Alveolar Septal Widening as an "Alert" Signal to Look Into Lung Antibody-mediated Rejection: A Multicenter Pilot Study

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Background. Antibody-mediated rejection (AMR) plays an important role in allograft dysfunction. Acute lung injury (ALI), endotheliitis, capillary inflammation, and C4d positivity have been described as morphological features conventionally associated with lung AMR. A multidisciplinary, international task force reviewed AMR cases in the context of four face-to-face meetings. Septal widening was a frequent, striking histological feature recognized first and easily at low-power magnification. This study aimed to evaluate whether septal widening could represent an "alert" signal for AMR. **Methods.** Following the face-to-face meetings that enabled the classification of cases as AMR or non-AMR, morphometry was performed on biopsies from 48 recipients with definite, probable or possible AMR, 31 controls (negative for any posttransplant injury) and 10 patients with nonimmune-related ALI. **Results.** Mean alveolar septal thickness was greater in AMR patients than in controls ($P < 0.001$). Septal thickness was not significantly different between AMR-ALI and non–AMR-ALI. Unexpectedly septal widening was the only histological change detected in some cases with probable or possible AMR that lacked the histological lesions conventionally associated with AMR. The thickness in these cases was similar to that observed in AMR cases with more severe histological injury such as ALI or neutrophilic capillaritis. **Conclusions.** Our data suggest that, even if unspecific as the other lesions conventionally associated with AMR, septal widening may represent an "alert" signal to look into lung AMR. A larger prospective study is mandatory to confirm the potential value of septal widening in the multidisciplinary approach of AMR.

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INTRODUCTION

Antibody-mediated rejection (AMR) is widely recognized in kidney and heart transplantation as a cause of graft dysfunction, and increasingly considered in pulmonary allograft failure.[1-3] The pathology of lung AMR still represents a diagnostic challenge for pulmonologists, immunologists, and transplant pathologists.[4] The morphologic interpretation of lung allograft biopsies with AMR is not yet well defined and is largely extrapolated from morphological findings of AMR in other solid organ allografts. The 2007 revision of the 1996 working formulation of lung rejection[5] speculated that subendothelial mononuclear cell infiltrates (small vessel intimitis) should raise the suspicion of AMR, thus a generic term of "capillary injury" was suggested. The International Society of Heart and Lung Transplantation (ISHLT) Pathology Council highlighted the importance of a multidisciplinary approach for the diagnosis of AMR using the "triple test": clinical allograft dysfunction, circulating donor-specific antibodies (DSAs) and abnormal histopathological findings.[6] In this statement, a critical discussion about C4d staining was included, reporting specific indications for a more accurate interpretation. C4d deposition has been widely used as a surrogate marker of AMR; however, conflicting results about its sensitivity and specificity have been shown in lung allografts.[7-15] The 2016 Banff study on the pathology of lung allografts from patients with circulating DSA reported that capillary inflammation, acute lung injury (ALI) and endotheliitis were morphological changes significantly associated with DSA.[4] These features can also be observed in other lung injuries (eg, infection, ischemia/reperfusion injury, drug toxicity, etc.), and a multidisciplinary approach is mandatory for accurate etiology. Different forms of capillary inflammation were the most frequently detected lesions. However, the authors reported a poor interpathologist agreement and cautioned about the use of C4d immunostaining due to infrequent diffuse staining.[4] The recent ISHLT AMR Consensus Report considers clinical allograft dysfunction, lung histology, C4d immunostaining, and DSA assessment as fundamental criteria to classify three levels of certainty in the diagnosis of AMR (definite, probable, and possible).[16] Moreover, it underlines the need for refining the morphological evaluation with potentially more sensitive and specific features and determining whether or not C4d positivity is indeed required for a diagnosis of AMR.[16] On the basis of the 2016 ISHLT consensus report and also on the fact that cases of definite AMR are relatively infrequent, particularly within a single center, a multi-institutional multidisciplinary task force was set up in 2016 involving several expert pathologists, pulmonologists, and immunologists from Europe and North America. The aim of the study was to review retrospective cases with clinical/pathological suspicion of AMR according to the ISHLT consensus report and to identify whether additional morphological features may be of help in the pathological diagnosis of AMR.

