several samples were repeatedly genotyped using different arrays. All samples were scanned by at least 1 of 3 arrays, namely, Illumina HumanHap610Quad, Omni2.5-4, and Omni2.5-8 (see Supplementary Table 1, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). A total of 394,239 markers that were common across the 3 arrays were used for the current study. Genotyping quality was controlled by excluding single-nucleotide polymorphisms (SNPs) with a call rate below 95%, with minor allele frequency below 5%, and deviating from Hardy-Weinberg equilibrium ($P < 1.0 \times 10^{-7}$). Excluded from the analysis were 162 samples with a call rate <95%, 295 individuals estimated to have kinship within this population (PI-hat more than 0.35), and 7 ancestry outliers identified by principal component analysis, with HapMap Phase 2, release 28, data set as reference. A total of 83 individuals were excluded, because of having or being suspected of having connective tissue diseases from their answers to the questionnaire. As a result, 3,170 samples were analyzed for GWAS. Logistic regression analyses were performed by using positivity of ACPA and RF as dependent variables, each SNP as an independent variable, and age and sex as covariates.

**HLA imputation.** Alleles for HLA–DRB1 were imputed based on genotypes in the GWAS by using HLA–IMP2 (31). We imputed HLA–DRB1 alleles for 589 patients with RA for a test set as reported previously (28). Imputed HLA–DRB1 alleles were compared with genotyped HLA–DRB1 alleles and an algorithm for determining HLA–DRB1 alleles was established (See Supplementary Appendix A, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). Next, HLA–DRB1 alleles were determined based on the same algorithm for 932 healthy individuals as described previously (32), and compared with the genotyped HLA–DRB1 alleles. The algorithms for HLA–DRB1 based on imputation provided more than 93.5% of sensitivity and 99.8% of specificity for SE. HLA–DRB1 alleles were inferred for the 3,170 individuals in the current GWAS using the same algorithm.

**Correlation analysis.** Effect sizes of SNPs in the logistic regression analysis for the autoantibody positivity in the healthy population were compared with those in the association study for RA susceptibility, recruiting 1,237 cases and 2,087 controls in Kyoto University and previously described elsewhere (19,32). The 259,249 SNPs that were common across the current study and the previous study were pruned by linkage disequilibrium of $r^2 > 0.3$. As a result, there were 82,445 SNPs remaining for further analysis. Correlation analysis was performed by using Pearson's correlation coefficients with 8 intervals, according to the $P$ values in each study.

**Power analysis.** Power analysis was performed by an online power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/).

**Statistical analysis.** Logistic regression analyses in genetic studies were performed by Plink, version 1.07 (33). Other statistical analyses were performed using the R statistic system (http://www.R-project.org) or SPSS (version 18). We regarded $P$ values less than 0.005 as significant to assess correlations in a conservative manner. A stringent cutoff value of $P < 5 \times 10^{-8}$ was adopted for the GWAS.

## RESULTS

**Characteristics of ACPA and RF.** In the current study, 1.7% and 6.4% of the study population (n = 9,575) showed positive ACPA and RF, respectively (Tables 1 and 2). The distribution of titers is shown in Table 1. We also found 0.44% of subjects being positive for both ACPA and RF, and a significant association between ACPA and RF positivity ($P = 2.0 \times 10^{-23}$ in chi-square test [odds ratio (OR) 5.19 (95% confidence interval [95% CI] 3.62–7.44)]) (see Supplementary Table 2, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). The individuals who were positive for both ACPA and RF showed a significant correlation of the titers of these autoantibodies ($\rho = 0.60$) (see Supplementary Figure 1, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). When we analyzed effects of candidates of correlates on positivity of these autoantibodies, we did not observe a significant difference in positivity for RF and ACPA between men and women. We found that ACPA positivity increased with respect to older age ($P = 0.00045$ in logistic linear regression analysis), especially for those in their 70s ($P = 0.00062$) (Table 2). While people in their 50s showed an increase of RF positivity ($P = 5.4 \times 10^{-5}$) (Table 2), no linear effect of age on RF positivity was observed ($P = 0.093$ in logistic linear regression analysis). The associations between age and ACPA or increase of RF for those in their 50s were observed mainly in women (see Supplementary Table 3, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). Next, BMI, smoking, alcohol consumption, and serum level of CRP were analyzed for associations with ACPA and RF positivity. While we did not find

**Table 1. Distribution of titers in ACPA and RF in the general population***

| | No. | Ratio, % |
|---|---|---|
| ACPA (units/ml) | | |
| <4.5 | 9,408 | 98.3 |
| 4.5–13.5 | 100 | 1.0 |
| >13.5–45 | 33 | 0.3 |
| >45 | 34 | 0.4 |
| RF (IU/ml) | | |
| ≤20 | 8,961 | 93.6 |
| 20–60 | 486 | 5.0 |
| >60–200 | 87 | 0.9 |
| >200 | 41 | 0.4 |

* ACPA = anti–citrullinated peptide antibody; RF = rheumatoid factor.

**Table 2. Distribution and correlates of ACPA and RF in the general population***

| | | ACPA | | | RF | | |
|---|---|---|---|---|---|---|---|
| | No. | Positivity, % | *P*† | OR (95% CI)† | Positivity, % | *P*† | OR (95% CI)† |
| All subjects | 9,575 | 1.7 | – | – | 6.4 | – | – |
| Sex | | | | | | | |
| Male | 3,168 | 1.7 | – | Reference | 5.7 | – | Reference |
| Female | 6,407 | 1.8 | 0.84 | 1.04 (0.74–1.45) | 6.8 | 0.040 | 1.21 (1.00–1.45) |
| Age, years | | | | | | | |
| 30–39 | 2,315 | 1.3 | – | Reference | 5.4 | – | Reference |
| 40–49 | 1,339 | 1.3 | 0.94 | 0.98 (0.53–1.80) | 5.8 | 0.67 | 1.06 (0.79–1.44) |
| 50–59 | 1,886 | 1.8 | 0.23 | 1.35 (0.82–2.25) | 8.7 | $5.4 \times 10^{-5}$ | 1.64 (1.29–2.10)‡ |
| 60–69 | 3,012 | 1.8 | 0.12 | 1.43 (0.90–2.26) | 6.3 | 0.13 | 1.20 (0.95–1.52) |
| 70–75 | 1,023 | 3.0 | 0.00062 | 2.46 (1.45–4.15)‡ | 5.7 | 0.67 | 1.07 (0.77–1.49) |
| BMI, kg/m$^2$ | | | | | | | |
| 18.5–25 | 6,876 | 1.8 | – | Reference | 6.6 | – | Reference |
| <18.5 | 902 | 1.2 | 0.37 | 0.75 (0.39–1.42) | 6.7 | 0.88 | 1.02 (0.76–1.37) |
| 25–30 | 1,567 | 2.0 | 0.71 | 1.08 (0.72–1.63) | 5.5 | 0.15 | 0.84 (0.66–1.07) |
| ≥30 | 230 | 1.3 | 0.66 | 0.77 (0.24–2.51) | 7.0 | 0.72 | 1.10 (0.65–1.86) |
| Smoking | | | | | | | |
| Never | 6,219 | 1.7 | – | Reference | 6.7 | – | Reference |
| Ex-smoker | 1,961 | 2.0 | 0.21 | 1.36 (0.83–2.20) | 5.8 | 0.97 | 1.00 (0.77–1.31) |
| Active | 1,395 | 1.6 | 0.49 | 1.22 (0.69–2.16) | 6.2 | 0.46 | 1.11 (0.83–1.49) |
| 0 < BI ≤ 200 | 1,056 | 1.5 | 0.65 | 1.14 (0.65–2.02) | 5.0 | 0.23 | 0.83 (0.61–1.13) |
| 200 < BI ≤ 600 | 1,254 | 1.7 | 0.32 | 1.34 (0.75–2.39)‡ | 5.3 | 0.82 | 1.04 (0.75–1.43) |
| 600 < BI | 1,044 | 2.2 | 0.32 | 1.42 (0.71–2.83)‡ | 7.5 | 0.018 | 1.58 (1.08–2.30)‡ |
| Alcohol§ | | | | | | | |
| Never or past | 3,193 | 2.1 | – | Reference | 6.3 | – | Reference |
| Current, light | 1,883 | 1.8 | 0.66 | 0.91 (0.58–1.41) | 7.3 | 0.049 | 1.26 (1.00–1.60) |
| Current, moderate/heavy | 3,396 | 1.3 | 0.025 | 0.60 (0.38–0.95)§ | 5.8 | 0.84 | 1.02 (0.81–1.29) |
| CRP, mg/dl | | | | | | | |
| <0.1 | 1,587 | 1.1 | – | Reference | 5.4 | – | Reference |
| 0.1–0.3 | 3,350 | 1.7 | 0.27 | 1.36 (0.78–2.37) | 6.4 | 0.17 | 1.20 (0.92–1.58) |
| >0.3–1.0 | 3,235 | 1.5 | 0.64 | 1.14 (0.64–2.06) | 6.4 | 0.30 | 1.16 (0.87–1.53) |
| ≥1.0 | 1,403 | 3.0 | 0.0078 | 2.26 (1.22–4.17)‡ | 7.5 | 0.0087 | 1.53 (1.11–2.12)‡ |

* ACPA = anti–citrullinated peptide antibody; RF = rheumatoid factor; OR = odds ratio; 95% CI = 95% confidence interval; BMI = body mass index; BI = Brinkman's Index; CRP = C-reactive protein.
† Logistic regression analysis adjusting for sex and age (statistics for BMI, alcohol, smoking, and CRP level).
‡ Suggestive or significant associations mentioned in the main text.
§ Those that drink more than once a week are categorized as moderate/heavy.

any significant associations for BMI, smoking, and alcohol consumption (Table 2), high alcohol consumption showed a suggestive protective effect with ACPA positivity, consistent with the previous report from European populations showing a protective effect of alcohol consumption against ACPA-positive RA (34). Smoking showed a suggestive dose-dependent effect on ACPA production, and this effect was strengthened in condition with alcohol consumption (Table 2 and Supplementary Table 4, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). A high level of CRP showed suggestive associations with ACPA and RF positivity ($P$ = 0.0078 and 0.0087, respectively) (Table 2). Because smoking is the established environmental risk factor for seropositive RA, especially in men, we separately analyzed effects of smoking on ACPA and RF production in men and women. As a result, while we found a slight increase of positivity in male ever-smokers, the associations did not reach a significant level and the ORs were much lower than those for seropositive RA (Table 3).

