2 test. Multivariate analysis using logistic regression models was performed to assess the associations among histologic subtypes, gender, smoking history, age, and mutational status. All analyses were performed using Stat View (version 5, SAS Institute Inc., Cary, NC) software on a Macintosh computer.

RESULTS

Patterns of PCR-RFLP for EGFR Mutations on Gel-Electrophoresis

Figure 1 presents the predicted gel-electrophoresis patterns for PCR-RFLP samples. We first confirmed the patterns of electrophoresis for EGFR mutations using vectors containing an EGFR exon 18 or 21 point mutation as well as wild-type or cell lines containing an exon 19 deletion mutation and wild-type genes. G719S and L858R mutant vectors were clearly distinguished from wild-type by PCR-RFLP (Figure 2*A* and Figure 2*C*, respectively). On the other hand, a shorter band from the deleted allele in exon 19 and a longer band from the wild-type allele were observed by reverse transcription-polymerase chain reaction from H1650 (Figure 2*B*).

Sensitivity of PCR-RFLP Analysis of EGFR Mutations in Exon 19 and Exon 21

We evaluated the sensitivity of our RFLP assay in exon 19 or in exon 21 by combining SK-MES-1 cells with H1975 or H1650, respectively, in different ratios (Figure 3*A*). In exon 19, a shorter band from the deleted allele was detected up to the level of 1×10^2-fold dilution. In exon 21, the mutant allele at the 154 bp band was also detected up to the level of 1×10^2-fold dilution. The band of 154 bp indicates digested mutant alleles, and the band of 98 bp indicates wild-type alleles.

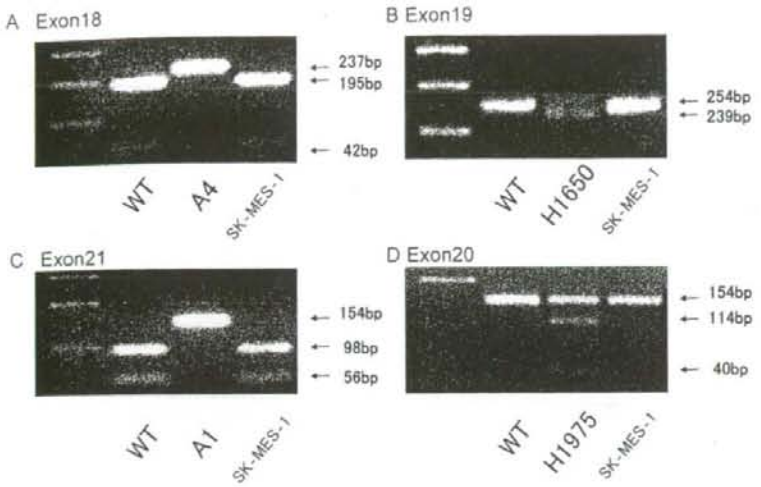

FIGURE 2. Demographic data of EGFR mutations in exon 18, 19, 21, and 20 by PCR-RFLP. *A*, The PCR products of exon18 treated with *Apa*I. *B*, The PCR products of exon19. *C*, The PCR products of the exon21 treated with *Msc*I and *Pvu*II, simultaneously. *D*, The PCR products of exon20 treated with *Nla*III. WT, EGFR (wild type) vector; A4, EGFR (G719S) vector; H1650, lung cancer cell line with EGFR mutation (del E746-A750); A1, EGFR (L858R) vector; H1975, lung cancer cell line with EGFR mutation (L858R and T790M); SK-MES-1, lung cancer cell line without EGFR mutation.

We also compared the sensitivity of cDNA and genomic DNA samples in pleural effusion from NSCLC patients for RFLP assay.

As malignant pleural effusion usually contains many hematopoietic cells such as macrophages and lymphocytes in addition to tumor cells, as a consequence, dilution of

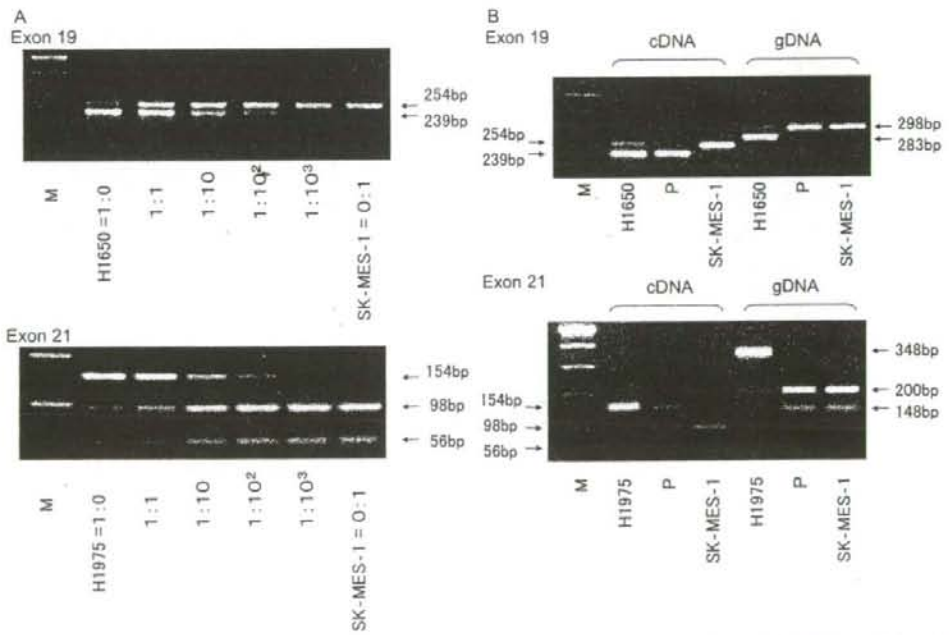

FIGURE 3. Sensitivity of PCR-RFLP analysis of EGFR mutations. *A*, In exon 19, H1650 was mixed with SK-MES-1 from 1- to 10^3-fold. A shorter band from the deleted allele was detected up to the level of 1×10^2-fold dilution. In exon 21, H1975 was mixed with SK-MES-1 from 1- to 10^3-fold. The mutant allele at the 154 bp band was detected up to the level of 1×10^2-fold dilution. SK-MES-1, lung cancer cell line without EGFR mutation; H1650, lung cancer cell line with EGFR mutation (del E746-A750); H1975, lung cancer cell line with EGFR mutation (L858R). *B*, PCR-RFLP was performed using either cDNA or genomic DNA (gDNA). The mutant allele in exon 19 was only detected by using cDNA in the case of malignant pleural effusion. In exon 21, the mutant allele (a 154 bp digested fragment) can be distinguished readily by using cDNA in the case of malignant pleural effusion of NSCLC. H1650; lung cancer cell line with EGFR mutation (del E746-A750), H1975; lung cancer cell line with EGFR mutation (L858R), SK-MES-1; lung cancer cell line without EGFR mutation, P; the case of malignant pleural effusion of NSCLC, M; marker.

genomic DNA derived from tumor cells occurs. To minimize the dilution caused by contaminated hematopoietic or other nontumor cells, we have chosen cDNA instead of genomic DNA for PCR/RFLP, taking into consideration that hematopoietic cells usually do not express the EGFR gene. Indeed, we could detect the mutation bands only when cDNA was used but not when genomic DNA was used from NSCLC patients with malignant pleural effusion (Figure 3*B*).