The pathologists reviewed transbronchial biopsies (TBBs) at multihead light microscope to reach a consensus diagnosis that was discussed with pulmonologists and immunologists, and classified patients as definite, probable, or possible AMR, or non-AMR.

The panelists noted that widening of alveolar septa was often a striking histological finding easily observed at low-power field in AMR cases before the high-power hunt for other features of AMR (capillary inflammation, ALI, and endotheliitis). The aim of the study was to evaluate whether objectively quantified septal widening could represent an "alert" signal for AMR, thus consisting of an additional parameter suggestive of AMR.

MATERIALS AND METHODS

Study Design and Population

The present study was a preliminary retrospective multicenter study of consecutive lung allograft recipients transplanted in the 2009–2016 period. It was based on the review of posttransplant TBB obtained from 48 patients with clinical/pathological diagnosis or suspicion of AMR. Only cases with complete clinical, immunological, and pathological records were considered (http://lungtransplant.dctv.unipd.it/amr/index.php; **Figure S1, SDC,** http://links.lww.com/TP/B709). In the context of the Pulmonary Pathology Working Group of the European Society of Pathology, we undertook the study to address histopathological uncertainties that still surround a firm diagnosis of AMR.

Following 4 face-to-face meetings (held in Birmingham, Padova, and Belgrade), expert lung pathologists reviewed the biopsies at multihead microscopy for any type of pathological lesion, particularly focusing on histological features conventionally associated with AMR (different forms of capillary inflammation, ALI, and endotheliitis) (**Table S1, SDC,** http://links.lww.com/TP/B709) diagnosed according to the Banff study.[4] In particular, ALI represents the most severe histological injury associated with AMR and is defined as a spectrum ranging from reactive pneumocytes with interstitial/alveolar edema to diffuse alveolar damage. Each case was extensively discussed by the panel which included experienced transplant pathologists (A.M.F., A.R., D.N., E.V., F.C., M.G., M.P.C., M.I., P.D., W.T.), pulmonologists (J.L.P., S.H.), and 1 immunologist (E.C.) and was classified according to the AMR ISHLT consensus report.[16] The participation of pathologists, possibly accompanied by their center's pulmonologists/immunologists, was on a voluntary basis. Two internationally reknown pulmonologists were also invited to join the task force (A.R., D.L.).

Ultimately, 9 cases were classified as definite, 17 as probable, and 22 as possible AMR (**Figure S2, SDC,** http://links.lww.com/TP/B709).

On the basis of the presence of alterations in pulmonary physiology, gas-exchange properties, radiologic features, or deteriorating functional performance, the majority of patients (41/48; 85%) were categorized as clinical AMR. The remaining 7 cases were patients with subclinical AMR.

In addition to these 48 cases, 31 DSA-negative patients with no posttransplant complications were recruited in the same time interval in all centers and had all the required information recorded in the study datasheet (**Figure S1, SDC,** http://links.lww.com/TP/B709). As ALI represents the most severe histological lesion responsible for septal widening, 10 cases of DSA-negative ALI related to ischemia/reperfusion injury were also included in the evaluations as "non–AMR-ALI" group. DSA analysis was negative in

the time interval from transplant to the TBB in the whole study population (89 patients).

The study was performed in accordance with the principles of the Declaration of Helsinki and the guidelines for Good Clinical Practice, and it was approved by the participating centre Ethic Committees. All patients provided written informed consent.

