Pulmonary nontuberculous mycobacteria disease and anti-neutrophil cytoplasmic antibody positivity: a retrospective analysis of long-term clinical outcomes including vasculitis onset

N. Oda¹, H. Matsui^{2,3}, N. Oya¹, T. Suzuki^{4,5}, R. Hazue¹, R. Rokutanda¹

¹Department of Rheumatology, Kameda Medical Center, Chiba; ²Department of Clinical Epidemiology and Health Economics, School of Public Health, The University of Tokyo; ³Clinical Research Support Division, Kameda Medical Center, Chiba; ⁴Department of Nephrology, Kameda Medical Center, Chiba; ⁵Department of Clinical Epidemiology, Graduate School of Medicine, Fukushima Medical University, Japan.

Abstract Objective

To investigate the positivity rate of anti-neutrophil cytoplasmic antibody (ANCA) and the incidence of ANCA-associated vasculitis (AAV) in pulmonary nontuberculous mycobacterial (NTM) disease.

Methods

We conducted a retrospective observational study using electronic medical records of patients diagnosed with pulmonary NTM disease who subsequently underwent testing for myeloperoxidase (MPO) or proteinase 3 (PR3) ANCA. We identified cases of vasculitis among these patients and documented those who developed AAV. Additionally, we reviewed the literature for previously reported cases of AAV following pulmonary NTM disease.

Results

We identified 63 patients with pulmonary NTM disease who subsequently underwent ANCA testing. Seven cases (11.1%) tested positive, predominantly for MPO-ANCA. Among them, five patients (71.4%) developed AAV, with three demonstrating concurrent NTM culture positivity at the time of AAV diagnosis. Three patients were diagnosed with microscopic polyangiitis, while the remaining two were diagnosed with granulomatosis with polyangiitis and eosinophilic granulomatosis with polyangiitis, respectively. In our case series of AAV, most patients developed glomerulonephritis. Additionally, a review of the existing literature revealed eight previously reported cases of AAV associated with pulmonary NTM disease.

Conclusion

This study is one of the largest to investigate the proportion of patients positive for ANCA with pulmonary NTM infection. Our findings suggest an association between NTM infection and AAV and highlight the importance of measuring ANCA in patients with NTM. Further studies are needed to clarify the underlying mechanisms and identify risk factors.

Key words

ANCA-associated vasculitis, pulmonary *nontuberculous mycobacterial* disease, MPO-ANCA, PR3-ANCA, retrospective study

Nobuhiro Oda, MD Hiroki Matsui, MPH Naoki Oya, MD Tomo Suzuki, MD, PhD Ryo Hazue, MD Ryo Rokutanda, MD Please address correspondence to: Nobuhiro Oda Department of Rheumatology, Kameda Medical Center, 929 Higashi-cho, Kamogawa, Chiba 296-8602, Japan. E-mail: oda.nobuhiro.2021@gmail.com Received on March 29, 2025; accepted in revised form on June 9, 2025. © Copyright CLINICAL AND EXPERIMENTAL RHEUMATOLOGY 2025.

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Introduction

Anti-neutrophil cytoplasmic antibody (ANCA) is commonly detected in ANCA-associated vasculitis (AAV) and plays a key role in its pathogenesis (1). However, it has been reported that ANCA can be present even in the absence of vasculitis (2). Among the factors inducing ANCA production, infections have been identified as a potential trigger (3). Notably, tuberculosis (TB) has been associated with a high ANCA positivity rate and is clinically regarded as a "great mimicker" of AAV, requiring careful differentiation (4). Moreover, there have been reports of cases where TB was associated with myeloperoxidase (MPO)-ANCA positivity and AAV development, and a history of TB has been suggested as a risk factor for systemic vasculitis (5, 6). However, there is limited data on ANCA positivity or AAV development in patients with nontuberculous mycobacteria (NTM), a condition closely related to TB. Previous studies have reported only false-positive ANCA results in NTM disease and a few cases of AAV complicating NTM disease (7, 8). Therefore, in this study, we conducted a single-center retrospective analysis to investigate the ANCA positivity rate and the development of AAV in patients with NTM disease.