Results of PCR-RFLP Analysis of EGFR Mutations in Exons 18, 19, 21, and 20 in Clinical Samples

Only 1 patient (patient 56) showed 2 fragments (237 bp and 195 bp, corresponding to mutant and wild-type alleles, respectively) by RFLP using *Apa*I for exon 18 (Figure 4*A*). We found a novel point mutation in codon 719 (2146G>C [G719D]) confirmed by direct sequencing.

In exon 19, we found 22 deletion mutations. Patients who had a deletion mutation showed two fragments corresponding to wild-type and deletion mutant alleles (Figure 4*B*).

In exon 21 using *Msc*I and *Pvu*II, we found 14 point mutations in codon 858 by RFLP. Patients who had a 2573T>G point mutation showed 3 fragments (154 bp and 98 bp, corresponding to L858R mutant and wild-type alleles, respectively) (Figure 4*C*). No L861Q mutation was observed in our specimens.

In exon 20 using *Nla*III, we found 2 point mutations in codon 790 (2369C>T [T790M]) by RFLP. Patients who had a 2369C>T point mutation showed 2 fragments (154 bp, 114 bp, and 40 bp, corresponding to T790M mutant and wild-type alleles, respectively) (Figure 4*D*).

Finally, we found 37 EGFR mutations (34%) in exons 18, 19, and 21 in the present study.

EGFR Mutations and Clinicopathologic Features

(Table 2) The mutation status was significantly correlated with pathologic subtype (adenocarcinoma including bronchiolo-alveolar carcinomas versus nonadenocarcinoma, odds ratio = 5.56, $p = 0.035$), smoking status (never-smokers versus ever-smokers; odds ratio = 4.34, $p = 0.007$) and age (65-year-old or younger versus older than 65 years; odds ratio = 2.64, $p = 0.037$) but not with gender (female versus male; odds ratio = 1.14, $p = 0.813$) by logistic multivariate analysis.

Indeed the female never-smoker patients with adenocarcinoma had a high mutation rate (18/27, 66.6%), whereas the young never-smokers with adenocarcinoma had a higher mutation rate (13/15, 86.7%). Moreover, the young female never-smoker patients with adenocarcinoma had the highest mutation rate (10/11, 90.9%), whereas the elderly male ever-smokers with nonadenocarcinoma had the lowest mutation rate (1/11, 9.1%).

EGFR mutations were not influenced by disease extent (TNM stage) (data not shown).

EGFR Mutations and Clinical Outcome in Patients Treated with Gefitinib

(Table 3) Among 109 patients, 36 were treated with gefitinib (250 mg/d) and evaluated for their response. Eight of 36 patients were treated with gefitinib as an initial treatment. The results of the evaluation showed that 10 patients responded to the treatment. Nine of 10 responsive patients had mutations in exon 19 or exon 21, whereas one had no mutations. The response rate to gefitinib in patients with EGFR deletion mutations in exon 19 and L858R point mutation in exon 21 was 58.3% (7 of 12) and 33.3% (2 of 6, 1 of 7 patients was not evaluable due to discontinuation of treatment because of side effect), respectively. The patient

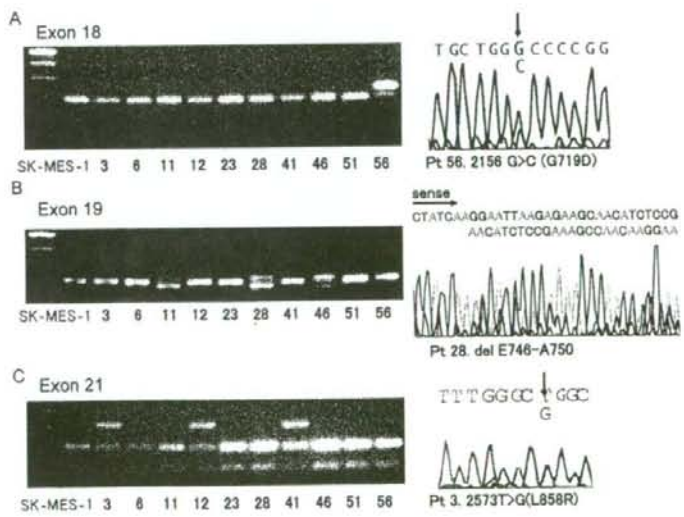

FIGURE 4. Result of PCR-RFLP analysis of EGFR mutations in exon 18, 19, and 21 in clinical samples. *A*, In exon 18, only patient 56 showed 2 fragments (237 bp and 195 bp, corresponding to mutant and wild type alleles, respectively). Direct sequencing analysis of PCR products is shown. Point mutation 2156G>C (G719D) (arrow) is detected in patient 56. *B*, In exon 19, patients 11, 28, and 46 showed 2 fragments corresponding to wild type and deletion mutant alleles. In-frame deletion was detected in patients 28. *C*, In exon 21, patients 3,12, and 41 showed 2 fragments (154 bp and 98 bp, corresponding to L858R mutant and wild type alleles, respectively). No L861Q mutation was observed in our specimens. Point mutation 2573T>G (L858R) was detected in patients 3, 12, and 41.

TABLE 2. Correlation of EGFR Mutations with Clinicopathologic Features

	EGFR Mutation		Odds Ratio	p
	+	−		
(A) Gender and EGFR mutation status				0.813
Female	22	28	1.14	
Male	15	44		
(B) Histology and EGFR mutation status				0.035
Adenocarcinoma (with BAC)	35	50	5.56	
Nonadenocarcinoma	2	22		
(C) Smoking habit and EGFR mutation status				0.007
Never smoker	21	16	4.34	
Ever-smoker	16	56		
(D) Age and EGFR mutation status				0.037
≤65	25	30	2.64	
>65	12	42		

EGFR, epidermal growth factor receptor; BAC, bronchiolo-alveolar carcinoma.

TABLE 3. Response to Gefitinib and EGFR Mutation Status

	EGFR Mutation		
Response	+	−	
CR	1	0	
PR	8	1	
SD	5	1	
PD	4	16	
CR/PR	9	1	
SD/PD	9	17	$p = 0.003$
CR/PR/SD	14	2	
PD	4	16	$p < 0.00001$

CR, complete response; EGFR, epidermal growth factor receptor; PD, progressive disease; PR, partial response; SD, stable disease.

with G719D mutation in exon 18 was not treated with gefitinib. On the other hand, 2 patients had T790M mutation and both also had activating mutations (data not shown). One patient who had L858R mutation was not treated with gefitinib. Another patient who had deletion mutation in exon 19 was resistant to gefitinib.

EGFR mutations were more frequently observed in samples from the patients who showed complete or partial responses (9 of 10 cases, 90.0%) than in samples from patients with stable disease or progressive disease (9 of 26, 34.6%; $p = 0.003$). Alternatively, the response rate to gefitinib in patients with EGFR mutations was 50% (9 of 18).

Comparison of Detection of Mutations by RFLP and Direct Sequencing

EGFR mutations were detected in 37 patients. They were detected by both RFLP and direct sequencing methods in 36 cases, whereas it was detected only by RFLP but not by direct sequencing in one case.

DISCUSSION

In the present study, we have described a reliable PCR-RFLP assay for the detection of mutations occurring in the EGFR TK domain. We have also analyzed a large series of NSCLCs for mutations in the TK domain of the EGFR gene by RFLP to assess the actual incidence of this genetic abnormality and its distribution according to histologic type, sex, smoking history, age, and TNM system parameters. In addition, we have analyzed the relationship between EGFR mutations and clinical outcome in patients treated with gefitinib.

