Treatment outcomes in ANCA-associated vasculitis

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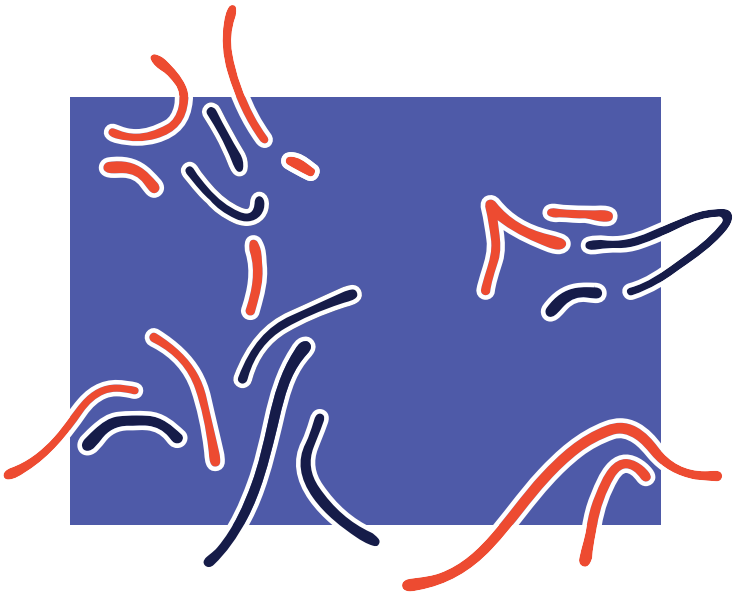
05 Chapter

Geographic differences in clinical presentation and outcome of antineutrophil cytoplasmic antibody-associated vasculitis: role of antibody specificity



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ABSTRACT

Objective
Clinical characteristics of ANCA-associated vasculitis (AAV) differ between geographic regions and ethnicities. Since ANCA-specificity varies between geographic regions and has been associated with differences in clinical picture of AAV, our objective was to investigate whether regional differences in clinical manifestations and outcomes might (partly) be explained by differences in ANCA-specificity.

Methods
ANCA specificity, organ manifestations at diagnosis, relapse-free survival and overall survival were compared between AAV patients from Dutch (n=264), Chinese (n=411) and Brazilian (n=97) observational cohorts.

Results
Frequencies of disease manifestations differed between countries. Mucosa/eye and otolaryngeal involvement were both associated with the presence of PR3-ANCA, irrespective of country. The differences of other organ manifestations between countries were independent of ANCA-specificity. In Cox regression, after correction for ANCA-specificity and organ manifestations associated with relapse risk, Chinese patients had an increased risk of relapse compared to patients from the Netherlands and Brazil (HR 1.9, 95% CI 1.3 to 2.8; Bonferroni-corrected P=0.03). Chinese patients had an increased mortality rate compared to patients from the Netherlands and Brazil (HR 15.5, 95% CI 6.7 to 36.0; P<0.001).

Conclusion
The lower frequencies of mucosa/eye and otolaryngeal involvement in China can be explained by a lower frequency of PR3-ANCA specificity. Chinese patients have a higher relapse risk than expected from lower frequencies of PR3-ANCA, GPA, and otolaryngeal involvement, and more frequent renal involvement. They also have a higher risk of mortality even after correction for baseline characteristics and treatment. This suggests that additional risk factors for relapse and mortality are present in this population.

INTRODUCTION

Clinical characteristics of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) differ between geographic regions and different ethnicities. Most importantly, while granulomatosis with polyangiitis (GPA) and PR3-ANCA specificity are most common in Northern Europe, microscopic polyangiitis (MPA) and MPO-ANCA specificity are more common in Southern Europe, Japan and China [1].

Previous studies found that PR3-ANCA specificity is a risk factor for relapse [2,3]. Also, PR3-ANCA positive patients had different genetic associations compared to MPO-ANCA positive patients in a Genome-Wide Association Study [4]. Therefore, differences in clinical characteristics and outcomes between populations might in part be explained by ANCA specificity. In two studies comparing the UK to Japan, ANCA type explained most phenotypic differences between GPA patients of both countries [5], while several population differences in organ manifestations of MPA patients could not be explained by ANCA specificity [6].

In this study, we sought to investigate the differences in disease characteristics and clinical outcome between a Brazilian, Chinese and Dutch cohort of AAV patients, spanning multiple continents and including long-term follow-up. Secondly, we aimed to investigate whether these differences might be explained by population differences in ANCA specificity.