The linear increase of ACPA positivity according to ages of individuals raised the possibility that the positive likelihood ratio (PLR) of having RA based on ACPA positivity differed according to the age groups. To address this point, we collected ACPA data from 2,067 patients with RA whose data on age at onset were available and calculated the PLR of having RA based on ACPA positivity. As a result, we found that the PLR of having RA decreased according to age (Supplementary Figure 2A, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). In particular, the group age >70 years demonstrated a significantly lower PLR of RA than the group ages 30–39 years ($P$ = 0.0033) (Supplementary Figure 2A, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/ acr.22385/abstract). When we analyzed RF positivity in the same manner, the PLR decreased in the group ages 50–59 years in comparison to the group ages 30–39 years, reflecting an increase of RF positivity in the general population ($P$ = 0.0013) (Supplementary Figure 2B, available in the online version of this article

**Table 3. Lack of significant association between smoking and ACPA or RF in men and women***

| All subjects | ACPA | | | | RF | | | |
|---|---|---|---|---|---|---|---|---|
| | No. | Positivity, % | *P*† | OR (95% CI)† | No. | Positivity, % | *P*† | OR (95% CI)† |
| Men | | | | | | | | |
| Never | 791 | 1.3 | – | Reference | 791 | 4.7 | – | |
| Ex-smoker | 1,399 | 2.1 | 0.25 | 1.62 (0.71–3.66) | 1,399 | 5.8 | 0.55 | Reference |
| Active | 978 | 1.5 | 0.38 | 1.50 (0.60–3.74) | 978 | 6.3 | 0.13 | 1.14 (0.74–1.76) |
| Ever (Ex and Active) | 2,377 | 1.9 | 0.27 | 1.54 (0.71–3.36) | 2,377 | 6.0 | 0.28 | 1.42 (0.91–2.23) |
| 0 < BI ≤ 200 | 436 | 1.6 | 0.67 | 1.31 (0.38–4.49) | 436 | 5.0 | 0.72 | 1.24 (0.84–1.84) |
| 200 < BI ≤ 600 | 943 | 1.5 | 0.53 | 1.35 (0.53–3.45) | 943 | 4.8 | 0.99 | 1.11 (0.62–2.00) |
| 600 < BI | 981 | 2.3 | 0.27 | 1.60 (0.70–3.69) | 981 | 7.6 | 0.10 | 1.00 (0.62–1.62) |
| Women | | | | | | | | 1.46 (0.93–2.29) |
| Never | 5,428 | 1.8 | – | Reference | 5,428 | 6.9 | – | |
| Ex-smoker | 562 | 1.8 | 0.60 | 1.22 (0.59–2.50) | 562 | 5.7 | 0.30 | Reference |
| Active | 417 | 1.7 | 0.51 | 1.31 (0.59–2.91) | 417 | 6.0 | 0.53 | 0.80 (0.52–1.22) |
| Ever (Ex and Active) | 979 | 1.7 | 0.44 | 1.25 (0.71–2.21) | 979 | 5.8 | 0.27 | 0.86 (0.53–1.38) |
| 0 < BI ≤ 200 | 620 | 1.5 | 0.73 | 1.13 (0.55–2.35) | 620 | 5.0 | 0.063 | 0.83 (0.59–1.15) |
| 200 < BI ≤ 600 | 311 | 2.3 | 0.20 | 1.68 (0.76–3.72) | 311 | 7.1 | 0.55 | 0.65 (0.42–1.02) |
| 600 < BI | 41 | 0 | 0.98 | NA | 41 | 7.3 | 0.73 | 1.15 (0.72–1.84) |

* ACPA = anti–citrullinated peptide antibody; RF = rheumatoid factor; OR = odds ratio; 95% CI = 95% confidence interval; BI = Brinkman's Index; NA = not applicable.
† *P* values and ORs in logistic regression analysis using age and alcohol drinking as covariates.

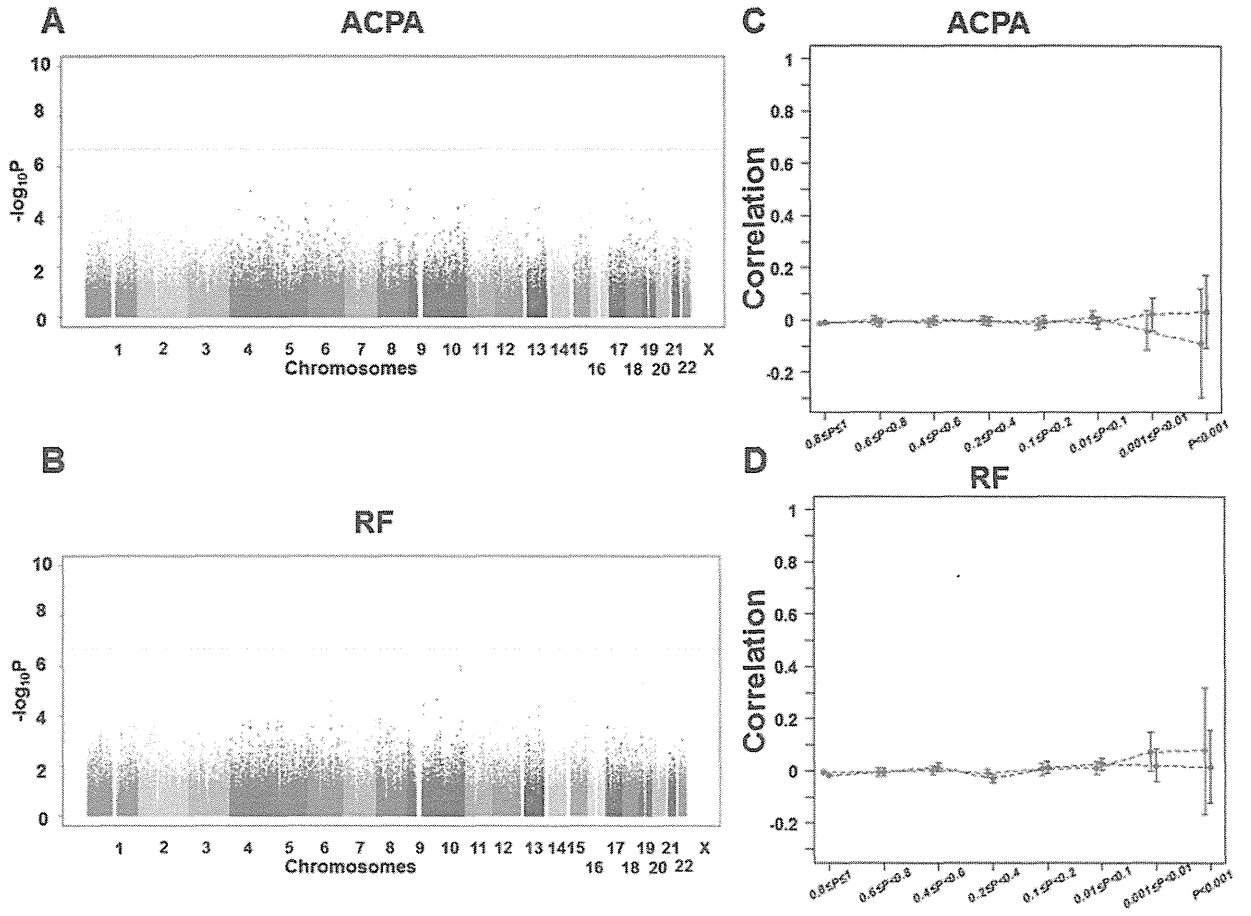


**Figure 1.** Genetic associations of single-nucleotide polymorphism (SNPs) and anti–citrullinated peptide antibody (ACPA) or rheumatoid factor (RF) positivity. Manhattan plot is shown for positivity of ACPA (**A**) or RF (**B**). No SNPs showed significant associations with positivity of ACPA or RF. Limited correlations of odds ratios in the SNPs of genome-wide association studies between RA susceptibility and positivity of ACPA (**C**) or RF (**D**). Blue broken lines indicate SNPs with *P* values in the range of x-axis for positivity of ACPA (**C**) or RF (**D**). Red broken lines indicate SNPs with *P* values in the range of x-axis for RA susceptibility. SNPs are pruned by $r^2 < 0.3$. The error bars indicate 95% confidence interval.

**Table 4. Lack of significant associations between positivity of ACPA or RF and combination of SE and smoking in the general population***

| | No. | ACPA | | | RF | | |
|---|---|---|---|---|---|---|---|
| | | Positivity, % | P† | OR (95% CI)† | Positivity, % | P† | OR (95% CI)† |
| SE (-) | 1,935 | 1.7 | – | Reference | 6.0 | – | Reference |
| SE (+) | 1,235 | 1.9 | 0.82 | 0.93 (0.47–1.81) | 6.8 | 0.95 | 1.01 (0.71–1.44) |
| All subjects | | | | | | | |
| SE (-) nonsmoker | 1,230 | 1.8 | – | Reference | 6.4 | – | Reference |
| SE (-) ex-smoker | 373 | 2.4 | 0.063 | 2.83 (0.94–8.46) | 4.3 | 0.16 | 0.58 (0.27–1.24) |
| SE (-) current smoker | 332 | 0.6 | 0.40 | 0.50 (0.10–2.47) | 6.0 | 0.64 | 0.84 (0.41–1.74) |
| SE (-) BI | – | – | 0.045 | 1.13 (1.00–1.27) | – | 0.24 | 1.05 (0.97–1.13) |
| SE (+) nonsmoker | 772 | 2.1 | 0.50 | 1.32 (0.59–2.98) | 7.0 | 0.89 | 0.97 (0.63–1.48) |
| SE (+) ex-smoker | 265 | 1.9 | 0.65 | 1.41 (0.31–6.33) | 7.2 | 0.75 | 0.88 (0.41–1.89) |
| SE (+) current smoker | 198 | 1.0 | 0.68 | 0.71 (0.14–3.63) | 5.6 | 0.44 | 0.68 (0.25–1.82) |
| SE (+) BI | – | – | 0.26 | 0.84 (0.62–1.14) | – | 0.48 | 0.96 (0.86–1.07) |
| Male | | | | | | | |
| SE (-) smoking (-) | 184 | 1.1 | – | Reference | 6.0 | – | Reference |
| SE (-) smoking (+) | 461 | 1.5 | 0.65 | 1.45 (0.30–7.07) | 5.2 | 0.51 | 0.75 (0.33–1.75) |
| SE (-) BI | – | – | 0.38 | 1.06 (0.93–1.20) | – | 0.24 | 1.05 (0.97–1.14) |
| SE (+) smoking (-) | 93 | 3.2 | 0.25 | 2.97 (0.48–18.44) | 2.2 | 0.20 | 0.36 (0.08–1.73) |
| SE (+) smoking (+) | 334 | 1.8 | 0.53 | 1.69 (0.33–8.52) | 6.0 | 0.51 | 0.74 (0.31–1.81) |
| SE (+) BI | – | – | 0.33 | 0.92 (0.78–1.09) | – | 0.23 | 0.93 (0.83–1.05) |
| Female | | | | | | | |
| SE (-) smoking (-) | 1,046 | 1.9 | – | Reference | 6.6 | – | Reference |
| SE (-) smoking (+) | 244 | 1.6 | 1.00 | 1.00 (0.32–3.15) | 4.9 | 0.28 | 0.61 (0.25–1.50) |
| SE (-) BI | – | – | – | – | – | 0.93 | 1.01 (0.80–1.27) |
| SE (+) smoking (-) | 679 | 1.9 | 0.81 | 1.10 (0.53–2.26) | 7.7 | 0.84 | 1.05 (0.65–1.69) |
| SE (+) smoking (+) | 129 | 0.8 | 0.44 | 0.44 (0.06–3.43) | 7.8 | 0.81 | 0.88 (0.30–2.61) |
| SE (+) BI | – | – | – | – | – | 0.24 | 1.18 (0.90–1.56) |