In our analysis of 109 cases, most of the EGFR mutations were present in adenocarcinomas. Approximately 41% of adenocarcinomas showed the EGFR mutation, whereas the corresponding figure was 8% for nonadenocarcinomas. In univariate analysis, EGFR mutations were also significantly more frequent in women, younger patients, and never-smokers (data not shown). However, when the histotype, sex, smoking history, and age were tested by multivariate analysis against the presence of mutations in EGFR as a dependent variable, histotype, history of never smoking, and younger age (≤65) remained significant, while female sex did not.

EGFR mutations were more frequently observed in the samples from younger patients (45.5%) than older patients (22.2%). Tomizawa et al. also reported that EGFR mutation was significantly more frequent in younger patients (38%) than in older patients (12%, $p < 0.0001$).[13] For lung cancer, young adults are generally defined as being 40 or 45 years and under, whereas elderly patients are defined as 65 or 70 years and older. When we used 45 years as a cutoff value for age, there was no significant difference between the younger and the older patients regarding EGFR mutations, either by univariate or multivariate analysis. On the other hand, when 65-year-old was used as the cutoff value, the younger, nonelderly patients had a significantly higher prevalence of the EGFR mutation. Indeed, we found that the nonelderly (≤65 years) female never-smoking patients with adenocarcinoma had the highest mutation rate (90.9%), however, this mutation rate was almost identical to the value found in the nonelderly never-smokers with adenocarcinoma independent of sex (86.7%). Moreover, we found only one mutation in the elderly ever-smoker patients with nonadenocarcinoma (1/16, 6.3%) or in males with the above 3 indexes (9.1%). Together with the findings of multivariate analysis, the results suggest that being female may not be a significant independent factor for predicting EGFR mutations, as has been reported elsewhere. This may be partially explained by the fact that female was the predominant sex in young and adenocarcinoma patients.[14]

EGFR mutations were more frequently observed in samples from patients who showed a complete or partial response than in samples from patients with a stable or progressive disease, supporting the findings of many previous reports (Table 3). The exon 19 deletion mutations are re-

ported to be more predictive of gefitinib response or demographics compared with the exon 21 mutation.[9,15,16] The response rate in our analysis was also higher in patients with the exon 19 deletion mutations (58.3%) compared with those with L858R (33.3%).

In tumors from patients not treated with either gefitinib or erlotinib, the 2369C>T mutation (T790M) seems to be extremely rare. We have identified only 2 cases of this mutation in 109 tumors (1.8%).

Large-scale screening requires a rapid and sensitive technique. At the present time, EGFR mutation detection is most commonly performed by direct DNA sequencing of the EGFR kinase domain. However, direct sequencing has several disadvantages with regard to clinical use. The most notable of these is a low detection rate when DNA from clinical samples is used, presumably because of the presence of high rates of contaminated normal and fibrous tissues in tumor samples. Detection of mutations by this method requires at least 30% of the mutated DNA in a sample.[17]

Some investigators have attempted to improve the sensitivity of detection of EGFR mutations in samples containing a mixture of tumor and normal cells. Wookey et al. reported that the ARMS method (Scorpion Amplified Refractory Mutation System technology) was superior to the direct sequencing method and introduced the WAVE method for detecting EGFR mutations.[18] Moreover, EGFR mutations were detectable using the ARMS method in serum DNA from patients with NSCLC.[19] One attempt involved the detection of EGFR mutations using a LightCycler PCR assay.[20] SSCP assay is more sensitive than direct sequencing and is a more rapid method.[21] Recently, 2 rapid and sensitive methods have been demonstrated: the peptide nucleic acid-locked nucleic acid PCR clamp method[22] and mutant-enriched PCR assay.[23] In these studies, EGFR mutations were detected in the presence of 1000-fold and 2000-fold wild-type EGFR genes, respectively.

Indeed, some of the above methods seem to be more sensitive than our method, however, our method does not require special equipment such as real-time PCR machines using multiple dyes like in the Scorpion ARMS and peptide nucleic acid-locked nucleic acid clamp PCR methods. Thus, compared with the other techniques, ours is relatively simple, cost-effective, fast, and reliable as we can see the mutated bands directly. The sensitivity for detection of mutations by our method is also sufficiently high as it is able to detect mutations in samples containing as few as 1% mutated cancer cells (Figure 3*A*). Although a variety of different mutations are seen spanning the entire EGFR TK domain, 94% reside in exons 18 (5%), 19 (48%), and 21 (41%).[24] Using the PCR-RFLP analysis, more than 90% of the mutations in the EGFR gene can be immediately recognized.

Specimens of lung tumor usually contain substantial proportions of normal cells, such as fibrous tissue and peripheral blood cells. Also, normal cells, such as inflammatory cells or mesothelial cells, are also contained in the pleural effusion of lung cancer patients, in addition to tumor cells. Contaminated wild-type DNA interferes with accurate analysis. Most epithelial cells express EGFR, whereas cells of hematopoietic origin are usually EGFR-negative.[25] Using cDNA instead of genomic DNA for the PCR-RFLP, we can minimize the influence of the contaminated hematopoietic or other nontumor cells, which have no EGFR expression. We compared the sensitivity of cDNA and genomic DNA samples in pleural effusion from NSCLC patients when using the RFLP assay. We were able to detect the mutation bands only when a cDNA sample was used (Figure 3*B*). Thus, PCR-RFLP using cDNA is more sensitive than using DNA, particularly when analyzing contaminated samples.

In conclusion, mutations in the EGFR TK domain define a new molecular type of lung carcinoma that is likely to respond to EGFR TK inhibitors. Good clinical independent predictive factors are suggested to be a nonsmoking history, younger age (≤65), and histotype of adenocarcinoma, but not female sex. The PCR-RFLP assay described here is a rapid and reliable method for the screening of EGFR kinase domain mutations in lung cancer patients with various types of samples, as we can minimize the influence of contaminating cells having no EGFR expression using cDNA. The sensitivity, cost, and simplicity of the procedure are satisfactory for genetic testing of lung cancer patients at the clinical laboratory level. RFLP will be one of the useful assays for predicting the sensitivity of NSCLC patients to EGFR-TKIs.

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CASE REPORT

A case report of surgical correction for congenital mitral regurgitation with subvalvular apparatus abnormality

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Abstract We report a successful complex mitral valve plasty using port access minimally invasive cardiac surgery for congenital mitral regurgitation that presented as an abnormality of the subvalvular apparatus. A 16-year-old male patient received a diagnosis of mitral regurgitation resulting from tethering of the anterior mitral leaflet and posterior mitral leaflet caused by an abnormality in papillary muscle insertion and a hypoplastic chordae tendineae. The posterior leaflet was closely tethered to the tips of the papillary muscle with essentially no chordae tendineae. The flexibility of the leaflet was restored by surgically removing the abnormal chordae, and reconstruction of chordae tendinae of the anterior leaflet was carried out using three loops and of the posterior leaflet using one loop with a loop technique method. As an additional procedure for persistent regurgitation, an edge-to-edge technique to the posterior commissure side was performed, after which the mitral regurgitation disappeared.

Key words Loop technique · Mitral valve plasty · Subvalvular apparatus abnormality · Port access minimally invasive cardiac surgery · MICS

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Introduction

Various surgical techniques have been reported for mitral valve plasty (MVP), all of which show good results.[1,2] MVP is enabled by adjusting various techniques in the case of a complicated lesion. We report a successful complex MVP using port access minimally invasive cardiac surgery (MICS)[3] for congenital mitral regurgitation that presented as an abnormality of the subvalvular apparatus.