Materials and methods

We conducted a retrospective observational study of patients diagnosed with pulmonary NTM disease who subsequently underwent MPO or proteinase 3 (PR3) ANCA testing at the Kameda Medical Center and Kameda Kyobashi Clinic from January 1, 2000, to January 24, 2024. Kameda Medical Center is a tertiary care center with approximately 2,000 daily patient visits, while Kyobashi Clinic serves as a satellite center. This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology guidelines. This study has been approved by the research ethics committee of Kameda Medical Center (Approval number: 23-107-250307). All participants provided informed consent through an opt-out process.

Outcome

This study had two primary objectives: firstly, to assess the positivity rates of MPO-ANCA and PR3-ANCA in patients with pulmonary NTM disease, and secondly, to determine the incidence of AAV among those who tested positive for ANCA in this cohort.

Exposure

Patients with respiratory specimens bronchoalveolar lavage (BAL) specimens, and other respiratory specimens) positive for mycobacteria and tested for MPO or PR3 ANCA were screened. Subsequently, patients with pulmonary NTM disease were identified, and those with ANCA measured after diagnosis were analysed. Pulmonary NTM disease was diagnosed based on the American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) diagnostic criteria, and AAV was diagnosed clinically based on the 2012 revised International Chapel Hill Consensus Conference Nomenclature of Vasculitides (9, 10). Clinical diagnoses of granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA) were made according to 2022 American College of Rheumatology/ European Alliance of Associations for Rheumatology Classification Criteria (11, 12, 13).

Data collection

Clinical and laboratory data were obtained from the medical records at the time of ANCA measurement. Clinical data included date of birth, sex, clinical course, autoimmune comorbidities, imaging findings, and laboratory data, MPO-ANCA, PR3-ANCA, and Mycobacterium species identified in respiratory cultures. Smoking status at the time of ANCA measurement and the reason for ANCA measurement were not included in this retrospective study because it was difficult to collect accurate data. Additionally, differentiating interstitial pneumonia or bronchiectasis from NTM-related lung lesions was challenging; therefore, these data were not reported for the overall study population (14).

The ANCA testing method at our institution changed over the study period. The details of how ANCA levels were measured between 2000 and 2012 are unknown. The upper limit of normal in the electronic medical records was 20, so that was adopted. From 2012 to 2018, ANCA was measured using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (Premmune, EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany). From 2018 onward, ANCA was measured using commercial Chemiluminescent Enzyme Immunoassay (CLEIA) kits (STACIA® MEBLux™ test, Medical and Biological Laboratories, Aichi, Japan). Due to a median diagnostic delay of approximately 3 months in Japan, we defined ANCA measured within 1 month before NTM diagnosis as "simultaneous" to account for real-world conditions (15). This cutoff ensures that ANCA measurements performed shortly before an official NTM diagnosis were included in the analysis. In patients with multiple ANCA measurements, only the first measurement was used. The diagnosis date of pulmonary NTM disease was defined as the date of submission of the first culture test confirming NTM. If NTM diagnosis was made at another hospital and the exact date was unknown, the 15th day of the following month was assigned. The follow-up period was defined as the date of NTM diagnosis to the date of the last recorded hospital visit. For patients still attending the hospital, the follow-up period ended on 24 January 2024.

Statistical analysis

Descriptive statistics were obtained for ANCA positive and ANCA negative groups. Continuous variables were summarised as means and standard deviations, while categorical variables were presented as the number of cases and proportions (%). The frequency of missing data for each variable was also presented. For AAV incidence, the number of cases and proportion (%) in each group were reported. A survival analysis evaluating the period from NTM diagnosis to AAV onset was conducted using the Kaplan–Meier curve

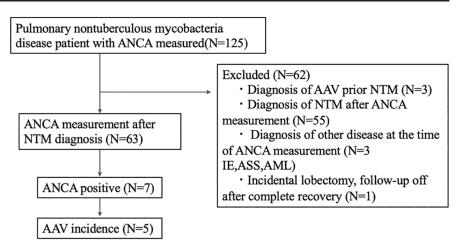


Fig. 1. Patient flow of this study.