* ACPA = anti–citrullinated peptide antibody; RF = rheumatoid factor; SE = shared epitope; OR = odds ratio; 95% CI = 95% confidence interval; BI = Brinkman's Index.
† Logistic regression analysis adjusting for age, sex, and alcohol consumption or age and alcohol consumption for the analysis of all subjects and men or women, respectively. Results of logistic regression analysis adjusting for only age were shown for ACPA analyses of men, women, and subgroup with <5 subjects positive for ACPA. Linear regression analysis of BI was applied for subsets with >5 smoking subjects.

at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract).

**Genetic components.** Next we performed a GWAS in 3,170 healthy subjects to estimate common variants associated with positivity of ACPA or RF. The GWAS did not show population stratification in both studies ($\lambda \leq 1.00$). Both GWAS did not demonstrate significant associations ($P < 5 \times 10^{-8}$) in any markers, including the HLA locus (Figures 1A and B). Due to limitations of sample sizes with positive ACPA or RF in the current study and the possibility of multiple variants with low effect sizes associated with the phenotypes, it could be that truly associated SNPs were enriched in the SNP group with suggestive associations, even if we did not find markers with significant associations. As previous genetic studies have suggested that SNPs with low to middle effect sizes contribute to RA phenotypes beyond ethnicity (19) even if they did not show significant associations, we hypothesized that multiple common variants with low to middle effect sizes contributing to RA would be associated with ACPA or RF production in the general population and vice versa. Therefore, we performed correlation analyses to compare the effect sizes of SNPs between the RA study and the 2 GWASs. We used data of the RA GWAS that recruited 1,237 cases and 2,087 controls in Kyoto University, which was a part of a previously published meta-analysis in a Japanese population (19). As a result, we did not find significant correlations between RA susceptibility and RF or ACPA positivity, even in a set of SNPs showing $P$ values less than 0.001 in each GWAS ($P \geq 0.40$) (Figure 1C and D).

**HLA–DRB1 and positivity of ACPA and RF.** Since the association between SE and positivity of ACPA and RF in patients with RA is well established, we analyzed whether these associations were observed in the general population. We imputed HLA–DRB1 alleles in the 3,170 individuals by HLA–IMP2 based on the genome-scanning data (details shown in Patients and Methods). Imputation of SE showed more than 93.5% of sensitivity and 99.8% of specificity for the genotyped SE in the 2 independent sets. The association studies showed that SE was not significantly associated with ACPA and RF positivity ($P$ = 0.82 and 0.95, respectively) (Table 4). Because previous studies showed that associations between SE and positivity for ACPA and RF in patients with RA were strengthened in the smoking population, we classified our subjects into 3 groups according to smoking status and assessed effects of

**Table 5. Significant associations between high ACPA level and smoking status or smoking quantity***

| | | RF high | | | ACPA high | | |
|---|---|---|---|---|---|---|---|
| | No. | Positivity, % | *P*† | OR (95% CI)† | Positivity, % | *P*† | OR (95% CI)† |
| Smoking (-) | 6,219 | 1.2 | – | Reference | 0.6 | – | Reference |
| Smoking (+) | 3,356 | 1.5 | 0.57 | 1.16 (0.70–1.90) | 1.0 | 0.0019 | 3.01 (1.50–6.03)‡ |
| Smoking BI | – | – | 0.0066 | 1.08 (1.02–1.15)‡ | – | 0.00011 | 1.14 (1.07–1.22)‡ |
| SE (-) | 1,935 | 1.3 | – | Reference | 0.5 | – | Reference |
| SE (+) | 1,235 | 1.0 | 0.35 | 0.75 (0.42–1.37) | 0.6 | 0.46 | 1.33 (0.62–2.84) |

* Nonsmoking subjects without rheumatoid factor (RF) or anti–citrullinated peptide antibody (ACPA) were set as reference for the analysis of smoking. Subjects without shared epitope (SE) and RF or ACPA were set as reference for the analysis of SE. OR = odds ratio; 95% CI = 95% confidence interval; BI = Brinkman's Index.
† Logistic regression analysis adjusting for age, sex, and alcohol drinking, or age and sex for the analysis of smoking or SE, respectively.
‡ Suggestive or significant associations mentioned in the main text.

SE. We did not find significant associations in any of the 3 groups (nonsmoking, ex-smoking, and currently smoking) (Table 4) and smoking quantity. As a previous study suggested that male subjects with SE are more sensitive to smoking in ACPA production (35), men and women were analyzed separately. We found an increase of ACPA positivity in SE-positive groups both for male nonsmoking and smoking groups, but the associations did not reach the significant level (Table 4).

**Association between high level of ACPA and smoking or SE.** Because the distribution of ACPA or RF levels in subjects positive for these antibodies is different between healthy people and patients with RA (see Supplementary Table 5, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract), we focused on those individuals with high levels of ACPA or RF. While the decreased number of positive subjects made it difficult to conclude the association, we observed a significant association between smoking and high levels of ACPA with a comparable effect size to patients with RA ($P = 0.0019$, OR 3.01 [95% CI 1.50–6.03]) (Table 5). Further, smoking showed a dose-dependent association with high levels of ACPA ($P = 0.00011$). Although smoking did not show association with high levels of RF, we observed a suggestive dose-dependent effect of smoking on high RF production ($P = 0.0066$) (Table 5). We found that the association trend between smoking and high ACPA levels was enhanced in male subjects (Supplementary Table 6, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). On the contrary, we did not find associations between SE and high levels of RF or ACPA.

## DISCUSSION

In the current study, we showed positivity of ACPA and RF in the general population, analyzed correlates of these autoantibodies, and assessed genetic effects alone and in combination with smoking. This is the first study to quantify ACPA and RF in a large-scale healthy population to assess correlates. Although the positivity of ACPA in this study was comparable to that in the previous Turkish study (1.0% in 941 subjects) (36), the positivity of RF was slightly higher than those in the previous study that were highly variable (21,25,36). This high positivity of RF in the current study may be explained by the high proportion of female subjects who showed a suggestive increase of RF positivity compared to men, and the high proportion of subjects in their 50s who showed the highest positivity of RF among the groups. The 201 subjects excluded due to possibly having connective tissue diseases showed positivity of 27.9% and 34.8% for ACPA and RF, respectively, reflecting that many of them have rheumatic diseases (data not shown). Considering the prevalence of RA patients in the Japanese population (0.5–1.0%), the frequency of excluded subjects in the current study (approximately 2% of study subjects) seems reasonable. Therefore, it is less likely that patients with RA were missed for exclusion and enriched in the remaining subjects. The cutoff values of 26 and 45 IU/ml for RF would give 95% and 98% specificity in the current study, respectively. ACPA showed more than 98% specificity with the current cutoff value.

The positivity of ACPA and RF showed correlations even in the general population. Although the OR of being positive for both autoantibodies is lower than that in patients with RA (the 2,067 patients in this study: OR 24.79 [95% CI 17.84–34.45]; data not shown), the titers in subjects positive for both autoantibodies also showed a good correlation. These might suggest that both autoantibodies share common genetic and/or environmental risk factors.

ACPA and RF positivity did not show strong association with sex. As approximately 80% of RA patients are women (37), the lack of association suggests that factors other than sex are essential to produce ACPA and RF. We detected an age-dependent increase of ACPA positivity. This result corresponds to a previous report suggesting that detectable levels of antibodies against fillagrin, one of the important targets of citrullination of RA, tended to be found in the older population (38). RF showed an inverse U pattern in association with age. Menopausal term seems to correspond to the peak of RF positivity in female subjects. However, when we divided female subjects ages 50–59 years into 2 groups based on menopause, we did not find a significant difference in the positivity of RF ($P = 0.31$ and OR 0.78; data not shown). The same tendency of

increase of RF positivity in men cannot be explained by menopause. A prospective study to follow the same participants to observe the level of RF and compare RF positivity before and after menopause may lead to more clues for mechanisms underlying RF production. Previous studies showed that the elderly population has high frequency of RF in Europe and the US (24,25). Men showed the suggestive association between aging and RF production in this study. The difference between populations may suggest that different environmental factors play a role in autoantibody production. In fact, a recent twin study analyzing ACPA revealed that large parts of variance of ACPA can be explained by nonshared environmental factors (39). Different PLRs suggest that when individuals were incidentally found to be positive for these autoantibodies, ACPA in particular, the likelihood of having RA or having risk of RA is different based on age.