Case report

A 16-year-old male patient who had been followed for 4 years at another hospital under a diagnosis of mitral regurgitation (MR) was referred to our hospital for a detailed examination because he became aware of breathlessness during exercise. On admission, his height was 159 cm, body weight was 57 kg, and blood pressure was 116/70 mmHg. On physical examination, a high-pitched systolic regurgitant murmur (Levine III/VI) at the lower left sternal border and a low-pitched early-diastolic rumble at the apex were detected. Electrocardiography showed left ventricular hypertrophy with normal sinus rhythm. Echocardiography revealed moderate MR resulting from tethering of the anterior mitral leaflet (AML) and posterior mitral leaflet (PML) caused by an abnormality in papillary muscle insertion and a shortening of the chordae tendineae. Notably, the PML was closely tethered to the tips of the papillary muscle with essentially no chordae tendineae (Fig. 1). Progression of MR was accepted in comparison with an echo provided by another hospital. LV end-diastolic and end-systolic dimensions (LVEDD/LVESD) were 58/38 mm, and the

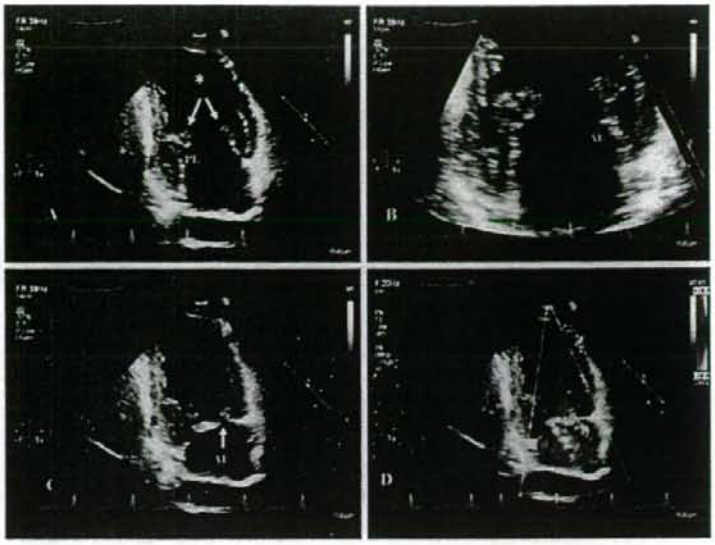

Fig. 1 Preoperative echocardiography. **A,B** Abnormality in papillary muscle insertion (*). The posterior leaflet was severely tethered to the tips of the papillary muscle with essentially no chordae tendineae. **C** A central part of the anterior leaflet was tethered by secondary chorda tendineae (*arrows*). **D** Doppler color flow image shows the regurgitation jet from the two junctions of the mitral valve. **A,B** Diastolic phase; **C,D** systolic phase. *PL*, posterior mitral leaflet; *AL*, anterior mitral leaflet

LV ejection fraction was 59%. The posteromedial papillary muscle insertion site and inferoposterior LV wall showed severe hypokinesis. Under cardiac catheterization, a pressure study showed that LV end-diastolic pressure was 13 mmHg and pulmonary artery wedge pressure was 12 mmHg. Furthermore, LVEDV showed 178.7 ml, showing potential for the enlargement of left ventricle volume.

Operative technique

Cardiac exposure was obtained by right anterior small thoracotomy by means of port access MICS. The mitral valve and subvalvular apparatus were observed via right-sided left atriotomy. The flexibility of the AML was maintained and the leaflet was approximately normal; however, two secondary chorda tendinaes were fixed to a portion of the central part of the AML. The PML presented a fixation such that it was drawn into the free wall of the left ventricle. The secondary chorda tendinae of the AML was removed, and the flexibility of the PML was restored by surgically removing the three short chordae tendinae that had been attached to it. Next, replacement of these chordae tendineae was performed using the loop technique (5-0 Gore-Tex polytetrafluoroethylene sutures).[4] After having fixed the neochordae with two loops to each papillary muscle, reconstruction of the AML was carried out using three loops and of the PML using one loop (Fig. 2). A coaptation line and movement of the bileaflet valve proved satisfactory in this plasty; however, a small degree of MR remained on the posterior commissure side. As an additional procedure for persistent MR, an edge-to-edge technique to the posterior commissure side was performed, after which the MR disappeared. Ring annuloplasty was carried out using a 32-mm Cosgrove annuloplasty ring. After this procedure, transesophageal echocardiography revealed a dramatic reduction of MR; however, extremely mild mitral stenosis remained (Fig. 3). The postoperative course was uneventful.

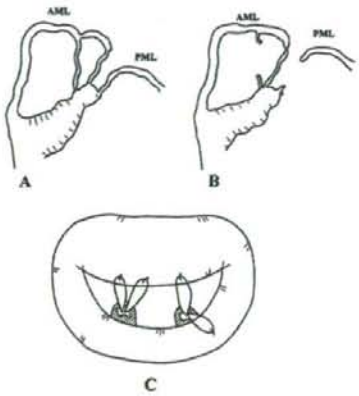

Fig. 2 Schematic presentation of the surgical procedure. Secondary chordae of the anterior leaflet and abnormal shortening of chordae of the posterior leaflet were divided to allow the flexibility of the leaflet (**A,B**). Reconstruction using a loop technique of the AML was carried out using three loops and of the PML using one loop. *AML*, anterior mitral leaflet; *PML*, posterior mitral leaflet

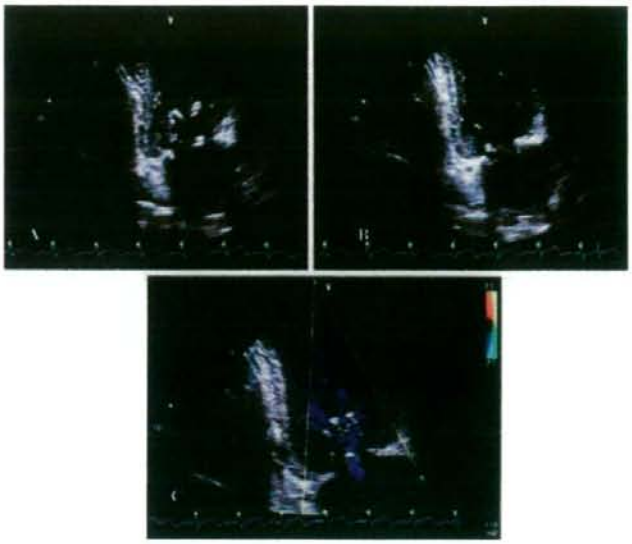

Fig. 3 Postoperative echocardiography showed physiological movement of both leaflets and no residual mitral regurgitation

Discussion

In this case, because the MR had resulted from abnormal papillary muscle insertion and marked foreshortening of the chordae tendineae, use of the standard MVP procedure was difficult. For valvuloplasty in younger patients, the ideal approach is to perform valve plasty while securing as wide a mitral valvular area as possible to allow for future physical growth. A valvular excision and resuture can cause partial degradation of leaflet mobility as a result of consolidation and cicatrization.[5] In this patient, therefore, the loop technique was employed in an attempt to preserve the mobility of the posterior mitral leaflet, to allow a broad range of plasty and to maintain as large a mitral valve area as possible.[6] The aberrant short chordae causing tethering were therefore surgically removed to permit recovery of the flexible leaflet. Because multiple neochordae tendineae were necessary in this case, artificial chorda replacement was employed using the loop technique. Using this method, a satisfactory coaptation line was successfully formed by reconstruction with neochordae, and a loop technique method that permitted multiple and simultaneous replacement proved effective.[4] A second congenital anomaly resulted in a papillary muscle being present in the vicinity of a mitral valve. It appeared likely that physiological movement of the leaflet would be inhibited by this papillary muscle because it was in close proximity to the leaflet edge. As a solution, the distance between the papillary muscle and the valve leaflet was increased by fixing the neochorda unit to the central portion of the papillary muscle. Preservation of physiological leaflet motion was thus enabled.