NTM: nontuberculous mycobacteria, ANCA: anti-neutrophil cytoplasmic antibody,

AAV: ANCA-associated vasculitis, IE: infectious endocarditis, ASS: anti-synthetase syndrome,

AML: acute myeloid leukemia

with a calculated 95% confidential interval. Statistical analyses were performed using R version 4.1.0.

Case presentation and review

We conducted a detailed chart review to describe the clinical characteristics of each AAV case. To integrate and describe previously reported AAV cases complicated by NTM with the cases confirmed in this study, we performed a PubMed literature review for reports of AAV complicated by NTM, covering publications up to February 20, 2025 (search terms are listed in the Supplement). We collected data on age, sex, period from NTM diagnosis to AAV onset, NTM species, ANCA serotype, ANCA titter, AAV subtype, biopsy site (results), and NTM treatment status before AAV onset.

Results

Patients with respiratory specimens (sputum, BAL or other respiratory samples) positive for mycobacteria who underwent MPO or PR3 ANCA testing were screened (N=165). A chart review was conducted, and patients meeting the ATS/IDSA diagnostic criteria for NTM disease were extracted (N=125) (Fig. 1). To investigate the ANCA positivity rate after NTM onset, patients who had ANCA tested before NTM diagnosis were excluded (N=55). Additionally, patients who underwent lobectomy were excluded due to potential surgical influence (N=1). Cases where

NTM developed after AAV onset were also excluded (N=3). Patients whose ANCA tests were performed during the diagnosis of other diseases were also excluded (N=3). Ultimately, 63 patients were included in our analysis.

Of the 63 patients with pulmonary NTM disease who underwent ANCA testing, seven (11.1%) were positive for ANCA, with five positives for MPO-ANCA and two positives for PR3-ANCA. The mean age was 71.21±10.76 years in the ANCA-negative group and 68.92±19.14 years in the ANCA-positive group. The proportion of male patients was 32.1% (18/56) in the ANCA-negative group and 57.1% (4/7) in the ANCA-positive group. The average duration from NTM diagnosis to ANCA testing was 562.32±979.01 days in the ANCA-negative group and 1699.00±1724.31 days in the ANCApositive group. In two patients with ANCA-positive, testing was performed at the time of NTM diagnosis. Five of seven (71.4%) patients had AAV at the time of ANCA measurement, and two did not develop AAV during the observation period (Table I). Among the seven patients, M. avium was identified in two, M. intracellulare in four, and M. kansasii in one.

Two patients did not develop AAV. In both cases, ANCA levels were measured at the time of NTM diagnosis, and neither patient was receiving treatment for NTM. One patient with M. kansasii tested slightly above the upper limit of

Table. I. Characteristics of patients with pulmonary NTM with and without ANCA positivity.

A	NCA-negativ	ve (mean (SD))	ANCA-positive (mean (SD))			
N	56		7			
Age (years old)	71.21	(10.76)	68.92	(19.14)		
Male (%)	18	(32.1)	4	(57.1)		
Comorbidities						
autoimmune disease	15	(26.8)	0	(0.00)		
Mycobacterium species (%)						
M.avium	26	(46.4)	2	(28.6)		
M.intracellulare	27	(48.2)	4	(57.1)		
M.gordonae	1	(1.8)	0			
M.kansassii	2	(3.6)	1	(14.3)		
ANCA						
ANCA Positive	0		7			
MPO-ANCA positive	0		5			
~2012 (unknown)	6		1			
2012~2018(ELISA)	13		1			
2018~(CLEIA)	37		3			
PR3-ANCA positive	0		2			
~2012 (unknown)	6		1			
2012~2018(ELISA)	11		0			
2018~(CLEIA)	37		1			
The period from NTM diagnos ANCA measurement (days)	is to 562.32	(979.01)	1699.00	(1724.31)		
AAV						
AAV onset	0		5			
Co-occurrence of AAV at diagr of NTM	_		0			