Analysis of candidates of correlates for ACPA and RF resulted in a positive association between high levels of CRP and ACPA or RF positivity. While this suggests an association between the production of these autoantibodies with preclinical inflammation, the current cross-sectional study could not conclude whether the production is a cause or a result. Other candidates for correlates were not associated with ACPA or RF. Many studies recruiting RA patients have shown that smoking is a strong environmental factor to produce ACPA and to develop RA (17,35). In our study, the associations between smoking and ACPA or RF positivity are not significant, while we observed significant or suggestive dose-dependent effects of smoking on high levels of ACPA or RF, respectively. A previous European study showed an association between active smoker and RF production in a healthy population (23). Since the median BI was 370 in the ever-smokers in the current study, equivalent to 1 pack a day for approximately 18 years, the less amount of smoking may contribute to a low effect of smoking status on autoantibody production in the current study. Previous studies revealed that ever smoking showed an OR of approximately 3 for male seropositive RA and 1.3 for female (18). Based on the seropositivity of nonsmokers, the current study is powered 100% in men and 68% in women to detect the effects with an OR of 3 and 1.3, respectively, at a level of $P = 0.05$. These results suggest that smoking is not associated with production of RF and ACPA at low levels but may be associated with the production of these antibodies at high levels in a healthy population. Although the current study cannot conclude that the association between smoking and high ACPA or RF is true due to the limited number of positive subjects, it is feasible to increase the number of healthy subjects. It will also be interesting to analyze smoking effects on low ACPA or RF levels in patients with RA. Isotypes of RF and ACPA were not quantified in the current study. Detailed classification of RF and ACPA would reveal specific associations of correlates, especially smoking.

While a recent twin study showed that heritability of ACPA was 0.23 (39), GWAS for positivity of RF or ACPA resulted in no significant signals, including the HLA locus. Our study had a power of 0.99 to detect an SNP with allele frequency of 0.4 (SE-positive ratio in healthy subjects) and an OR of 2.0 associated with 7% of frequent phenotype at a level of $P = 0.00001$. Our study also had a power of 0.42 to detect an SNP associated with 1.6% frequent phenotype at a level of $P = 0.01$. The imputed SE showed an OR of 1.15 for both ACPA and RF. Considering an OR of 2.0–3.0 in patients with RA for positivity of RF or ACPA in the previous studies, the current study indicates that SE was not similarly associated with ACPA and RF production in the general population as in RA patients. Furthermore, we did not observe associations between SE and high ACPA or RF. ACPA and RF production may need other factors than SE, such as chronic inflammatory stimulation. While the male population showed suggestive associations between SE and ACPA production, the limited number of the positive subjects did not allow us to draw any conclusions. Common direction of SE and smoking for ACPA production in men suggests that men are more sensitive to these risk factors than women. Although previous studies reported that HLA–DRB1*09:01 had a lowering effect of ACPA in the Japanese (27), we did not find a significant effect of *09:01 on ACPA positivity (Supplementary Table 7, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract). HLA–DR13, especially DRB1*13:01, shows a negative association with ACPA-positive RA in the European population (40). Although we did not find ACPA-positive subjects with DRB1*13:01 in the current study (Supplementary Table 7, available in the online version of this article at http://onlinelibrary.wiley.com/doi/10.1002/acr.22385/abstract), low frequency of DRB1*13:01 made it difficult to conclude the association between ACPA production and DRB1*13:01. No associations were detected between DR13 and ACPA production either. The negative results of genetic correlation analyses suggest that RA susceptibility and ACPA or RF production in the general population share limited genetic components in spite of wide confidence intervals of SNPs due to low power of the current study.

Because disease-specific autoantibodies, including ACPA and RF, were shown to appear several years before the diagnosis of the diseases (21,41–45), it will be interesting to follow the current study population to observe whether or not they will develop RA. It will also be very interesting to validate our results in other populations and compare the associations among the different populations.

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### AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Terao had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Terao, Ohmura, Ikari, Kawaguchi, Takahashi, Setoh, Nakayama, Kosugi, Sekine, Tabara, Taniguchi, Momohara, Yamanaka, Yamada, Matsuda, Mimori.

**Acquisition of data.** Terao, Ohmura, Ikari, Kawaguchi, Takahashi, Setoh, Nakayama, Kosugi, Sekine, Tabara, Taniguchi, Momohara, Yamanaka, Yamada, Matsuda, Mimori.
**Analysis and interpretation of data.** Terao, Kawaguchi.

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# Histological Changes of Pulmonary Arteries Treated by Balloon Pulmonary Angioplasty in a Patient With Chronic Thromboembolic Pulmonary Hypertension

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A 41-year-old man was referred to our hospital for the treatment of chronic thromboembolic pulmonary hypertension (CTEPH). On admission, he was in World Health Organization functional class III. Right heart catheterization demonstrated that pulmonary arterial pressure (systolic/diastolic/mean) was 140/42/71 mm Hg, cardiac index was 1.6 L/min/m2 and pulmonary vascular resistance was 1663 dyn ·s·cm$^{-5}$. The patient had severe pulmonary hypertension and was considered inoperable because of peripheral organized thrombi and coexisting seminoma; therefore, balloon pulmonary angioplasty (BPA) was performed. After BPA, pulmonary angiography showed improvement of pulmonary arterial flow (Figure 1A). Pulmonary arterial pressure was 108/42/67 mm Hg and cardiac index was 2.5 L/min/m$^2$ when he returned to the cardiac care unit after BPA. His condition temporarily improved; however, 2 hours later, it deteriorated because of reperfusion pulmonary injury and gastrointestinal bleeding. He required mechanical ventilation and percutaneous cardiopulmonary support. To improve hemodynamics, another session of BPA was tried 9 days later (Figures 2A and 3A) and pulmonary arterial pressure seemed to improve to 83/48/58 mm Hg. However, he died from right heart failure on day 26 after BPA despite intensive care.

Histological analysis of the autopsy specimens showed recanalized thrombi in the bilateral elastic pulmonary arteries (Figure 4). Diffuse pulmonary arterial medial and intimal thickening were also observed in muscular pulmonary arteries. These findings are consistent with CTEPH and the diagnosis was confirmed. The arterial media was dissected near the lamina elastica interna by BPA (Figures 1B, 2B, and 3B). The organized thrombi were forced to one side and the dissection formed pseudovascular spaces that configured new lumina, which were larger than the original channels. Newly formed intima was observed on the inner surface of these pseudovascular spaces (Figures 1B-c, 2B-b, and 3B-b, black arrowheads).

According to the guidelines for the diagnosis and treatment of pulmonary hypertension, pulmonary endarterectomy is a standard therapy for patients with CTEPH with proximal thrombi.[1] However, not all patients can undergo this curative surgery because of the presence of thrombi in distal pulmonary arteries, difficulty of the operation, or comorbidities. We have reported that BPA could improve hemodynamics in inoperable patients with CTEPH.[2] However, it is unknown how pulmonary arteries are changed by BPA and the mechanism by which hemodynamic improvement is achieved, although thrombi are not removed from the affected arteries. We have recently reported a case in which we were able to examine a pulmonary artery in a single lobe after BPA.[3] A pathological examination of the dilated lesion showed that the vascular lumen was dilated by a small incision and compression of the thrombi without dissection. In contrast, the histology of the present case demonstrated that the lumina were dilated by the dissection at a plane in the media (Figures 1B, 2B, 3B, and 5). Dissection at this plane is exactly what expert surgeons do in pulmonary endarterectomy. Importantly, this dissection was not obvious and was not recognized by pulmonary angiography just after BPA (Figures 1A-b, 2A-b, and 3A-d).

There was also a lesion where vascular lumen was dilated by a small incision without dissection in the present case (Figure 3B-b, white arrow), which is similar to our previously reported case. There is a difference in the angiographic lesion types between the pulmonary arteries where dissection occurred and did not occur. Lesion types of each pulmonary artery in CTEPH are determined by abnormal angiographic patterns: pouching defects, webs or bands, intimal irregularities, abrupt vascular narrowing, and complete vascular obstruction.[4] Although we do not know whether mechanism of dilating pulmonary artery differ depending on angiographic lesion type, at least, web lesions in the present case (Figures 1A, 2A, and 3A, black arrows) were dilated by dissection made by BPA and band lesion was dilated without dissection.

In BPA, complete removal of organized thrombi is impossible but the present case suggested that dissection and compression

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of the thrombi would help improve blood flow and ultimately reduce pulmonary arterial pressure and vascular resistance. This case also suggests that in performing BPA, we need to be aware that we might dissect the pulmonary arteries, as in pulmonary endarterectomy, and take care not to make a tear in the arteries, which could cause massive hemorrhage. Taking it into consideration, it would be better not to use a balloon with a large diameter compared with the lesion diameter. This case provides insight into the mechanisms responsible for improvement of hemodynamics and the risk of causing vascular injury in BPA.

## Disclosures

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KEY WORDS: angioplasty ■ pathology ■ pulmonary hypertension ■ thrombosis

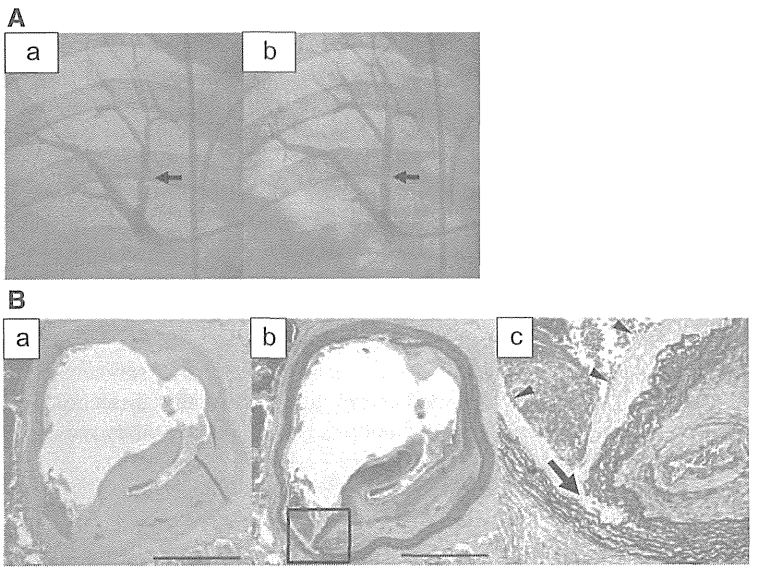


**Figure 1.** Representative images of pulmonary angiography and histology of pulmonary arteries of the upper lobe of the right lung. **A**, Angiographic images before (a) and after (b) BPA. A web lesion of the right pulmonary artery (arrow) was treated by BPA. Angiography after BPA showed a dilated vessel and increased flow in the distal arteries. Arrows indicate the areas where specimen for **B** was obtained. **B**, Histology of a pulmonary artery treated by BPA (arrow in **A**). This lesion was treated by BPA at the initial session and second session (26 and 17 days before death). (a) Hematoxylin–eosin stain, low magnification. A large lumen was formed in a pulmonary artery by BPA. Bar, 1 mm. (b) Elastic tissue stain revealed that the dissection occurred in the media. Bar, 1 mm. (c) Elastic tissue stain. High magnification of the dissection site (square in **B**-b). Dissection occurred in the middle of media (arrow). Intima was newly formed on the inner surface (arrowheads). One of recanalized lumina in organized thrombi was also seen on the right.