Valve replacement can now be performed safely with relatively low morbidity and mortality. However, the MVP is preferable in young patients because it reduces the need for long-term anticoagulation treatment and provides a more physiological correction of the lesion. MVP in MR caused by congenital anomaly appears to be feasible by adopting various techniques, as was demonstrated in this case. In addition, initial operations using port access MICS will enable safe reoperation using standard procedures in the future.

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Clinical Engineering 別冊

心臓手術の実際

外科医が語る術式，臨床工学技士が語る体外循環法

[編集]
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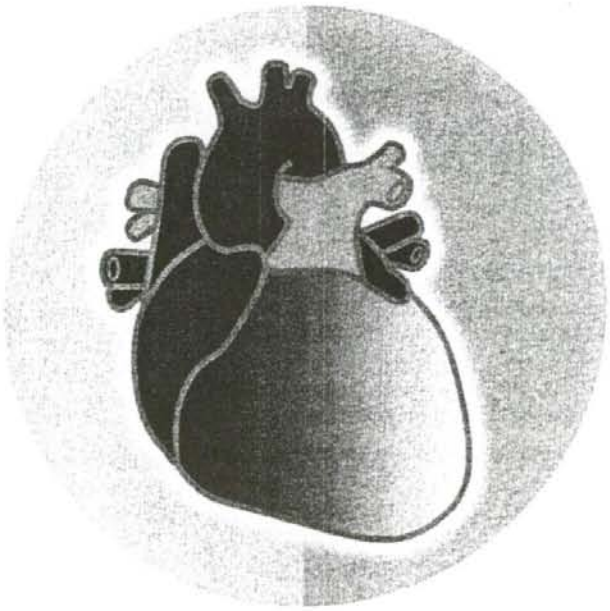

秀潤社

Ⅲ-4. 心内膜床欠損症に対する手術と体外循環法
－慶應義塾大学－

心内膜床欠損症(ECD)は，近年では atrioventricular canal defect または atrioventricular septal defect(AVSD，房室中隔欠損症)と表記されることが多い．ここでは，ファロー四徴症(TOF)などの合併心疾患がなく，かつ二心室修復が可能なものを対象とする．また本疾患の半数以上はダウン症候群を合併している．ここでは，慶應義塾大学における心内膜床欠損症手術の術式について医師が，その術式に対応した体外循環法について臨床工学技士が解説する．

医師

心内膜床欠損症に対する手術

心内膜床欠損症の解剖学，病態生理

心内膜床欠損症(ECD)は，心室間交通量の度合により部分型，移行型，完全型に分類され，完全型はさらに共通前尖の形態により Rastelli A，B，C 型に分類される．C 型は共通前尖が分葉せずに心室中隔と腱索の連続がないものをいう．A 型は共通前尖が分葉している部分において多数の細かな腱索によって支持されている．B 型はまれである．しかし，心内膜床は発生学上，心房中隔と心室中隔とともに，僧帽弁や三尖弁に相当する左右房室弁を形成する基になる(図1)ので，ECD は形態上も病態生理上も症例ごとで異なり，分類することよりも1つのスペクトラムとしてとらえるほうが理解しやすい．

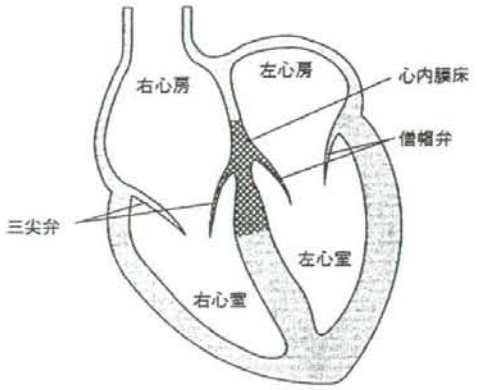

図1　心内膜床の位置

部分型は，心房位での左右短絡のみで心室間交通のないものをいい，一次孔欠損(ostium primum)と表記されることもある．左側房室弁(僧帽弁に相当)の前尖には cleft といわれる裂け目があり，これは腱索付着による左室自由壁からの支持がないという点で，正常僧帽弁交連の弁接合と大きく異なっている．左右房室弁は心室中隔の尾根と接合することにより連続しているが，それらの弁口は互いに独立している．修復術の適応が心房間交通のみである場合は，二次孔型心房中隔欠損と同様，3歳以上が手術時期となる．しかし，左側房室弁閉鎖不全による左心不全を呈する場合には早期に手術する．

移行型は，心室間交通が少量のみ存在するもので，一般的に部分型よりも心房位での左右短絡・僧帽弁閉鎖不全ともに高度で，しばしば，より早期に修復術が必要になる．また部分型と異なり共通前尖と共通後尖は連続していないが，

ごく短い腱索により心室中隔の尾根に固定されている.

完全型は, 心房位での左右短絡に加えて心室間交通が大きく, unrestrictive(血行力学上無制限な交通)になっているものを指す. 乳児期に多量の心室間左右短絡による左心不全症状, または肺血管閉塞性病変の進行をきたすことが多いため, 乳児期早期が修復術の適切な時期である. 特にダウン症候群の場合には肺血管閉塞性病変の進行が速いので, 特に修復術時期を遅らせないことが重要である. 左右房室弁は共通となっていて, その形態も症例ごとで異なる.

外科的に最も重要な解剖学的ポイントは左側房室弁の形態であり, 一般に左側弁尖の形成が良好なほど, 左側方尖が付着している弁輪周囲に占める角度が大きいほど, また左側共通前後弁尖を支持する腱索が豊富なほど, 修復が容易である.

2 ECD に対する手術

修復術の至適時期については前述の通りである. なお, 肺動脈 banding(絞扼)術の適応は, 現在では人工心肺の絶対的禁忌(脳出血や RS ウイルス感染など)に限定されている. 手術術式は完全型を中心に記述する.

まず, 胸骨正中切開し, 胸腺部分切除の後, 心房中隔パッチ用に心膜を採取し, 動脈管は体外循環前に閉鎖しておく. 人工心肺のカニュレーションは, 上行大動脈に送血用を, 上下大静脈に脱血用を挿入し, 中等度低体温高流量体外循環とする. 左室ベントを右上肺静脈より挿入する. 上行大動脈を遮断し, 順行性心筋保護液注入により心停止とする. 房室間溝に平行に大きく右房切開をする. 房室弁の形態を歪ませないように配慮して吊り糸をかけて視野を展開する. この手術では, 十分な時間をかけて心内解剖を評価し, 修復の全体的計画を決定してから修復の針糸をかけ始めることが特に肝要である.