SD: standard deviation; WBC: white blood cell; RBC: red blood cell; Cre: creatinine; CRP: C-reactive protein; ANCA: anti-neutrophil cytoplasmic antibody; AAV: ANCA-associated vasculitis; MPO: my-eroperoxidase; PR3: proteinase3; MPA: microscopic polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; GPA: granulomatosis with polyangiitis.

normal for PR3-ANCA (3.5 U/mL by CLEIA; normal range: <3.5 U/mL), and another with M. intracellulare had

elevated MPO-ANCA (102, unknown method), which turned negative on follow-up examination and no vascu-

litis developed during the observation period (104 days in the former and 322 days in the latter).

Five patients developed AAV (Table II, III). Of these, four tested positive for MPO-ANCA and one for PR3-ANCA. The subtypes of AAV were MPA in three patients (Cases 1~3), EGPA in one (Case 4) and GPA in one (Case 5). Of the five patients, all had Mycobacterium avium complex (MAC), with M. avium identified in two cases and M. intracellulare in three cases. In three patients (Cases 1, 2, and 5), NTM culture positivity was confirmed at the time of AAV diagnosis. Two patients developed AAV while undergoing treatment for NTM. Only one patient developed AAV without having received any treatment for NTM. The remaining patients developed AAV after completing treatment. In total, four patients were either receiving treatment or were in the post-treatment phase when AAV was diagnosed. Notably, all patients demonstrated lung lesions on chest CT at the time of AAV diagnosis, which resembled those observed at the time of NTM diagnosis. The median duration from NTM diagnosis to AAV onset was 1,176 days (interquartile range, 510-2,352 days) and Kaplan-Meier analy-

Table II. Fulfillment of the 2022 ACR/EULAR classification criteria for MPA, GPA, and EGPA in patients from this study.

	case1 MPA	case2 MPA	case3 MPA		case4 EGPA		case5 GPA
p-ANCA/MPO-ANCA (+6)	1	1	1	obstructive airway disease (+3)	0	bloody nasal discharge, nasal crusting or sino-nasal congestion (+3)	0
lung fibrosis or interstitial lung disease (+3)	1	0	0	nasal polyps (+3)	0	cartilaginous involvement	0
pauci-immune glomerulone- phritis (+3)	1	0	1	mononeuritis multiplex/motor neuropathy not due to radiculopathy (+1	1	conductive or sensorineural hearing loss (+1)	0
sino-nasal symptoms or signs (-3)	0	0	0	eosinophilia (+5)	1	c-ANCA/PR3-ANCA (+5)	1
c-ANCA/PR3-ANCA (-1)	0	0	0	extravascular eosinophilic predominant inflammation (+2)	1	pulmonary nodules, mass or cavitation on chest imaging (+2)	1
eosinophilia (-4)	0	0	0	cANCA/PR3-ANCA (-3) haematuria (-1)	0 0	granuloma or giant cells on biopsy (+2) inflammation or consolidation of the nasal/paranasal sinuses on imaging (+1) pauci-immune glomerulonephritis (+1) p-ANCA/MPO-ANCA (-1) eosinophilia (-4)	
Points ≥5	12	6	9	Points ≥6	8	Points ≥5	7

MPO: myeroperoxidase; NTM: nontuberculous mycobacteria; PR3: proteinase3; MPA: microscopic polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; GPA: granulomatosis with polyangiitis; ANCA: anti-neutrophil cytoplasmic antibody; ACR: American College of Rheumatology; EULAR: European Alliance of Associations for Rheumatology.