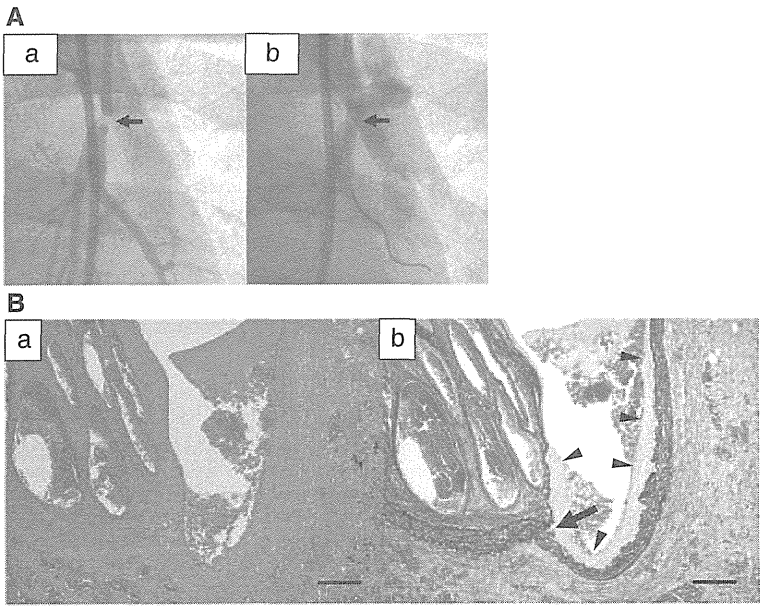


**Figure 2.** Representative images of pulmonary angiography and histology of pulmonary arteries of the lower lobe of the left lung. **A**, Angiographic images before (a) and after (b) BPA. After BPA, a web of the pulmonary artery (arrow) was successfully treated and increased flow is shown in the distal arteries. **B**, Histology of a pulmonary artery treated by BPA (arrow in **A**). This lesion was treated by BPA at the second session (17 days before death). (a) Hematoxylin–eosin stain. A large lumen in the middle and several small lumina are observed in the left. A large lumen is thought to be made by BPA. Bar, 200 μm. (b) Elastic tissue stain. Dissection occurred in the media near the lamina elastica interna (arrow). A pseudovascular space, made by BPA, was enlarged and compressed microchannels against the arterial wall. Newly formed intima (arrowheads) was observed on the inner surface of the dissection lumen. Bar, 200 μm.

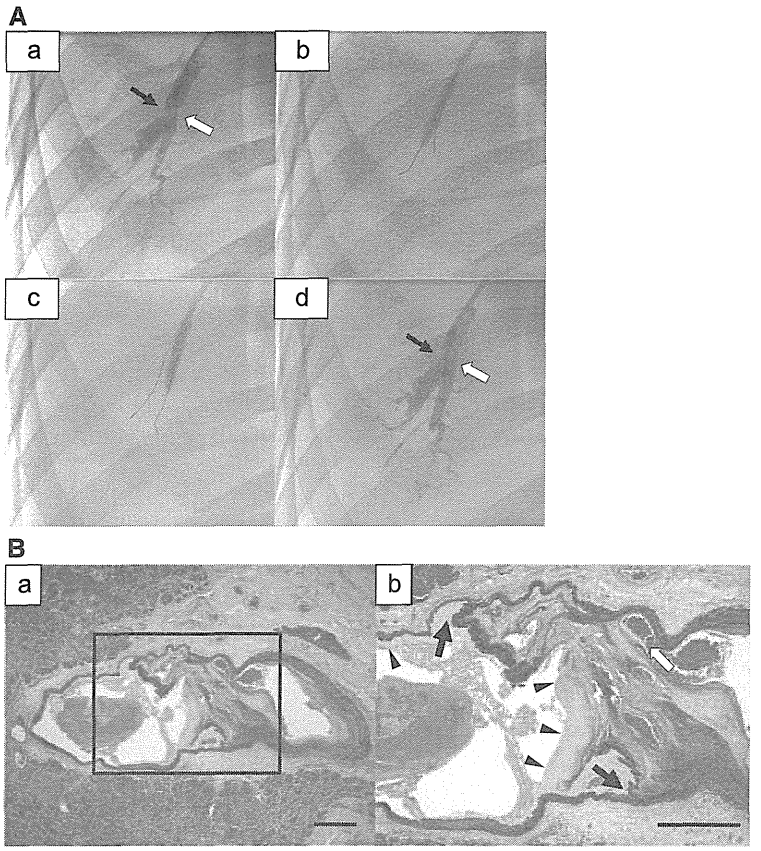


**Figure 3.** Representative images of pulmonary angiography and histology of pulmonary arteries of the lower lobe of the right lung. **A**, Angiographic images before (a), during (b and c) and after (d) BPA. A web (b) and band (c) lesions of the right pulmonary arteries were treated by balloon pulmonary angioplasty (BPA). Angiography after BPA showed vessels were dilated at the site of angioplasty. Arrows indicate the areas where specimen for **B** was obtained. **B**, Histology of a pulmonary artery treated by BPA 17 days before death. (a) Elastic tissue stain, low magnification. A specimen was made at bifurcation by a horizontal sectional view. Bar, 1 mm. (b) High magnification of a square in **B**-a. In a pulmonary artery with web lesion treated by BPA (on the left), dissection occurred in the media under the lamina elastica interna (black arrows). Newly formed intima (arrowheads) was observed on the inner surface. A large pseudovascular space was made by BPA and pre-existing microchannels are compressed toward an adjacent artery. The pulmonary artery with band lesion (on the right) was treated by BPA. A small incision (white arrow) is observed. This artery was dilated not by dissection but by enlargement of a preexist lumen. Bar, 1 mm.

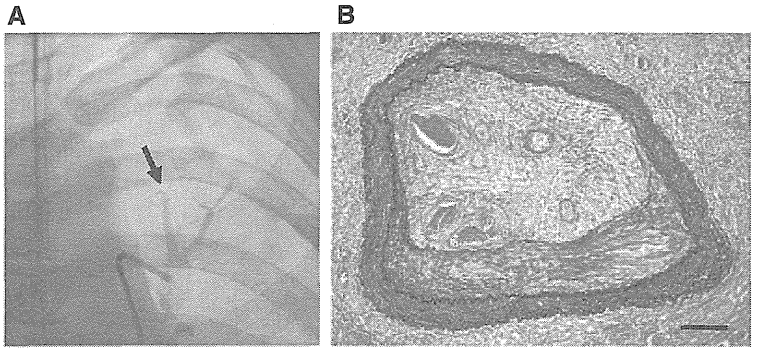


**Figure 4.** Representative images of pulmonary angiography and histology of pulmonary arteries of the upper lobe of the left lung. **A**, An angiographic image of pulmonary arteries where BPA was not performed. Complete obstruction (arrow) is shown in the distal artery. **B**, Histology of a pulmonary artery (arrow in **A**). Elastic tissue stain. The elastic pulmonary artery, where BPA was not performed, exhibits luminal stenosis and an organized thrombus with small recanalized channels. Bar, 200 μm.

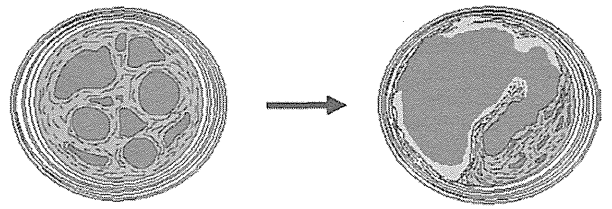

**Figure 5.** Schematic diagram of proposed mechanism of balloon pulmonary angioplasty (BPA) dilating pulmonary occlusive lesions. Structure of pulmonary artery with organized thrombus (green) and recanalized channels (**left**) is thought to be changed by BPA (**right**). The lumen is opened wide by dissection in the medial wall and the organized thrombus is compressed to one side. Newly formed intima (blue) covers the inner surface of the dissected pulmonary artery.

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INTERNAL MEDICINE

□ CASE REPORT □

# Pulmonary Microvascular Remodeling after Balloon Pulmonary Angioplasty in a Patient with Chronic Thromboembolic Pulmonary Hypertension

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## Abstract

We herein report a case of peripheral type chronic thromboembolic pulmonary hypertension treated with medical therapy and subsequent balloon pulmonary angioplasty (BPA). After a series of BPA procedures, the patient's hemodynamics almost completely normalized. The patient was later diagnosed with lung carcinoma, and the vasculature of the resected lung demonstrated intimal thickening and luminal stenosis in the pulmonary arteries in both the areas where BPA was performed and not performed, in spite of a marked reduction in pulmonary arterial pressure. The present case is the first report on the histology of the pulmonary vasculature following BPA.

**Key words:** chronic thromboembolic pulmonary hypertension, pathology, angioplasty

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## Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH) is a disease caused by elevation of pulmonary vascular resistance, primarily due to the presence of organized thrombi in the pulmonary arteries. Although patients with thrombi in the main pulmonary arteries are good candidates for pulmonary endarterectomy, the outcomes of this potentially curable surgery are poor in patients in whom obstruction is present in the distal subsegmental pulmonary arteries. The microvascular changes that occur in vascular lesions of CTEPH are similar to those observed in pulmonary arterial hypertension (PAH) (1). Based on these findings, various PAH-specific drugs have been used to treat CTEPH; however, the results have so far been unsatisfactory (2). Balloon pulmonary angioplasty (BPA) has been reported to be effective in reducing the pulmonary artery pressure (PAP) in affected patients (3). We previously refined the procedure of the reported BPA and recently reported its efficacy in inoperable patients with CTEPH (4).