DSA ASSESSMENT

Before transplantation, all patients were screened for the presence of anti-HLA antibodies with the LABScreen SAB assay (One Lambda, Canoga Park, CA). Peripheral blood DSA analysis was performed on all index patients at or close to the time of the reference biopsy. Anti-HLA IgG reactivity was analyzed in each center with validated bead-based assays using the LABScreen mixed kit (One Lambda, Canoga Park, CA) and the single-antigen class I and class II kits. Analyses were performed using One Lambda software (HLA Fusion Version 2.0). In the mixed assay, results above a cutoff value of 3.0 (ratio) were considered positive, according to a beta test performed on each laboratory's samples. To identify HLA specificity, single-antigen assays (One Lambda) were performed, using mean fluorescence intensity (MFI) as a measure of antibody reactivity. We considered DSA-positive patients those with MFI values ≥1400.[17] For statistical analyses, in the cases with more than one DSA, the DSA with the highest MFI (immunodominant DSA) was selected. Only 1 immunologist (E.C.) was included in the panel, but he reviewed all immunological datasheets and discussed with the immunologists from the different centers, when needed.

MORPHOMETRIC ANALYSES

Morphometric analyses were performed by a highly experienced and expert biologist (F.L.) who was blinded to the patient categorization in the AMR or control groups. Morphometry, performed using a Zeiss light microscope connected to a digital camera and Image ProPlus 6.0 (Media-Cybernetics, Inc., Warrendale, PA), was used for image analysis. Four fields for each section were observed and captured at a final magnification of ×200, including in most cases roughly a total sample area of 0.5 mm^2. Thickness of alveolar septa was measured between the epithelial cell surface on opposite sides of the septum in well-aerated lung parenchyma far from areas with acute cellular rejection or organizing pneumonia. At least 20 measurements were performed per field, with a total number of at least 80 measurements per patient. Morphometrical evaluation of each case was performed twice in an independent session by the same reader using the same software to reduce the probable random reading error. Data are expressed in microns as the mean value of all measurements and sessions for each patient.

Statistical Analyses

Statistical differences between AMR patients and controls were tested by using parametric or nonparametric approach, as appropriate. To explore the relationship between septal widening and the main clinical and morphological features (native disease, DSA, capillaritis, ALI, acute cellular rejection) simple and multiple linear models (general linear model procedure) were applied and the adjusted mean difference (95% confidence interval) of septal thickness between groups was estimated on the subgroup of AMR patients (n = 48).

The receiver operating characteristic (ROC) analysis was applied to evaluate the performance of the septal thickness in classifying AMR patients. The optimal cutoff value was identified by ROC analysis and Youden index. The impact of the time elapsed since transplantation on alveolar septal thickness was assessed in 4 time intervals: <2, 2–6, 6–12, and >12 months using a 2-way analysis of variance with multicomparisons tests. Statistical analyses were performed with SAS 9.4 software (SAS Institute, Inc.). Significance level of 2-tailed tests was set at 0.05.

RESULTS

Study Population

The main characteristics of the study population are reported in Table 1 (AMR and controls) and Table 2 (AMR-ALI and non–AMR-ALI). Most patients were DSA positive (43/48), with a prevalence of class II HLA DSA (Table 1; **Table S2, SDC,** http://links.lww.com/TP/B709).

Histology, Septal Widening, and Diagnosis of AMR

ALI was detected in only 35% of AMR cases, while different grades of capillary inflammation were present in the majority of the patients (with rare cases of neutrophilic capillaritis).

Only 3 cases did not show any lesion conventionally associated with AMR (absence of ALI, capillary inflammation, endotheliitis). These cases showed interstitial edema with dilated capillaries and mild lymphomonocyte infiltrate and thus the absence of the conventional histological aspects lead to the definition of "negative histology" and consequently to the category downgrading to probable (n = 1) and possible AMR (n = 2; due also to the absence of C4d) (**Table S3, SDC,** http://links.lww.com/TP/B709).

Widening of alveolar septa was easily and diffusely observed at low-power field in biopsies of AMR cases before the high-power hunt necessary to detect other AMR suggestive features (capillary inflammation, ALI, endotheliitis).

The normality of septal thickness distribution was confirmed both in AMR samples (Shapiro-Wilk, $P = 0.30$) and in controls (Shapiro-Wilk, $P = 0.22$) (**Figure S3, SDC,** http://links.lww.com/TP/B709). The mean septal thickness was significantly greater in AMR than control cases (9.0 µm ± 2.1 µm versus 5.3 µm ± 1.7 µm; $P < 0.001$) (Figure 1A, C, D). There was no significant difference in septal thickness between definite, probable, and possible AMR cases.