Table III. Details of patients with AAV diagnosed after NTM disease in our study and previously reported cases.

Case/Reference	age/sex	The period rom NTM diagnosis to AAV onset	Speicies of NTM	ANCA serotype	Titer	AAV subtype	Biopsy site (findings)	NTM treatment status before AAV onset	Mycobacterial culture status at the time of AAV diagnosis	CT findings from the initial presentation of NTM lung disease at the time of AAV diagnosis
Our cases case 1	80M	9 months (278 days)	M.avium	MPO	≥300U/mL (CLEIA)	MPA	Kidney (Crescentic glomerulonephritis)	Under treatment (CAM,EB)	Positive	Unresolved
case 2	76F	6 years (2,386 days)	M.intracellulare	MPO	≥200.0RU/mL (ELISA)	MPA	No	Untreated	Positive	Unresolved
case 3	74F	10 years (3,894 days)	M.intracellulare	MPO	≥300U/mL (CLEIA)	MPA	Kidney (Necrotising crescentic glomerulo- nephritis, Medullary angiitis),skin (Perivascular neutro- philic infiltration)	6 months after completion of treatment (CAM,RFP,EB)	Negative	Unresolved
case 4	74M	10 years (3,878 days)	M.avium	MPO	163U/mL (CLEIA)	EGPA	Skin (Epidermal intracellular oedema and intradermal and eosinophilic periva- scular dermatitis)	3 years after completion of treatment (CAM,RFP,KM)	Negative	Unresolved
case 5	67F	3 years (1,450 days)	M.intracellulare	PR3	34(unknown)	GPA	Kidney (mild vasculitis, interstitial nephritis)	Under treatment (CAM,RFP)	Positive	Unresolved
Previous reports Chaiamnuay, et al. (16)	75F	20 months	M.Avium Intracellae	MPO	29(unknown)	GPA	Lung, skin	2months after completion of treatment (CPFX,AZM,EB)	Unknown	-
Aneet, et al. (17)	59F	12 months	MAC	c-ANCA	1;40	GPA	No	Completion of treatment (AZM,RFP,EB)	Unknown	-
Asano, et al. (18)	73F	1 year	MAC	MPO	7.9 U/mL	MPA	Kidney (crescentic glomerulonephritis, interstitial nephritis with immuno deposition)	Treatment (CAM, REF, EB) preceded but stopped after 2 days	Unknown	-
Addy, et al. (19)	70F	3 months	M.abscessus	MPO	8 AI	MPA	No	Simultaneous treatment (IV Cefoxitin/Amikacin/ CAM/ Minocycline) started	Positive	-
Alnaser, et al. (20)) 27M	Simultaneous	M.kansasii	MPO	150U/mL	MPA	Kidney (Necrotising crescentic glomerulonephritis)	Simultaneous treatment (NTM treatment RFP,EB,INH,PZA)	Positive	-
Kato, et al. (21)	65F	2 years	MAC	MPO, GBM	14.1U/mL	MPA	Kidney (crescentic glomerulonephritis, Medullary angiitis, interstitial nephritis)	Untreated	Unknown	-
Tomoshima, et al. (8)	81F	Simultaneous	M.avium	МРО	107IU/mL	OMAAV	No	Simultaneous treatment (CAM, RFP, EB)	Positive	-
Yoshii, et al. (22)	72M	7years	M.intracellulae	MPO	611U/mL	MPA	Kidney (Necrotising crescentic glomerulonephritis)	Under treatment (CAM,RFP,EB)	Unknown	-

M: male; F: female; MPO: myeroperoxidase; NTM: nontuberculous mycobacteria; ANCA: anti-neutrophil cytoplasmic antibody; PR3: proteinase3; MPA: microscopic polyangiitis; EGPA: eosinophilic granulomatosis with polyangiitis; GPA: granulomatosis with polyangiitis; OMAAV: otitis media with anti-neutrophil cytoplasmic antibody-associated vasculitis; PSL: prednisolone; RTX: rituximab; AZA: azathioprine; PE: plasma exchange; CYC: cyclophosphamide; MAC: Mycobacterium avium complex; CAM: clarithromycin; RFP: rifampicin; INH: isoniazid; EB: ethambutol; AZM: azithromycin; KM: kanamycin.

sis demonstrated that the AAV-free survival rate among patients with NTM gradually decreased over time. None of the patients with a negative initial ANCA had chart-confirmed AAV onset during the observation period.