## Case Report

A 53-year-old man began to experience dyspnea on exertion in 2001. Right heart catheterization revealed a mean PAP of 45 mmHg, and he was diagnosed with idiopathic PAH at a nearby hospital (Fig. 1A). The patient's dyspnea deteriorated, and the continuous intravenous infusion of epoprostenol was started in 2002. In August 2008, the patient was admitted to our hospital due to exacerbation of dyspnea and hemosputum. A chest CT scan revealed ground grass opacity in the left lower lobe, and alveolar hemorrhage was suspected (Fig. 2A). Prior to this time point, the patient had been admitted for alveolar hemorrhage several times due to thrombocytopenia and likely as a result of the high dose of epoprostenol. Patients are reportedly susceptible to alveolar hemorrhage when the epoprostenol dose is >28 ng×kg$^{-1}$×

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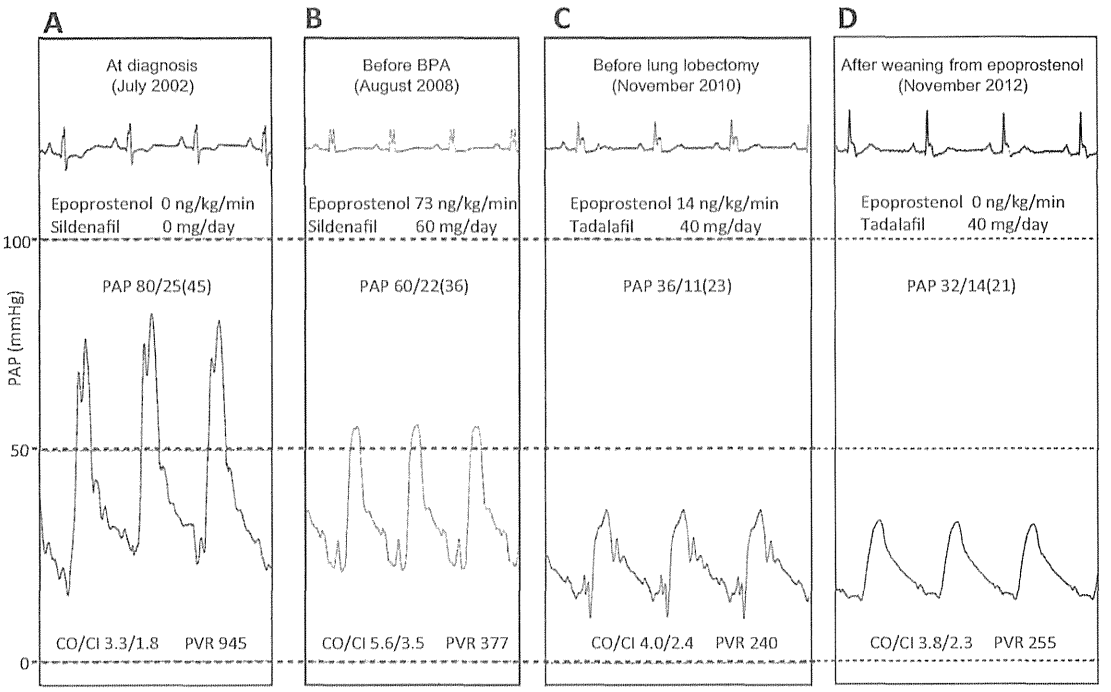


**Figure 1. Treatment and hemodynamics in the present case. The pulmonary artery pressure (PAP) was partially decreased by medical treatment and almost normalized with balloon pulmonary angioplasty (BPA). PAP (systolic/diastolic/mean) (mmHg); CO/CI (L/min/L/min/m$^2$), cardiac output/cardiac index; PVR (dyne/sec/cm$^5$), pulmonary vascular resistance**

$min^{-1}$ because epoprostenol has a potent inhibitory effect on platelet aggregation (5). Exercise avoidance, oxygen inhalation and the administration of carbazochrome sodium sulfonate hydrate and tranexamic acid improved the patient's condition and CT findings. After the alveolar hemorrhage had resolved, no abnormal shadows were detected on CT scans, and the patient underwent right heart catheterization. The mean PAP decreased from 45 to 36 mmHg following treatment with epoprostenol and sildenafil (Fig. 1B). The patient's desaturation and exertional dyspnea were severe, and the diagnosis was reevaluated. A perfusion lung scan revealed multiple segmental defects (Fig. 2Ba). Pulmonary angiography disclosed multiple lesions of obstruction and narrowing of the pulmonary arteries, compatible with a diagnosis of CTEPH (Fig. 2Ca, c, e). Because the lesions were limited to segmental or smaller arteries with no thrombi in the bilateral main pulmonary arteries, the diagnosis was corrected to peripheral type CTEPH. We then performed BPA, targeting A7 and A9 of the left lobe (Fig. 2Cb, d). The mean PAP decreased to 30 mmHg immediately after angioplasty. BPA was repeatedly performed, targeting A1, 2, 8, 9 and 10 of the right lobe (Fig. 2Cf) and A1+2, 3 and 4 of the left lobe in a total of four sessions. Finally, after performing BPA to A1 and 2 of the right lobe (Fig. 3A), the mean PAP decreased to 23 mmHg and the dose of epoprostenol was gradually reduced to 14 $ng \times kg^{-1} \times min^{-1}$ due to the patient's high cardiac output with a low mean PAP (Fig. 1C).

In November 2010, after another session of BPA, a chest CT scan was performed as a routine examination to check for reperfusion pulmonary injury, a complication of BPA. The scan revealed a nodular lesion in S3 of the right upper lobe (Fig. 2D). A lung biopsy specimen demonstrated squamous cell carcinoma, and lobectomy of the right upper lobe was successfully performed. A lung specimen demonstrated organized thrombi with recanalized channels in the segmental arteries, compatible with a diagnosis of CTEPH (6). The organized thrombi were forced to one side, and the arterial lumen was dilated after BPA (Fig. 3B). Arterioles of <100 μm in diameter showed intimal and medial thickening, although no complete obstruction was observed. Such pulmonary microvascular remodeling was found not only in S3, where BPA had not been performed, but also in S1 and S2, where BPA had been performed (Fig. 3C, D). Due to the sufficient improvement in the patient's hemodynamics after another session of BPA and the improvement in the perfusion lung scan findings (Fig. 2Bb), the patient was finally weaned off from epoprostenol in April 2012. The pulmonary hypertension was well controlled eight months after weaning from epoprostenol therapy (Fig. 1D).

## Discussion

The present case is the first report of the histology of the pulmonary vasculature after BPA that resulted in a remarkably decreased mean PAP to near the normal range. The immediate decrease in the PAP led us to believe that there was no small vessel disease in this patient. However, the analysis

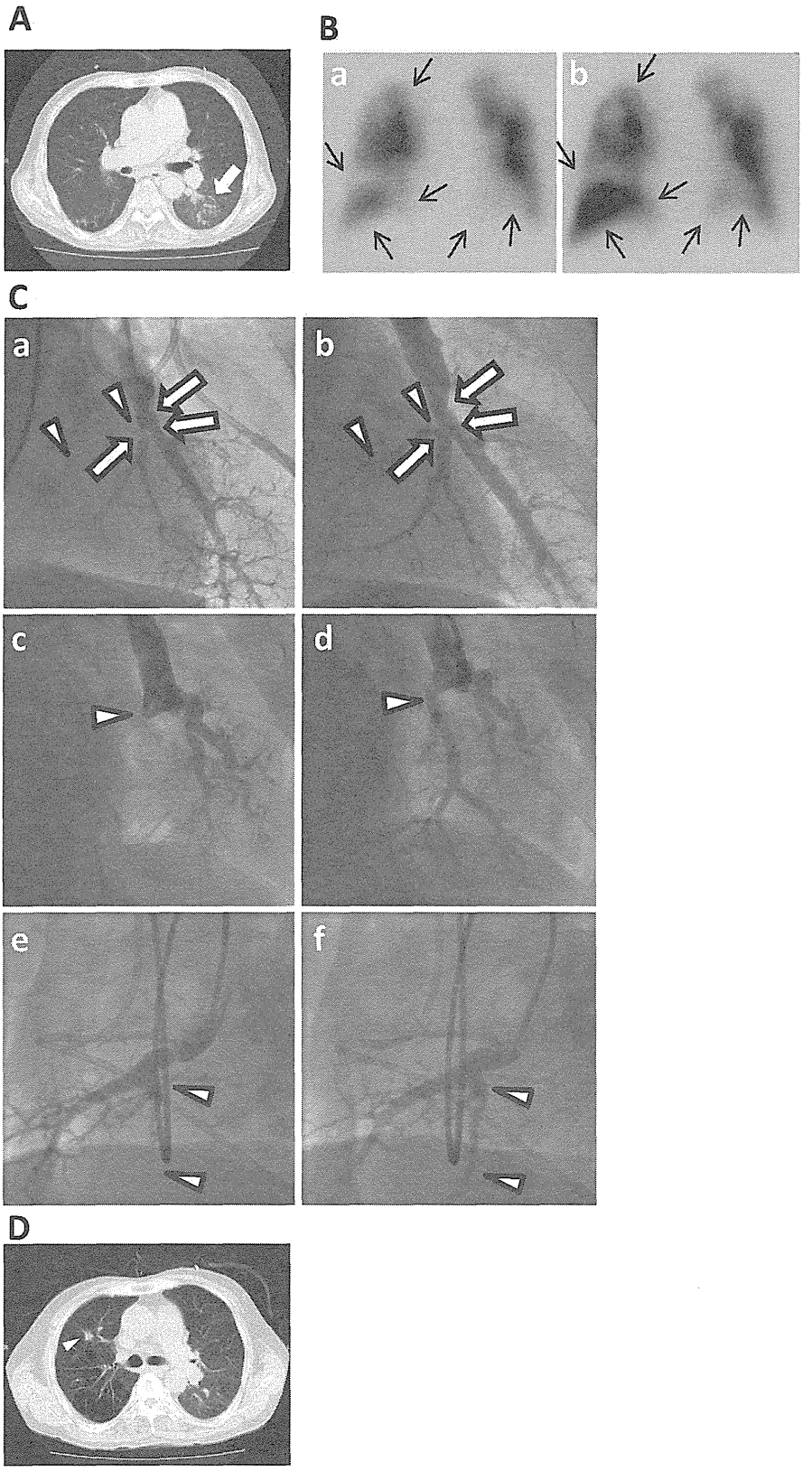


**Figure 2.** **Imaging tests in the present case. A: Ground glass opacity in the left lower lobe (arrow) suggested alveolar hemorrhage in August 2008. B: Perfusion lung scans obtained before (a) and after (b) balloon pulmonary angioplasty (BPA). Segmental defects (arrows) improved after BPA. C: Pulmonary angiography before (a, c, e) and after (b, d, f) BPA. Ring-like stenosis (arrows) was treated with BPA, and the flow was increased. Complete obstruction of the pulmonary arteries (arrowheads) was visualized on angiography after BPA. D: A nodular shadow (arrowhead) appeared in November 2010.**