Septal widening was effective in distinguishing the AMR cases from negative patients, as shown by the ROC curve of area under curve of 0.93 (95% confidence interval: 0.88–0.99; Figure 1B). According to the cutoff of 6 µm, selected on the basis of Yuden's index to dichotomize alveolar septal thickness, 46 (96%) AMR and 9 (29%) control patients had a septal thickness >6 µm ($P < 0.001$).

Unexpectedly, the widening was also detected in each of the 3 cases with probable/possible AMR without histological signs conventionally associated with AMR (7.8 ± 2.0 µm). The thickness in these cases was similar to that observed in AMR cases with more severe injury as ALI

TABLE 1.

Study population: main recipient and donor characteristics

	AMR patients (N = 48)	Controls (N = 31)	*P*
Age, y (mean ± SD)	42.5 ± 18.4	45.3 ± 16.7	0.50
Sex (F:M), n (%)	24:24 (50:50)	12:19 (40:60)	0.39
Native disease, n (%)			0.47
CF	10 (21)	12 (38)	
IPF	15 (31)	7 (23)	
PPH	10 (21)	3 (10)	
COPD	9 (19)	7 (23)	
Others	4 (8)	2 (6)	
Surgical procedure (SLT:BLT)	5:43	1:30	0.24
Donor age, y (mean ± SD)	35.9 ± 17.3	35.1 ± 16.6	0.86
Donor sex (F:M), n (%)	19:29	12:19	0.87
Donor smokers:nonsmokers, n (%)	15:33	8:23	0.65
Ischemic time, min (mean ± SD)	270.9 ± 71.9	258.5 ± 69.8	0.48
Immunology[a]			
DSA at the time of reference TBB, n (%)	43 (90)	–	–
DSA Class I, n (%)	6 (12.5)	–	–
DSA Class II, n (%)	26 (54)	–	–
DSA Class I and II, n (%)	11 (23)	–	–
DSA Max MFI (median, range)	5736 (1465–22 836)	–	–
Pathology			
Capillary inflammation, n (%)			
Score 1	18 (38)	–	–
Score 2	7 (15)	–	–
Score 3	3 (6)	–	–
Acute lung injury, n (%)	17 (35)	–	–
Endotheliitis, n (%)[b]	2 (4)	–	–
Negative histology, n (%)[c]	3 (6)	–	–
Acute cellular rejection, n (%)			
A0	31 (65)	–	–
A1	9 (19)	–	–
A2	6 (12)	–	–
A3	2 (4)	–	–
A4	0	–	–
Lymphocytic bronchiolitis, n (%)	1 (2)	–	–
Infections, n (%)	3 (6)	–	–
Posttransplant time[d], days (median, Q1–Q3)	90 (51–366)	137 (86–349)	0.26

[a]DSA negativity was an inclusion criteria for controls.
[b]Endotheliitis was concomitantly present in one patient with ALI and one with capillary inflammation.
[c]Absence of morphological lesions suggestive of AMR.
[d]Post-transplant time is the interval time from transplantation to the considered TBB.
ALI, acute lung injury; AMR, antibody-mediated rejection; BLT, bilateral lung transplantation; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; DSA, donor-specific antibodies; F, female; IPF, idiopathic pulmonary fibrosis; M, male; MFI, mean fluorescence intensity; n, number; PPH, primary pulmonary hypertension; Q, quartile; SD, standard deviation; SLT, single lung transplantation; TBB, transbronchial biopsies.

(9.5 ± 2.8 μm) or in cases with neutrophilic capillaritis (8.2 ± 2.0 μm). When comparing nonimmunological ALI (ie, ALI related to ischemia-reperfusion injury) to AMR-ALI, the mean septal thickness was not different (10.4 ± 2.7 μm versus 9.5 ± 2.8 μm).