左室内を晶液で充満させて共通房室弁を閉鎖位とし, 心室中隔尾根上で共通前後弁尖の kissing point(接合点)を見付けて, これを中心として分割線を決める. 分割線の約 80%の長さを長径とした半弧形 GORE-TEX® パッチ(ジャパンゴアテックス(株))を作成して心室中隔欠損閉鎖用とする. まずこのパッチの弧状下縁を心室中隔右側面に連続縫合で縫着する. 腱索付着を可及的に温存する. 次に, GORE-TEX® パッ

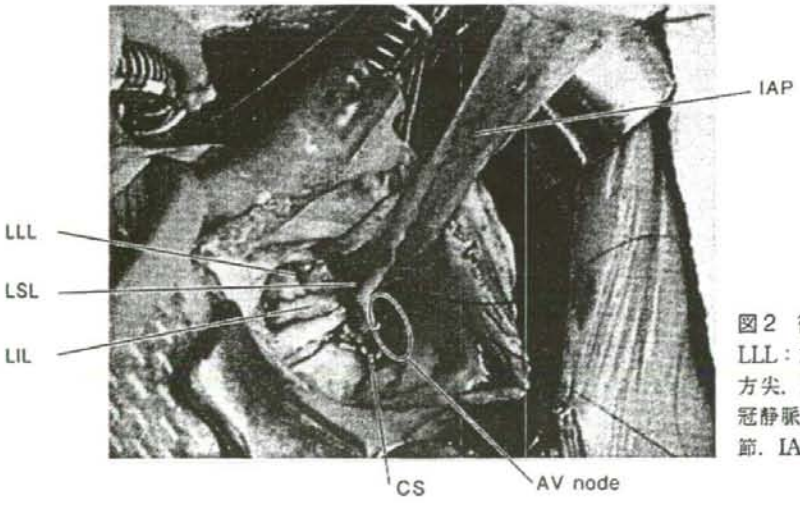

図2 術中写真
LLL:左側方尖, LSL:左上方尖, LIL:左下方尖, CS:冠静脈洞, AV node:房室結節, IAP:心房中隔パッチ.

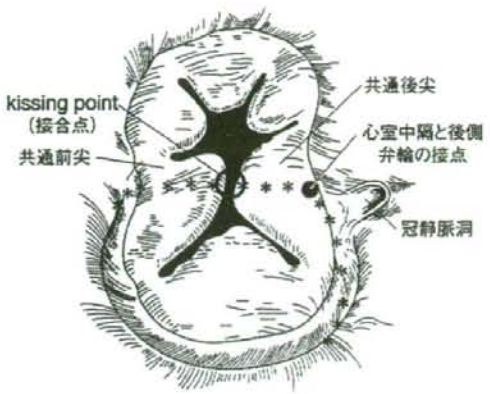

図3 共通房室弁と慶應義塾大学における心房中隔パッチ縫着線(＊)

チの上縁，共通前後弁尖の分割線，心房中隔パッチ下縁の順でU字縫合をかけ固定する(図2)．心房中隔パッチの後側縫合線をとるときの房室結節の避け方は右房側と左房側の2法があり，我々は後者を採用している(図3)．なお，房室結節の位置の指標はあくまでも心室中隔と後側弁輪の接点であり，しばしば左房側変位がみられる冠静脈洞開口部ではない．

次に，左側房室弁形成のために再び左室を充満させる．弁形成の内容はcleft閉鎖と必要に応じてKay-Reed弁輪縫縮である．原則としてcleftは全長にわたり閉鎖する．理由は，この部分の残存/再発閉鎖不全が再手術の原因のほとんどであるからである．最後に，心房中隔パッチを連続縫合で縫着して心内操作を終了する．心拍動再開を待って右房切開を閉鎖し，体外循環から離脱する．心房と心室にペーシングリード，肺動脈カテーテルを挿入して閉胸する．

移行型，部分型においては，基本的に心室中隔欠損を除いた後半部分と同等と考えてよい．

術式に関連した周辺知識

体外循環離脱後，血行動態が不安定なときには，経食道または経心表面心エコーで特に左側房室弁機能を評価することが肝要である．ECDは術後数日までの間に肺高血圧crisis(急激な発症：PH crisis)を引き起こすリスクが高いので，肺動脈圧を連続的に監視しながら，高濃度酸素，高換気量，筋弛緩による呼吸管理を必要な時間行うことにより，そのリスクを最小限とする．もしPH crisisを引き起こした場合には，一酸化窒素ガス吸入療法を行う．

体外循環

心内膜床欠損症手術における体外循環

1 標準的小児体外循環システム

1-1 人工心肺システム構成

Sorin Group Deutschland社(旧・STÖCKERT社)製人工心肺装置「S Ⅲ(インファントモデル)」(ポンプコンソール3基ベース)にて小児から成人の体外循環まで対応可能なシステムを構成している．ローラポンプはすべてダブルヘッドポンプ(直径85 mm)を使用する．送血ポンプは症例によりポンプヘッド部のチューブ径1/4インチ(流量≦1.5 *l*/min)と5/16インチ(流量≦2.0 *l*/min)を使用し，流量＞2.0 *l*/minの症例より遠心ポンプを使用する．脱血方法は全症例，陰圧吸引補助脱血とし，充填量の削減，操作性・視認性の向上を図っている．表1に体外循環の回路サイズと充填量を示す．また，このほかにV-V MUF用に「JMSマルチフローポンプMF-01」((株)ジェイ・エム・エス)を2基搭載する(図4)．

1-2 特徴

新生児・乳幼児は，成人に比べて毛細血管の透過性が高く，体外循環において水分・血漿成分が間質へ漏出しやすい状況にある．よって，

表1 慶應義塾大学における体外循環の回路サイズと充填量

患者区分(体重)	≦10 kg	≦15 kg	≦20 kg	≦30 kg	≦40 kg	>40 kg
回路区分	SSS	SS		S	M	L
ポンプ種類	ローラポンプ			遠心ポンプ		
ポンプチューブ[インチ]	1/4	5/16				
送血回路 [mm]	4.5	6	6	6	8	10
脱血回路 [mm]	6	6	6	8	10	10
充填量 [ml]	185	230	375	450	650	850
充填薬剤組成	血液充填(体重<7 kg)*			無輸血・無血液製剤充填(10 kg<体重)		
	赤血球濃厚液-LR®(RCC-LR) 140 ml			重炭酸リンゲル 適量		
	新鮮凍結血漿-LR®(FFP-LR) 120 ml			サリンヘス注射液®(ヒドロキシエチルデンプン7000) 5～10 ml/kg (最大500 ml)		
	20%アルブミン溶液 25 ml					
	20% D-マンニトール溶液 4 ml/kg			20% D-マンニトール溶液 4 ml/kg (最大300 ml)		
	ヘパリンナトリウム 2 ml					
	アルブミン充填(7 kg<体重<10 kg)					
	5%アルブミン溶液 150 ml					
	重炭酸リンゲル 50 ml					
	20% D-マンニトール溶液 4 ml/kg					
	ヘパリンナトリウム 2 ml					

＊ 重炭酸リンゲル液500 ml＋αで洗浄限外濾過処理を行う.