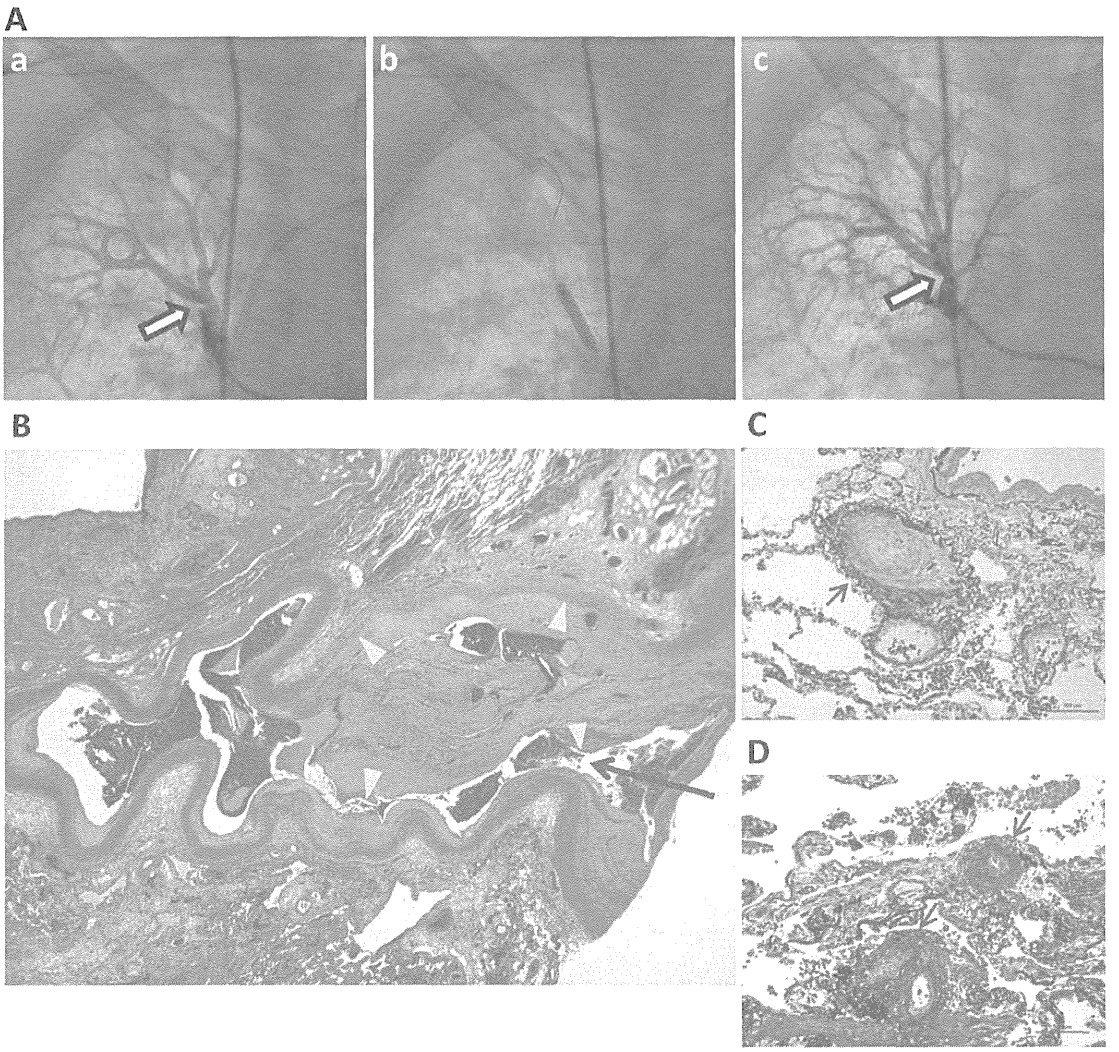


Figure 3. A: Angiograms obtained during balloon pulmonary angioplasty (BPA). a: Stenosis in the segmental branch of A1 (arrow). The red and blue circles indicate the areas from which the specimens presented in Fig. 3C, D were obtained. b: Balloon inflation at A1. c: An angiogram obtained immediately after BPA shows a dilated A1 (arrow) and improved runoff of the peripheral arteries. B-D: Histology of the resected lung lobe. B: A pathological image of the dilated lesion indicated by the arrow in Fig. 3A. The hilar pulmonary artery exhibits an organized thrombus (yellow arrowheads) with recanalized channels forced to one side of the vascular lumen and a lumen newly formed by BPA (blue arrow)(Hematoxylin and Eosin staining, original objective magnification ×4). C: One of the muscular-type pulmonary arteries in the right S1 (red circle in Fig. 3Aa) where BPA was performed (diameter: approximately 100 μm, blue arrow). Luminal stenosis and multiluminal recanalization were observed (Elastic tissue stain, original objective magnification ×20). D: The muscular type pulmonary arteries (blue arrows) of the right S3 (blue circle in Fig. 3Aa), where BPA was not performed, also showed similar changes (Elastic tissue stain, original objective magnification ×20).

of the lung specimen revealed that pulmonary microvascular remodeling was still present in the pulmonary arteries in the resected right upper lobe, in both the areas where BPA was and was not performed. One reason for this apparent discrepancy may be the mild degree of vascular remodeling. Although intimal and medial thickening was observed, no complete obstruction was observed in the small pulmonary arteries in the present case. Furthermore, we examined the pulmonary vasculature in the single resected lobe. It is possible that the degree of microvascular remodeling varies among lobes in the same patient. Another possibility is the difference in the type of cells constituting the vascular remodeling and mechanisms underlying CTEPH and PAH (7, 8). Distal pulmonary vasculopathy in patients with CTEPH develops in both the occluded and non-occluded pulmonary vascular bed and is characterized by lesions considered typical for idiopathic PAH pathologically (9). However, this may differ in the physiological setting due to the

distinct characteristics of cells constituting the vascular wall.

Vascular remodeling reportedly remains at autopsy in patients who have died of other causes years after undergoing successful pulmonary endarterectomy (10). Considering that pulmonary endarterectomy cannot directly resolve microvascular remodeling, immediate hemodynamic normalization after surgery is due to the removal of stenosis in the proximal pulmonary arteries. Along the same line, BPA ameliorates hemodynamics by eliminating stenosis and obstruction in the subsegmental pulmonary arteries, irrespective of the presence of microvascular remodeling. The mechanism by which pulmonary microvascular remodeling occurs in CTEPH has not been fully elucidated. Further investigation is needed to understand the precise mechanisms and time course of development of pulmonary vascular remodeling and its resolution following successful treatment.

**Author's disclosure of potential Conflicts of Interest (COI).**
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# Balloon pulmonary angioplasty: a treatment option for inoperable patients with chronic thromboembolic pulmonary hypertension

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In chronic thromboembolic pulmonary hypertension (CTEPH), stenoses or obstructions of the pulmonary arteries due to organized thrombi can cause an elevation in pulmonary artery resistance, which in turn can result in pulmonary hypertension. CTEPH can be cured surgically by pulmonary endarterectomy (PEA); however, patients deemed unsuitable for PEA due to lesion, advanced age, or comorbidities have a poor prognosis and limited treatment options. Recently, advances have been made in balloon pulmonary angioplasty for these patients, and this review highlights this recent progress.

**Keywords: thrombosis, pulmonary hypertension, angioplasty, pathology, lung injury**

## INTRODUCTION

Chronic thromboembolic pulmonary hypertension (CTEPH) is a form of pulmonary hypertension classified as Group 4 (1). In CTEPH, stenoses or obstructions of the pulmonary arteries due to organized thrombi can cause an elevation in the pulmonary artery resistance, which may in turn result in pulmonary hypertension (**Figure 1**). Previously, the prognosis of patients with CTEPH whose mean pulmonary arterial pressure (PAP) is >30 mmHg was very poor, if left untreated, at only 10% at 3 years (2).

The only established and potentially curative treatment currently available for CTEPH is pulmonary endarterectomy (PEA) (3); however, PEA can only be performed at a limited number of institutions at the present time, as the surgical technique requires proficiency and intermittent total circulatory arrest under deep hypothermia (4, 5). Although the postoperative outcome was reportedly worse in patients with distal thrombi, at expert centers, the outcomes of both proximal and distal cases are recently reported to be excellent (5, 6). However, because patients of an advanced age, with comorbidities, and with a poor general condition are ineligible for PEA, not all patients can undergo the surgery. Based on the data from an international registry, 63% of the patients with CTEPH were considered operable, 36% inoperable, and 57% actually underwent surgery (7).

Although patients who are unsuitable for PEA are treated with pulmonary hypertension-specific drugs, the efficacy of these drugs for lowering the mean PAP in patients with CTEPH had not been established (8). Riociguat, a stimulator of soluble guanylate cyclase, has recently been reported to be effective for inoperable CTEPH or persistent/recurrent pulmonary hypertension after PEA (9). The riociguat group was observed to have a significant improvement in the primary endpoint of 6-min walk distance. Pulmonary vascular resistance significantly decreased and the NT-proBNP level and WHO functional class were significantly improved. It is now approved by the U.S. Food and Drug Administration, the European Medicines Agency, and in Japan. However, its long-term efficacy is not yet established. Moreover, medical therapy in CTEPH should not be considered as a replacement for PEA (3) and riociguat is approved only for selected patients as described above.

There is another treatment option for inoperable patients with CTEPH, balloon pulmonary angioplasty (BPA). The latest guideline for CTEPH states that there are numerous concerns and unanswered questions about this technique and its role in CTEPH remains uncertain and requires further evaluation before it can be recommended as an established treatment for CTEPH (3). Recently, advances have been made in BPA and this review highlights its recent progress.

## HISTORY OF REFINED BALLOON PULMONARY ANGIOPLASTY

Balloon pulmonary angioplasty is an interventional treatment that uses a balloon catheter to dilate pulmonary stenoses (**Figure 2**). BPA was first developed in the field of pediatric cardiology for treating congenital hypoplastic and stenotic pulmonary arteries (10), and since 1988, BPA has been performed for CTEPH cases that are ineligible for PEA (11). A study published in 2001 summarizing the outcomes for 18 CTEPH cases suggested that BPA was effective (12). However, the procedure required improvement, as the treatment effects were less than those obtained by PEA. Further, BPA was found to be frequently associated with pulmonary edema, which can be fatal. Later, two more cases with inoperable CTEPH were reported to be improved by BPA (13). However,

more than 20 years after the first report of BPA for CTEPH, BPA is still not widely accepted as a therapeutic option for inoperable patients.