The multivariable analysis based on the adjusted models indicates that only the native disease (pulmonary hypertension [PPH]) was independently associated with septal thickness but explained only 25% of the whole septal thickness variability (R^2 = 0.25) (Table 2; **Table S4, SDC,** http://links.lww.com/TP/B709).

Focusing more in depth on DSA-positive AMR patients, those with PPH (n = 10) showed a higher mean MFI values than patients with other native diseases (n = 33), even if such data are not statistically significant (9182.1 ± 7372 versus 6368 ± 4416; *P* = 0.1).

Septal Widening and Posttransplant Time

The median time (d) elapsed from transplantation did not significantly differ between AMR cases and controls (Table 1). Alveolar septa were significantly thicker early after transplantation when compared to later time points (*P* = 0.03). At all time points, septal thickness was significantly greater in AMR patients than in controls except in biopsies taken <2 months posttransplantation, likely due to the limited number of available cases at that time point (n = 3; Figure 1E).

TABLE 2.

AMR-ALI vs non–AMR-ALI: principal recipient and donor characteristics

	AMR-ALI (n = 17)	Non–AMR-ALI (n = 10)
Age, y (mean ± SD)	40.8 ± 18.9	44.8 ± 16.2
Sex (F:M), n (%)	10:7 (59:41)	4:6 (40:60)
Native disease, n (%)		
CF	4 (23.5)	2 (20)
IPF	5 (29)	1 (10)
PPH	4 (23.5)	2 (20)
COPD	3 (18)	1 (10)
OTHERS	1 (6)	4 (40)
Surgical procedure (SLT:BLT)	2:15 (12:88)	0:10 (0:100)
Donor age, y (mean ± SD)	39.9 ± 18.9	40.8 ± 16.1
Donor sex (F:M), n (%)	9:8 (53:47)	7:3 (70:30)
Donor smokers:nonsmokers, n (%)	7:10 (41:59)	4:6 (40:60)
Ischemic time, min (mean ± SD)	294.9 ± 66.6	311.6 ± 111.1
Posttransplant time,[a] days (median, Q1–Q3)	66, 21–189	62, 31–324

[a]Post-transplant time is the interval time from transplantation to the considered TBB.

ALI, acute lung injury; AMR, antibody-mediated rejection; BLT, bilateral lung transplantation; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; F, female; IPF, idiopathic pulmonary fibrosis; M, male; MFI, mean fluorescence intensity; n, number; PPH, primary pulmonary hypertension; Q, quartile; SD, standard deviation; SLT, single lung transplantation.

DISCUSSION

This multi-institutional and multidisciplinary pilot study points out for the first time diffuse septal widening as an additional morphological finding frequently detected in AMR cases. Definitely at multihead microscopy, septal widening unanimously emerged as a striking finding that was confirmed by subsequent blind computerized morphometric analysis. This latter procedure is time consuming but by far more objective. The detection of septal widening was overall an intriguing finding in some cases lacking the histological lesions conventionally associated with AMR but presenting clinical dysfunction, DSA and in one case C4d positivity. Indeed, it is our contention that the presence of septal widening should not be rapidly dismissed, even if unspecific as the other conventionally lesions associated with AMR. On the contrary, we believe that such an alert signal (easily recognized at low power magnification) should be included among the histological aspects suggestive of AMR, avoiding underestimation of AMR certainty and undue delays in patient clinical management.

Diffuse/multifocal septal widening compromises the alveolar capillary structure, the most important lung functional unit, providing a potential pathogenetic mechanism for the dysfunction, which appears poorly responsive to current available treatments.