Case presentation

Case 1: An 80-year-old man with a history of diabetes mellitus presented with worsening kidney function and haematuria 9 months after diagnosis and 6 months after treatment for pulmonary NTM disease with *M. avium*. Blood tests revealed positive MPO-ANCA, CT showed infiltrating shadows with cavities, and kidney biopsy showed crescentic glomerulonephritis, leading to a diagnosis of MPA.

Case 2: A 76-year-old woman with a history of pulmonary NTM disease due to M. intracellulare presented with fatigue, anorexia, and low-grade fever for 2 months. She had worsening kidney function with an elevated inflammatory response, proteinuria, hematuria, and MPO-ANCA positivity, leading to a diagnosis of MPA. She did not undergo biopsy.

Case 3: A 74-year-old woman with a history of pulmonary NTM disease of M. intracellulare had pain in her extremities for 6 months, purpura on her lower legs for 2 months, anorexia, and low-grade fever. Further examination revealed kidney impairment with hematuria and proteinuria, and positive MPO-ANCA. Kidney biopsy revealed necrotising crescentic glomerulone-phritis and medullary angiitis, leading to a diagnosis of MPA.

Case 4: A 74-year-old man with pulmonary NTM disease by M. avium, previously treated but currently untreated, developed new lung consolidations and elevated inflammatory markers 1 month before AAV diagnosis. He was treated for bacterial pneumonia but remained refractory. Simultaneously, he began to notice purpura, an abnormal sensation in both lower extremities, and muscle weakness. Further examination revealed MPO-ANCA positivity and increased eosinophilia. A skin biopsy of the purpura revealed intradermal and eosinophilic perivascular dermatitis, leading to a diagnosis of EGPA.

Case 5: A 67-year-old woman with pulmonary NTM of M. intracellulare treated with antibiotics was diagnosed with AAV after a 3-month history of fever of unknown origin, proteinuria, and positive PR3-ANCA. Kidney biopsy showed mild vasculitis and interstitial nephritis. Despite the absence of granulomatous lesions in the biopsy, the patient was diagnosed with GPA based on 2022 diagnostic criteria retrospectively.

Literature review

We screened 97 articles in PubMed and identified 8 case reports of AAV complicated by pulmonary NTM disease (8, 16-22) (Table II). The mean patient age was 65.3 years, and 75% were female. Seven patients were positive for MPO-ANCA, while one was positive for c-ANCA. AAV subtypes included 2 cases of GPA, 5 of MPA, and one of otitis media with AAV (OMAAV). Vasculitis developed concurrently with NTM culture positivity in 38% (3/8) of cases, while the remaining patients developed vasculitis between 3 months and 7 years after NTM diagnosis. 75% of patients had pulmonary NTM disease with MAC. 50% (4/8) patients who developed AAV was either undertreated or had completed treatment for NTM.