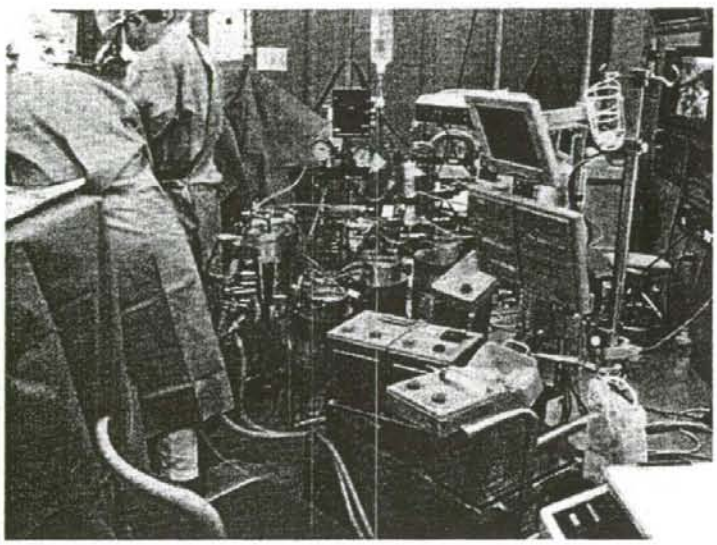

図4 慶應義塾大学における体外循環システム

体外循環中あるいは体外循環後の浮腫を軽減するためには，①希釈率を低くすること，②異物接触面積を少なくし，凝固線溶系，補体系，キニン・カリクレイン系，血小板，白血球，サイトカインなどの活性化あるいは亢進を抑えるために体外循環回路を小型化すること，③充填液中あるいは血中の有害物質を除去することが必要である[1]．当施設では，分離型ローラポンプの使用や陰圧吸引補助脱血を用いて他の脱血方法に比べ回路を短縮することで体外循環回路を小型化し，充填液の洗浄限外濾過処理，DUF，MUFを行うことで充填液中や血中の有害物質の

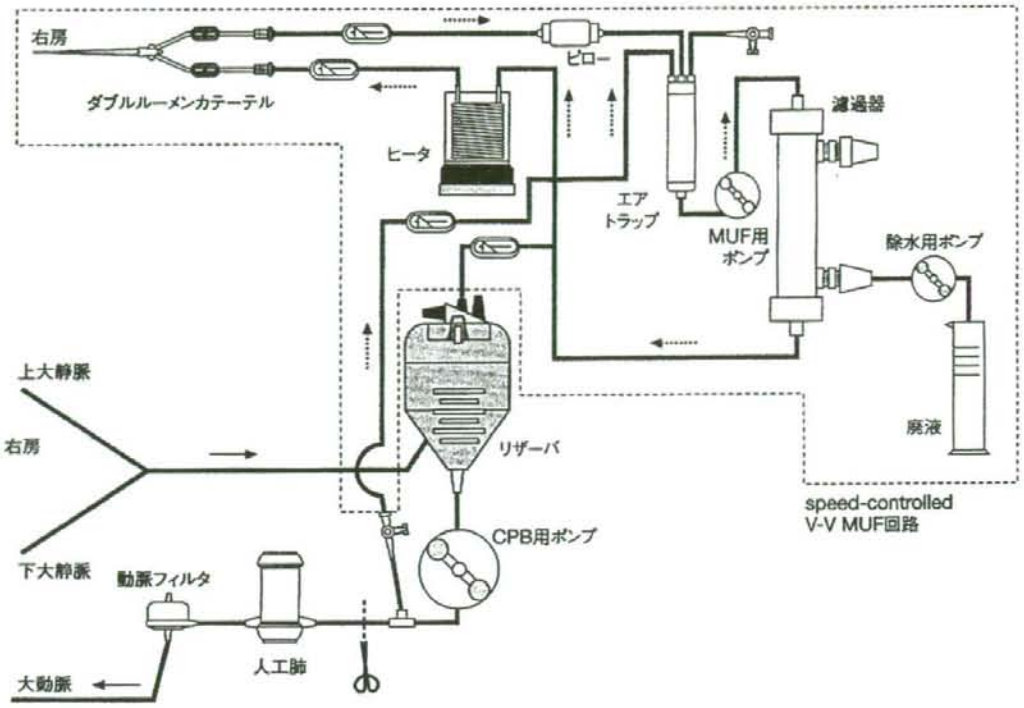

図5　MUF回路図

除去を行っている．

1）充填血洗浄・血液充填組成

赤血球濃厚液-LR®(RCC-LR)1単位(140 ml)，新鮮凍結血漿-LR®(FFP-LR)1単位(120 ml)，20%アルブミン溶液25 ml，20% D-マンニトール溶液4 ml/kg，ヘパリンナトリウム2 ml，重炭酸リンゲル500 mlで体外循環回路の充填を行う．充填液が十分に撹拌されたことを確認した後，ヘマトクリット(Ht) 25～30%になるように500 ml＋αの限外濾過を行い，カリウムをはじめとする電解質の補正および有害物質の除去を行う[2]．

2）陰圧吸引補助脱血

当施設では，1996年より成人体外循環に陰圧吸引補助脱血を導入し[3]，1999年以降，小児を含むすべての体外循環で陰圧吸引補助脱血を行っている．小児体外循環においては，落差脱血に比べ1～2サイズ細身のカニューレを使用し，体外循環開始時の陰圧を－30 mmHgとし，脱血量に合わせて適宜増減させる．最大陰圧を－80 mmHgとしているが，ほとんどの症例では－40～－50 mmHgの陰圧で必要脱血量を得ることができる．このとき，脱血回路へのエアの混入とコラップス*1に留意する．また，血液にかかる陰圧を正確に測定するために，脱血回路において陰圧の測定を行っている．

3）speed-controlled V-V MUF

当施設では，心房中隔欠損症(ASD)を除く20 kg未満の体外循環症例にV-V MUFを施行している．MUFの血液ポンプのほか，濾過にもローラポンプを使用しているため，濾液のスピードコントロールが容易[4]である(図5)．V-V MUF

＊1　コラップス
過陰圧などにより，脱血カニューレが血管壁に吸い付いてしまう現象．

用のカテーテルは透析用ダブルルーメンカテーテル(12 Fr)を右房より挿入し，血流量(Q_B)，濾過流量(Q_F)は体重に関係なく，Q_B 120 ml/min，Q_F 40 ml/min とし 15 分間行う．体外循環離脱と同時に MUF を開始し，置換液は体外循環回路内残血を使用，必要に応じて濾過型人工腎臓用補液，アルブミン製剤，重炭酸リンゲル液などを追加する．

2 心内膜床欠損症根治手術に対する体外循環

心内膜床欠損症(ECD)手術時の体外循環は次のように行う．

2-1 体外循環開始前

①充填血液の洗浄限外濾過処理の確認(Ht，カリウム濃度，pH など)．

②中心静脈圧(CVP)のラインよりヘパリンナトリウムを 3 ml/kg 投与する．2 分後，活性化凝固時間(ACT)の測定を行う．

③ACT が 200 秒を超えたら，サクションポンプを回し始め，体外循環中は ACT を 480 秒以上に維持する．送血のカニュレーションを開始する．

④送血回路接続後，拍動チェック，送りテストを行う．以降，血圧に留意しながら，脱血カニュレーション中の出血時など，必要に応じ適宜送血を行う．

表2 体外循環血流量

患者体重 [kg]	血流量 [ml/kg/min]
< 5	200 以上
5 ～ 8	180
8 ～ 10	160
10 ～ 12	150
12 ～ 15	130
15 ～ 20	120
20 ～ 30	100
30 ～ 40	80 ～ 100
40 ～ 50	70 ～ 90
50 <	60 ～ 80

2-2 体外循環開始時

①急激かつ異常な送血圧の上昇に注意しながら体外循環を開始する．

②陰圧を－ 30 mmHg から適宜増加させながら，予定灌流量まで血流量を上げていく(表 2)．予定流量が得られなければ術者に報告し，脱血カニューレの位置を調整する．

③呼吸(換気)の停止．

2-3 完全体外循環(図 6)