At present, we do not have an adequate mechanistic explanation of how septal widening develops nor what the key actors are. Alveolar septa were significantly wider early posttransplant compared with later time points, probably due to the contribution of ischemia-reperfusion injury. However, at all time points septal thickness was greater in AMR cases than in controls. The plausible mechanisms underlying septal widening in AMR may be mainly related to 3 factors, namely, inflammatory burden, endothelial/epithelial cell swelling, and edema. Several in vitro and in vivo studies have demonstrated that, following DSA binding and complement activation, there is a critical early step characterized at electron microscopy by endothelial swelling with cytoplasmic blebs, detachment of interendothelial junctions, and subsequent edema followed by inflammation. The anatomy of the alveolar septum is complex[18] and the full comprehension of the mechanisms of injury is even more intricate. This is especially true if one takes into consideration that the morphological and biological responses of endothelial cells to injury vary due to the well-known microvascular endothelial cell heterogeneity.[19] Therefore, the lung vascular system and especially endothelial cells should represent an important area of indepth morphological and molecular investigation to identify underlying mediators of septal widening and the identification of specific targeted treatments. Both airway and alveolar epithelial cells, particularly type II pneumocytes, constitutively express class I and II major histocompatibility molecules, especially HLA-DR. In vitro studies have shown that binding of HLA antibodies to lung epithelial cells triggers the release of different mediators ultimately leading to severe cellular alterations and death.[20,21] Such morphological and molecular changes in lung AMR represent another intriguing and novel field to investigate. The inflammatory burden in lung AMR is a further interesting area of research. Several recent studies have demonstrated that, following endothelial injury/activation, there is the infiltration of different types of inflammatory cells, including natural killer (NK) cells. The presence of high NK transcripts in many AMR renal biopsies supports the concept of a central role of NK cells in mediating allograft injury.[22] Even though fibrosis could represent an additional underlying factor in septal widening, our data to date exclude the contribution of such a process, as demonstrated by conventional collagen special staining (elastic Van Gieson), used in scheduled posttransplant TBBs.

Our study, however, presents several limitations. First, this was a retrospective study with a relatively small number of patients; although septal widening was consistently identified, further longitudinal evaluation in larger case series is required to confirm the value of this lesion. Second, our series includes a limited number of cases with definite AMR. C4d-negative immunostaining, probably due to poor sensitivity or due to complement-independent AMR, was the main cause of AMR category downgrading