Since 2004, we have been attempting to improve the BPA procedure at our hospital. Because we had seen many patients with CTEPH who were diagnosed as inoperable and suffered from increasing disability in spite of treatment with pulmonary hypertension-specific drugs. In Japan, there had been no specific drug available for treating CTEPH until September 2014 when riociguat was approved. Considering the high mortality of these patients when untreated, we needed an alternative therapeutic option. We had performed BPA on 68 patients by 2011 and reported efficacy of BPA (14). With only one perioperative death, the safety of BPA was also improved compared with that previously reported (12). With the publishing of BPA studies from other Japanese institutions (15, 16), BPA was covered at the Fifth World Symposium on Pulmonary Hypertension (3). Now, attempts are being made outside of Japan as well, and the efficacy of BPA has been confirmed in selected patients (17–19).

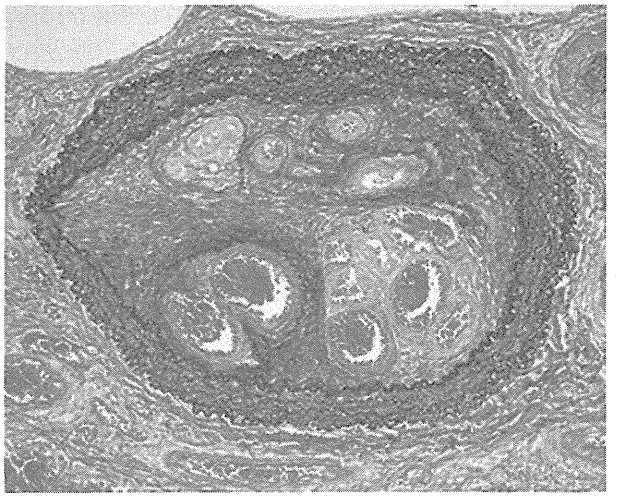

**FIGURE 1 | Organized thrombi in chronic thromboembolic pulmonary hypertension.** The histopathological findings indicated luminal narrowing due to thrombi organized by fibrous intimal hyperplasia, containing characteristic recanalized channels (known as colander lesions) of the pulmonary artery (elastic tissue stain).

## INDICATIONS OF BPA

### PATIENT FACTORS INFLUENCING THE INDICATION FOR BPA

Since PEA is the gold standard therapy for CTEPH, patients who are not eligible for PEA are considered as candidates for BPA. Furthermore, patients with residual or recurrent pulmonary hypertension after PEA are also candidates in case repeated surgery is judged to be difficult. Most importantly, the patients should be informed about and understand the risks and benefits of both PEA and BPA before undergoing either treatment.

Contraindications of BPA include iodine allergy, as the use of a contrast medium is essential in BPA. Additionally, in cases with renal dysfunction, the benefits of performing BPA must be weighed against the risks. Severity of pulmonary hypertension may not necessarily be a contraindication of BPA. Although previous reports have indicated a higher mean PAP at baseline is associated with more frequent complications, the patient prognosis will be worse without effective treatment in cases with severe hemodynamics. BPA can be expected to have more powerful effect in these patients. Indeed, recent studies, including mainly severely ill patients with high mean PAP and low cardiac index, reported successful treatment outcomes with BPA (14–16, 20, 21). Age is also often considered, and the safety and efficacy of BPA in elderly patients have already been reported in recent studies (14–16), with the hemodynamic improvements being comparable between younger and elderly patients (22). The prevalence of complications was also comparable between these two groups.

According to the Japanese Circulation Society's statement regarding BPA, the candidates for BPA are described as follows: (1) unsuitable cases for PEA (surgically inaccessible lesions, surgically accessible but inoperable because of comorbidities, and cases of residual or recurrent pulmonary hypertension after PEA); (2) cases

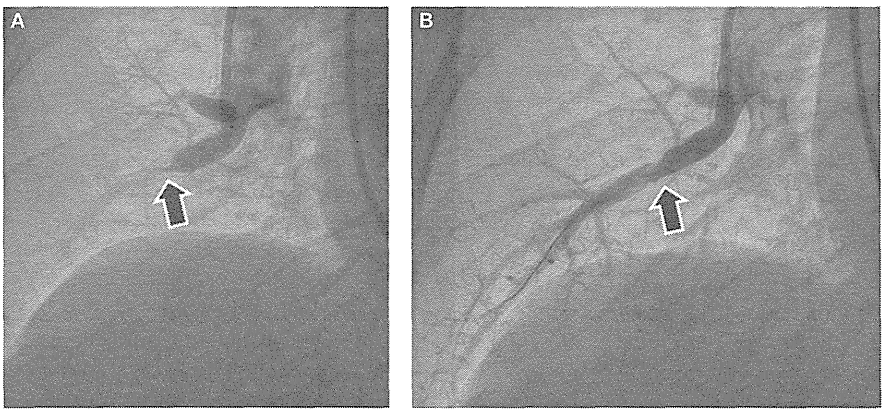


**FIGURE 2 | Pulmonary angiography before and after balloon pulmonary angioplasty (BPA). (A)** Subtotal obstruction was noted in the pulmonary angiography before BPA (arrow). **(B)** Pulmonary angiography after BPA showed blood flow to the peripheral arteries after balloon dilatation. The arrow indicates the same site as the arrow in **(A)**.

in which conventional therapy is insufficient (WHO functional class ≥III after conventional therapy, mean PAP ≥30 mmHg, or PVR ≥300 dyne · s · $cm^{-5}$); (3) patients who provide informed consent (patients who want to be treated with BPA after fully understanding the risks and benefits of both BPA and PEA); and (4) cases without serious complications, multiorgan failure, or iodine allergy (23).

### INDICATION FOR EACH LESION

The most distinct difference between PEA and BPA is the different perception of the indication for lesions. In PEA, the organized thrombi are considered as a whole, and the indication for surgery is made depending on the extent and location of the organized thrombi. In contrast, in BPA, each individual artery is evaluated for indication of BPA based on the lesion type and distribution/location of the lesion.

In patients with CTEPH, lesions are generally present in most arteries, and there is usually a mixture of various lesion types. The classification of CTEPH is currently based on the endarterectomized tissue removed by PEA (24). This is useful as it is easy to understand the distribution of the organized thrombi. However, in performing BPA, the angiographical classification is more useful in evaluating the lesion types of each target lesion for BPA, including pouching defects, webs or bands, intimal irregularities, abrupt vascular narrowing, and complete vascular obstruction (25).

When large amounts of organized thrombi are found in the proximal portion of the pulmonary artery, BPA is not recommended, as the thrombi cannot be removed using this method and surgically easily accessible. Moreover, complete obstructions at the orifices of the segmental arteries that do not show traces of distal arteries or pouching defects are also unsuitable for BPA, as it is extremely difficult for the guidewire to pass through these arteries, although BPA for proximal thrombi has been attempted (26).

Regarding the lesion location, most studies have reported on the treatment of lesions in the segmental or subsegmental arteries in peripheral-type CTEPH patients. In PEA, the more distal the lesion is located, the more difficult the surgery is, and this is why BPA is mainly performed in the distal pulmonary arteries. However, when the lesions are located too distally in the subsegmental pulmonary arteries for the balloon catheter to pass the lesion, BPA may also be unsuitable.

The success rate for each lesion varies among the lesion types. In cases of ring-like stenosis and web and abrupt narrowing, a success rate of almost 100% is achieved, while in cases of the subtotal obstruction type, the success rate is reported to be approximately 90% (23). Conversely, in pouching defects, the success rate is <50%.

## THE PROCEDURE OF BPA

### THE GENERAL PROCEDURE OF BPA

The BPA procedure is approached either through the right internal jugular vein or the right femoral vein, with the internal jugular vein route being better for manipulating the guiding catheter into either the left or right pulmonary artery (12, 14). Using this approach, two operators are needed: one to manipulate the guiding catheter and one to manipulate the guidewire. On the other hand, the femoral vein route has the advantage of one operator being able to manipulate both the guiding catheter and the guidewire; however, manipulating the guiding catheter via the right pulmonary arteries is extremely difficult. A 9-French (Fr) sheath is inserted into the vein, through which a 6-Fr long introducer sheath is advanced into the pulmonary artery. After the sheaths are inserted, heparin is administered to reach an activated clotting time of around 200 s, and an additional 500–1,000 units of heparin are administered every hour. Subsequently, a 6-Fr guiding catheter (multipurpose type or the Amplatz type) is advanced into the pulmonary artery being treated. After performing selective pulmonary angiography, a 0.014″ guidewire is used to cross the lesion. When the treatment is not performed in the upper lobe of the lung, the pulmonary artery must be stretched as much as possible via deep inhalation from the patient, as this facilitates the passage of the guidewire. When the wire has successfully crossed the lesion, a balloon catheter of an appropriate diameter (1.5–10 mm) is selected to dilate the lesion.

Initially, since it was believed that reperfusion itself was the cause of BPA-related lung injury, the target area for one treatment session was limited to two segments on the same side until the mean PAP fell below 30 mmHg (14). There is also an attempt to reduce the rate of lung injury by restricting the treatment area (27). However, the targets and areas for treatment need to be decided according to the operator's experience and the patient's lesion type and distribution/location. For example, arteries in the middle or lingular lobes or lesions in the distal to subsegmental arteries are particularly difficult to treat. Because the effect of treating each lesion depends on the size of perfusion area of the segmental arteries containing the lesions, there is an expectation that it is more effective to prioritize treatment of the lesions in the branches of the inferior lobe. Currently, at our institution, there is no limitation regarding the number of lobes targeted in one session but the maximum time of radiographic fluoroscopy in a single session is limited to 60 min. As a consequence, lesions from 4–10 sites are generally treated in a single session. To enhance the therapeutic effect of BPA, it is important to make the area of reperfusion large, which requires repeated treatment with three to four sessions per patient depending on the treatment goal (14–16).

### HOW TO EVALUATE EACH LESION

It is critical to evaluate each lesion type and vessel diameter in order to determine the appropriate balloon size for performing angioplasty in BPA. There are several different modalities that have been recently reported as useful, with some of these modalities providing us with clearer images of the lesion. However, we need to consider the feasibility of the modality to be performed in all patients and during all sessions of BPA, as well as the cost and time involved in performing each imaging modality.

Pulmonary angiography is considered the conventional and standard method. To evaluate the lesions in more detail, selective pulmonary angiography should be performed by injecting contrast medium from a catheter inserted into the segmental artery rather than the main pulmonary artery. Intravascular web and band lesions in subsegmental pulmonary arteries are often invisible, and subtotal occlusion with a faint trail of contrast medium in the peripheral arteries can be easily overlooked by ordinary angiography. Accordingly, selective pulmonary angiography should be routinely performed (**Figure 2A**).