①上大静脈をスネアし，脱血量に変化がないか確認する．脱血量が減少するようであれば，上大静脈のカニューレの位置を調整する．

②下大静脈でも同様のテストを行う．上下大静

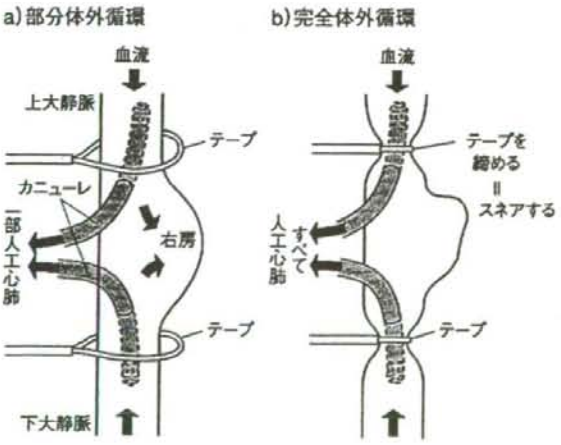

図6 部分体外循環と完全体外循環
人工心肺の開始時および離脱時には，生体での循環と人工心肺による循環の両方が行われる．このように，循環の一部分を人工心肺で担っている状態を部分体外循環(a)という．完全体外循環(b)は，開心操作を行うために上大静脈・下大静脈にそれぞれ通したテープで上下大静脈を締め(スネアするという)，上大静脈・下大静脈の血流を人工心肺に導き，体循環を人工心肺で維持する状態．

脈とも問題なければ完全体外循環に移行する.

2-4 大動脈遮断

①大動脈遮断時の送血圧の上昇に注意する.

②心筋保護液を注入(10 ml/kg)し, 心停止を得る. 20 kg 未満の症例では, 術野にてシリンジで注入する.

③局所心冷却法を施行する. 以降 10 分ごとに局所心冷却を行う.

④必要な薬液の投与, 輸液, 輸血などを行う.

⑤大動脈遮断解除直前に, 心筋浮腫軽減の目的で 20% D-マンニトール 2 ml/kg を投与する.

⑥体外循環離脱時, 直腸温で 35.5℃以上となるように, ゆっくりと復温・保温を開始する.

2-5 大動脈遮断解除～部分体外循環

①キシロカイン®(塩酸リドカイン, 4 mg/kg)を投与する.

②拍動の再開を確認・観察し, 必要であれば除細動を行う.

③徐脈であればペーシング, あるいはイソプレナリン塩酸塩 0.01 mg を投与し, 心拍数を観察する.

④経食道心エコー (TEE)ガイド下でルートおよびベントよりエア抜きを行う.

⑤必要であればカテコラミン・血管拡張剤などの投与を開始する.

2-6 体外循環離脱

①呼吸(換気)再開.

②塩化カルシウムを投与し, 体血管側に容量負荷を行う.

③TEE による壁運動, 房室弁の評価を行う.

④血圧, CVP を観察しながら徐々に血流量を下げる. 血流量が半分になったら下大静脈のカニューレを抜去する.

⑤MUF を準備する.

2-7 体外循環離脱後

①上大静脈のカニューレを抜去し, そこから MUF 用のダブルルーメンカテーテルを挿入し, 速やかに MUF を開始する.

②MUF の効果を観察しながら, 徐々に CVP を下げる. MUF 施行中は体温の低下に注意する.

③MUF 終了後, 投与ヘパリンナトリウムと等量のプロタミン硫酸塩を投与する.

3 ECD の体外循環のポイント

ECD における体外循環のポイントは次の通りである.

①ECD は完全型, 不完全型に大別されるが, その中間型や移行型も存在し, ECD のタイプ・症状により, 手術時期・患者体重に幅がある.

②当施設においては ASD, 心室中隔欠損症(VSD), ECD, ファロー四徴症(TOF)などの軽症例では, 体外循環中の最低 Ht 値を 20% 以上としているため, 7 kg を無輸血充填の限界としている.

③ECD の手術では, 完全型, 不完全型にかかわらず, 僧帽弁の逆流テストを行うことが多い[5]. 逆流テストの水分により体外循環中の血液の希釈が進むため, 患者の体重によっては積極的に除水を行う必要がある.

④体外循環中の灌流圧は 30 ～ 40 mmHg とし, クロルプロマジン塩酸塩を 1 ～ 6 mg/kg 分割投与するが, 30 mmHg を下回る場合は血流量を上げて対処する. 昇圧剤は使用しない. このことは尿量の確保においても重要である.

⑤小児開心術においては, 腎臓の未熟性ゆえに術後腎不全の危険性が高い. 術後腎不全の予防・術後の浮腫の予防のため尿量の確保は重要である. 体外循環開始時にフロセミド 5 ～ 10 mg を投与し, 10 ～ 15 分後, 尿量が 10 ml/kg/hr を下回るようであれば追加投与を行う.

⑥ECD の体外循環は, 軽度低体温～常温で行う.

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CIRCULATION VISUAL BEST

心臓血管外科テクニック

Ⅰ 弁膜症編

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CIRCULATION VISUAL BEST

MC メディカ出版

7. 肺動脈弁狭窄症，肺動脈弁閉鎖不全症

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1) 肺動脈弁狭窄症

病態

肺動脈弁狭窄（pulmonary stenosis：PS）症は，単独の先天性心疾患としてはその7～10%を占める．むしろ心房中隔欠損や末梢性PS症などの他の先天性心疾患との合併疾患として見られることが多く，右心系の閉塞性病変で最多の病変である．弁尖の癒合が成因であることが多い．肺動脈弁は円錐状またはドーム状の形状であり，その先端の開放部が狭小となっている．弁尖は癒合し成人例においては石灰化が見られる場合がある．弁尖が非常に厚くなっているだけで癒合がない場合には変性（dysplasia）による狭窄である．まれにリウマチ性の炎症やカルチノイド病変の波及がPSの原因である場合があり，またNoonan，Williams，Alagille症候群などの遺伝性，染色体性疾患と合併していることもある．また心房中隔欠損症などに代表されるような他の心房間，または心室間レベルでの短絡病変があった場合には機能的なPSを呈することがある．これは肺動脈弁を通過する血流が増加していることによって相対的に右心室からの血流駆出の障害となる現象であり，次第に右室圧が増加するので，心拍出量を維持するために右心室が肥大し，さらなる肺動脈弁および弁下レベルでの圧較差を生じていくことがある．

臨床経過

新生児期や乳児に有症状となるものの多くはcritical PSと呼ばれ，特有の病態生理，臨床経過および外科治療戦略を有し，弁膜症の範囲から逸脱するので，本稿では幼児期以降に発症したPSを対象とする．PSは通常は大動脈弁狭窄に比較すると耐性が高く，より良好な臨床経過を取る．現代では青年期成人期に達した患者寿命は健康人と同等と考えられる．Hayes[1]らの592人のさまざまな程度のPSの患者に対し，15年以上にわたって予後の追跡調査を行った報告によれば，手術を施行されなかったごく軽度の狭窄であれば全経過中を通じて無症状で有意な狭窄の進行は見られず，最大収縮期圧較差が25～49mmHgの患者のうちの20%と，圧較差が50mmHgの患者のうちの大部分が狭窄解除を要した．小児期にPSの診断がなされた患者が成人期に達している場合には，それが手術を受けているいないにかかわらず，無症状すなわちNew York Heart Association（NYHA）心機能分類でⅠないしⅡ度である[2]．

右心室から肺動脈への血液の駆出に対する障害は二次性の右室肥大をもたらす．右室肥大は右心室の構造上の特性から特に流出路で顕著になりやすいので，dynamicな狭窄としてこれを助長することがある．経過につれて運動耐性の低下，呼吸困難，右心不全徴候を呈することがある．右心不全は重度のPS症患者の死因のうち最多のものであり，多くは手術を行われなかった患者が30歳を過ぎてから見られるようにな