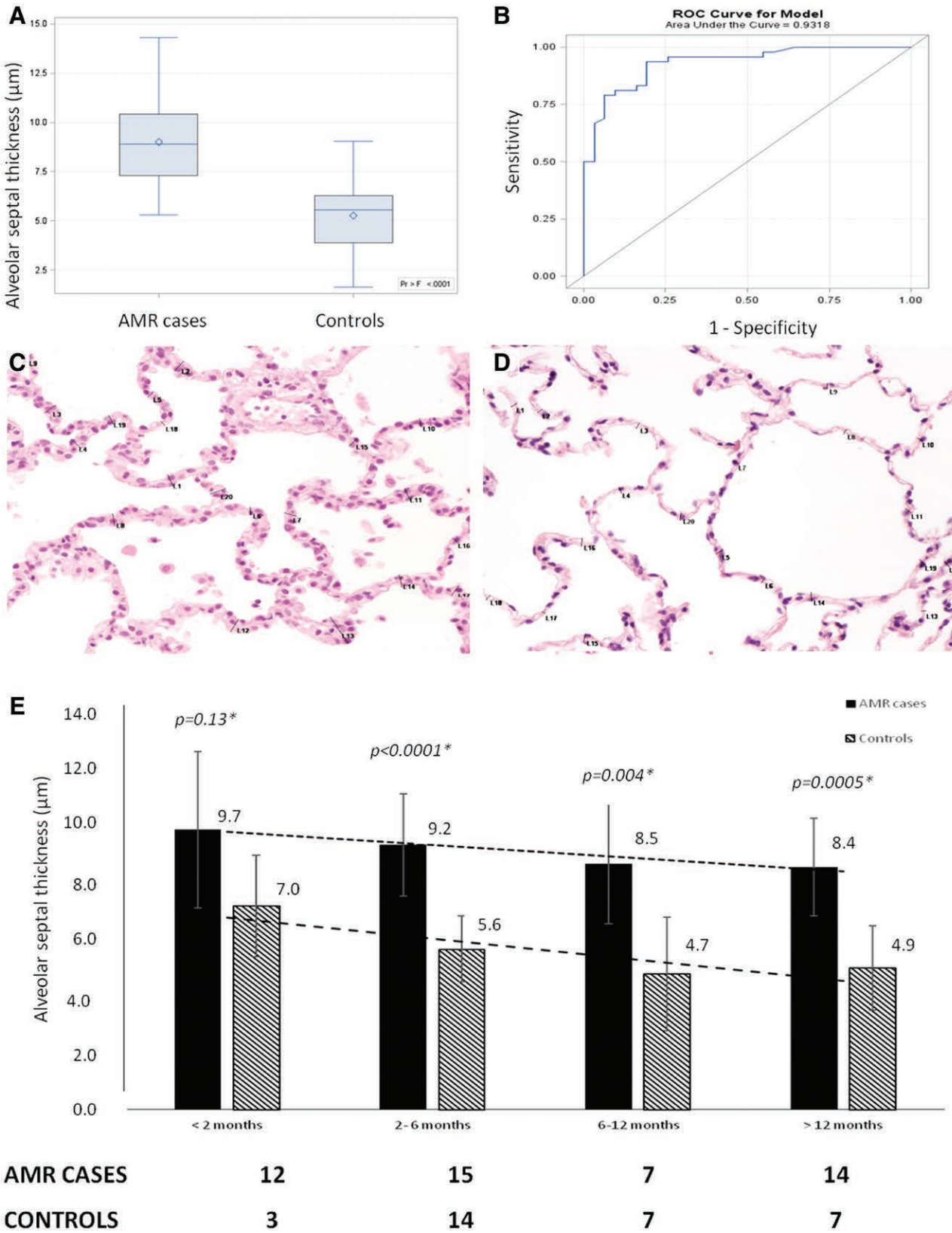

FIGURE 1. Alveolar septal thickness in AMR and control cases. A, Box plot showing that mean alveolar septal thickness was greater in AMR cases than in controls ($P < 0.0001$). B, ROC curve showing that septal widening is a reliable marker in detecting AMR cases. C and D, Emblematic images highlighting wider septal thickness in an AMR case compared with a control case, respectively. Final magnification: ×200. E, Mean alveolar septal thickness (µm) (vertical bars indicate standard errors) for the AMR groups and posttransplant time. Adjusted effect of posttransplant time ($P = 0.08$). Adjusted comparison of AMR vs controls ($P < 0.0001$). *Significance level observed by comparing AMR cases and controls at each specimen age. AMR, antibody-mediated rejection.

TABLE 3.

Comparisons of alveolar septal thickness by clinical, pathological, and immunological subgroups on AMR subjects (n = 48)