Discussion

This study is one of the largest to examine the proportion of ANCA-positivity with pulmonary NTM disease. We found that ANCA was positive in 11.1% of patients, predominantly MPO-ANCA. Two patients who tested positive for ANCA but did not develop vasculitis were weakly positive or measured using previous assays and improved spontaneously. 71.4% of patients demonstrating ANCA positivity developed AAV, predominantly MPA. Most patients had MAC infections. In three patients, NTM culture positivity persisted at the time of AAV diagnosis, and all patients had lung lesions consistent with prior NTM infection. Finally, we identified 8 previous reported cases of AAV complicated by pulmonary NTM disease. The prevalence of ANCA positivity in NTM has

been rarely reported in the literature. Only one case of false-positive ANCA results has been reported in a patient with NTM (23). In contrast, there have been eight reported cases of AAV occurring in association with NTM. (8, 16-22). This skewed report supports our observation that most patients with ANCA positivity developed systemic vasculitis. This study found that two patients who were positive for ANCA did not develop vasculitis. In the general Japanese population, ANCA positivity has been reported in approximately 0.9% of individuals (24). In comparison, our study found that even among NTM patients without AAV, 3.2% were ANCA-positive, suggesting a potential association between NTM infection and increased ANCA production.

In our study, we identified 5 cases of AAV in patients with pulmonary NTM disease. Furthermore, according to our PubMed search, eight cases of AAV in patients with pulmonary NTM have been reported to date (Table II). In previous reports, six of eight cases of NTM with AAV involved female patients, consistent with our findings where three out of five patients were female, demonstrating a female preponderance. Furthermore, most patients in our study were MPO-ANCA positive. Similarly, most previously reported cases were MPO-ANCA positive, suggesting a potential association between NTM and MPO-ANCA. Reflecting this antibody trend, the majority of AAV subtypes in our study and previous reports were MPA with glomerulonephritis. On the other hand, we diagnosed one case each of EGPA and GPA, and previous reports included one case each of GPA and OMAAV. This suggests that, while MPA is the most common subtype, other forms of AAV can also occur in association with NTM. The time from NTM disease onset to AAV onset varied widely from 9 months to 10 years in our study, and a similar trend was observed in previous reports ranging from several months to 7 years (Fig. 2). Some cases of NTM disease and AAV were diagnosed simultaneously in previous reports. As for the bacterial strains, in our study, M. avium was identified in two cases and

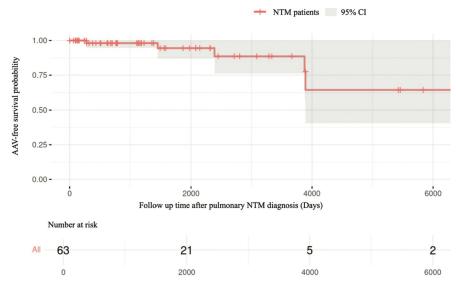


Fig. 2. Kaplan-Meier curve showing AAV-free survival in patients with NTM, with 95% confidence intervals

NTM: nontuberculous mycobacteria; AAV: anti-neutrophil cytoplasmic antibody -associated vasculitis; CI: confidence interval.

M. intracellular in three. Similarly, four previously reported cases involved M. avium, which may reflect the predominance of MAC among NTM infections (25). It is also noteworthy that all cases in this study involved pathogenic NTM species. The most commonly affected organ in vasculitis was the kidney, and many cases were accompanied by fever. Therefore, when an unexplained fever or glomerulonephritis/acute kidney injury occurs during an NTM infection, the possibility of concurrent AAV should be considered.

While the role of infections in autoantibody induction in connective tissue diseases is known, our research proposes a potential link between NTM infection and AAV development, potentially through ANCA production, especially MPO-ANCA, through three proposed mechanisms (26). First, ANCA production may arise from pulmonary NTM infection. MPO-ANCA is known to be pathogenic in inducing nephritis, with its generation linked to MPO exposure via NETosis and impaired NET clearance (27-29). Necrotizing granulomas and NETosis are documented in NTMinfected lungs, mirroring potential pathways in TB where NETosis in caseous necrosis has been implicated in ANCA production (30-32). The established association between infections and AAV, particularly the increased