	N	Mean (SD)	Range	*P*	Mean difference	95% CI
Sex						
Men	24	9.30 (2.39)	5.30–14.30	0.37	*ref*	
Women	24	8.75 (1.79)	5.42–12.50		−0.55	−1.77 to 0.68
Age[a] (y)						
<48	24	8.77 (2.06)	5.30–14.05	0.35	*ref*	
≥48	24	9.28 (2.16)	6.10–14.30		0.57	−0.65 to 1.80
Donor sex						
Men	29	8.87 (2.16)	5.30–14.30	0.35	*ref*	
Women	19	9.44 (1.92)	6.72–14.05		0.58	−0.65 to 1.82
Donor age[a] (y)						
<35	24	9.29 (1.88)	5.30–12.50	0.71	*ref*	
≥35	24	8.40 (2.28)	6.10–14.30		−0.70	−1.30 to 1.15
Donor smoking						
No	16	7.70 (2.66)	2.58–14.30	0.83	*ref*	
Yes	32	7.85 (2.69)	2.80–12.50		0.15	−1.21 to 1.51
Native disease						
CF	10	7.62 (1.43)	5.30–10.10	**0.05**	*ref*	
IPF	15	9.32 (1.85)	6.42–12.50		1.70	−0.60 to 4.00
PPH	10	10.13 (2.67)	7.33–14.30		2.50	0.00 to 5.03
COPD	9	9.40 (1.63)	6.34–11.80		1.78	−0.80 to 4.37
Others	4	7.80 (2.69)	5.42–11.40		0.19	−3–15 to 3.52
DSA						
Negative	5	9.81 (1.97)	7.3–12.5	0.38	*ref*	
Positive	43	8.93 (2.13)	5.30–14.30		−0.88	−2.89 to 1.13
ALI						
No	31	8.77 (1.74)	5.30–11.53	0.27	*ref*	
Yes	17	9.48 (2.76)	5.42–14.30		0.71	−0.57 to 1.99
Capillary inflammation						
No	20	9.23 (2.73)	5.42–14.30	0.75	*ref*	
Yes	28	8.88 (1.56)	5.30–11.53		−0.35	−1.60 to 0.91
Capillary score						
0	20	9.23 (2.73)	5.42–14.30	0.78	*ref*	
1	18	8.82 (1.34)	6.34–11.30		−0.40	−2.27 to 1.47
2	7	9.30 (2.02)	5.30–11.40		0.08	−2.45 to 2.61
3	3	8.22 (1.98)	6.42–10.35		−1.00	−4.57 to 2.56
C4D						
No	33	9.35 (1.95)	6.10–14.05	0.11	*ref*	
Yes	15	8.31 (2.33)	5.30–14.30		−1.04	−2.34 to 0.26
ACR						
No	31	8.61 (1.71)	5.30–11.03	0.07	*ref*	
Yes	17	9.77 (2.58)	6.34–14.30		1.16	−0.09 to 2.41
Infection						
No	45	9.05 (2.14)	5.30–14.30	0.78	*ref*	
Yes	3	8.69 (1.96)	6.50–10.28		−0.35	−2.91 to 2.20
Histology						
No	3	7.76 (2.47)	6.10–10.60	0.34	*ref*	
Yes	45	9.11 (2.09)	5.30–14.30		1.35	−1.18 to 3.88
Posttransplant time (mo)						
0–2	14	9.68 (2.70)	5.42–14.30	0.41	*ref*	
2–6	15	9.16 (1.77)	5.30–12.50		−0.53	−2.621 to 1.57
6–12	7	8.50 (2.07)	6.10–11.53		−1.18	−3.79 to 1.44
>12	12	8.39 (1.69)	6.34–11.80		−1.29	−3.51 to 0.92

Bold indicates significant *P* value.

[a]Age and donor's age dichotomized on its own median values.

ACR, acute cellular rejection; ALI, acute lung injury; AMR, antibody-mediated rejection; CI, confidence interval; COPD, chronic obstructive pulmonary disease; CF, cystic fibrosis; DSA, donor-specific antibodies; IPF, idiopathic pulmonary fibrosis; PPH, primary pulmonary hypertension; SD, standard deviation.

to probable or possible.[16] The identification of novel, more sensitive surrogate markers of AMR is therefore an important area for future research. Third, the control group included fewer cases than the index group. This is mainly related to the fact that, in some centers, DSA testing was not systematically performed at each protocol biopsy but only on clinical/pathological demand. As a consequence, many potential control patients lacked posttransplantation DSA information and could not be enrolled. In future studies, such a limitation will be easily overcome as many centers have now adopted a scheduled protocol for DSA screening. The results obtained in multivariable analysis concerning the relation between alveolar septal widening and AMR patient's native disease (PPH) need to be confirmed in larger case series. However, PPH patients showed a trend of higher DSA MFI values than those with other native diseases. Other authors have demonstrated the association of some native diseases with lung graft AMR[3,23] and patients with PPH are often bridged to transplantation with extracorporeal membrane oxygenation, which was reported at a high risk of anti-HLA sensitization,[24] thus PPH patients could be at higher risk to develop AMR and consequently suggestive pathological signs.

In conclusion, our study suggests that widening of alveolar septa represents a morphological feature that may help in the multidisciplinary diagnosis of AMR in addition to the conventionally accepted morphological lesions. An essential advantage of this new finding is that it is easily recognized thus acting as an "alert" signal possibly reinforcing the suspicion of AMR and more timely and adequate patient management. Additional studies are eagerly awaited to validate this novel marker.

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