incidence of MPO-ANCA-positive AAV with infections (3, 33) and the correlation between pneumonia and MPO-ANCA-associated AAV, further supports this hypothesis. Animal models demonstrating MPA-like disease with co-induced influenza infection in MPO-ANCA injected mice also highlight this relation (34). Our observation of NTM excretion in over half the patients at the time of ANCA positivity or AAV diagnosis, along with the tendency for longer NTM disease duration in ANCA-positive patients, suggests a role for NTM lung infection and chronic inflammation in MPO-ANCA production and AAV onset. Second, ANCA production and AAV onset may be secondary to NTM-associated interstitial pneumonia or bronchiectasis. ANCA has been reported in patients with interstitial pneumonia, some of whom later develop AAV. Given that interstitial pneumonia can be a complication of NTM infection, its impact cannot be excluded (14, 35). Bronchiectasis, another complication of NTM, is also a recognized risk factor for MPO-ANCA-positive AAV (36, 37). In our study, AAV was observed many years after NTM diagnosis in several patients, including two with persistent pulmonary infiltrates despite the absence of ongoing bacterial excretion. Taken together with previous reports linking tuberculosis to systemic vasculitis, these findings suggest that NTM infection itself, particularly when persisting for more than 10 years may induce structural pulmonary changes that contribute to vasculitis development (6). Third, drug-induced ANCA is a possibility. Although ANCA production has been associated with anti-tuberculosis drugs, and most AAV patients in our study were exposed to such treatments, at least one AAV case occurred without any prior anti-TB therapy (38). Based on these findings, we propose the potential existence of a distinct clinical entity, "NTM-AAV", representing a subgroup of patients in whom chronic NTM infection may play a role in the pathogenesis of AAV. However, further research is warranted to definitively establish the causal relationship and elucidate underlying mechanisms.

Our study has some limitations. Firstly, although it represents the largest study of its kind to date, it was conducted at a single-center and involved a small number of cases. Therefore, generalizability of the findings may be limited, and due to insufficient statistical power, we were unable to perform statistical analyses to identify risk factors for ANCA positivity. Furthermore, since ANCA measurement was left at the discretion of the treating physician, the possibility of selection bias cannot be denied. Secondly, in many of the patients who developed AAV, a period of time had elapsed since their NTM diagnosis, and questions remain regarding the correlation. Whether the presence of NTM causes AAV requires studies including appropriate healthy control groups. Thirdly, the mechanisms underlying ANCA induction are not well understood, raising the possibility of confounding factors. Notably, our study did not examine the presence of interstitial pneumonia or bronchiectasis; therefore, the possibility that these factors preceded the development of NTM and AAV cannot be ruled out. Lastly, there was a period during which the ANCA testing method was unknown, and the results from this period may not be generalizable. In Japan, PR3-ANCA testing by ELISA became available in

1993, followed by MPO-ANCA testing in 1998. Since 2012, CLEIA has been widely used (39). Although ANCA measurement methods have evolved over time in Japan, a study comparing ELISA (commonly used before 2012) and CLEIA (widely adopted thereafter) found that both methods had comparable diagnostic accuracies for AAV (40). Since only a small number of cases in this study were tested before 2012, and the accuracy of the test method at that time was likely comparable to the current one, we did not consider the impact to be significant. Finally, as this was a single-center retrospective study, the prevalence of asymptomatic ANCA positivity among NTM patients remains unclear. These limitations can be resolved in prospective studies.

Conclusion

This study is one of the largest to show the proportion of patients demonstrating ANCA positivity in pulmonary NTM disease. ANCA was positive in 11.1% of patients, predominantly MPO-ANCA. Additionally, 71.4% of cases with positive ANCA developed AAV, predominantly MPA. These findings suggest a potential link between NTM infection and AAV. Based on our results and the consideration of possible underlying mechanisms, we propose the existence of a distinct subgroup of patients with NTM-AAV. Since NTM-AAV frequently involves glomerulonephritis, monitoring ANCA should be considered in patients under NTM follow-up, particularly in patients presenting with haematuria or unexplained inflammatory responses. Further large-scale studies are needed to elucidate the association between NTM infection, ANCA production and the development of AAV.

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