PATIENTS AND METHODS

Study populations

For this retrospective cohort study, 264 patients were recruited from the departments of Internal Medicine/Nephrology and Rheumatology of the University Medical Center Groningen in the Netherlands, 411 from the Institute of Nephrology, Peking University First Hospital in China, and 97 from Rheumatology divisions of the following centers in Brazil: Hospital Federal dos Servidores do Estado do Rio de Janeiro (HFSE-RJ), Universidade do Estado do Rio de Janeiro (UERJ), State University of São Paulo, and. Universidade Federal de São Paulo - Escola Paulista de Medicina. Consecutive GPA and MPA patients diagnosed between 1987 and 2015 (Brazil), between 1996 and 2012 (China), and between 1990 to 2015 (Netherlands), respectively, were considered for inclusion in the study. Patients were classified according to the 2012 updated Chapel Hill Consensus Conference definitions [7]. Patients were treated according to previously described guidelines used in the respective countries [8-10]. All patients signed informed consent for collection of their data for the study. In the Netherlands, approval was given by the local Medical Ethical Committee of the University Medical Center Groningen (METc 2010.057). In China, approval was provided by the Ethics Committee of the Peking University First Hospital (IRB00001052-16049). In Brazil, approval was granted by the Ethics Committee on Research (process nr. 0147/2016). The research was conducted according to the principles from the declaration of Helsinki.

Data collection

Baseline patient characteristics were age, sex and geographic origin. The following disease characteristics at baseline were collected: diagnosis (GPA, MPA or renal limited vasculitis), disease activity and organ involvement using the Birmingham Vasculitis Activity Score (BVAS) [11], and ANCA status and specificity by Indirect Immune Fluorescence (IIF) or ELISA, if available. Modality of induction therapy was collected as treatment information. As follow-up data, relapses (new onset of disease activity attributable to vasculitis [12]) and mortality were collected for all centers.

Statistical analysis

Statistical analyses were performed using SPSS version 23 (IBM) and R version 3.4.2. A two-sided P-value <0.05 was considered statistically significant. Data are presented as mean (SD) or median (IQR) for continuous variables as appropriate, and as n (%) for categorical variables. Univariable analyses were performed to compare baseline variables between geographic origins. These were Kruskall Wallis test for continuous variables and Fisher's Exact test for categorical variables. In case of significant group differences, post-hoc tests were performed. Multivariable Cox regression was performed for relapse-free survival using geographic origin and previously reported factors associated with relapse-free survival as predictors [2,13]. Geographic origin, in addition to previously reported factors associated with mortality in AAV [14], were used as predictors in multivariable Cox regression analysis of overall survival. The proportional hazards assumption was tested using the scaled Schoenfeld residuals test. Due to the large number of tests performed in this study, Bonferroni-corrected P-values were calculated by multiplying all P-values by the number of tests performed (i.e., 74). P-values greater than 0.99 after Bonferroni correction are shown as P>0.99. A Bonferroni-corrected P-value <0.05 (corresponding to an unadjusted P-value of $6.8*10^{-4}$) was considered statistically significant.

RESULTS

Baseline differences

Several differences existed between patients from the three countries. Chinese patients were significantly older at diagnosis than Dutch and Brazilian patients. Chinese patients were less frequently PR3-ANCA positive and less frequently diagnosed with GPA compared to Dutch and Brazilian patients. See **Table 1**.

Disease characteristics per population are also shown in **Table 1**. Chinese patients, compared to both Dutch and Brazilian patients, less frequently had eye/mucosa (i.e., mouth ulcers, episcleritis) and ENT involvement (i.e., nasal complaints, sinusitis, otitis media) and more frequently had renal involvement (i.e., proteinuria, hematuria, elevated serum creatinine). Dutch patients less frequently had pulmonary involvement (i.e., nodules on chest imaging) compared to patients from other countries, and less frequently had abdominal involvement (i.e., abdominal pain/bloody diarrhea) compared to Chinese patients. Brazilian patients more frequently had skin involvement (i.e., ulcers, purpura) compared to Chinese patients, and less frequently had systemic involvement (i.e., malaise) compared to both other countries.

Table 1. Baseline characteristics.

Variable	NL (n=264)	CN (n=411)	BR (n=97)	Overall P†	P CN vs NL†	P BR vs NL†	P CN vs BR.†
Male	138 (52)	194 (47)	43 (44)	>0.99			
Age (years)	52 (40-63)	66 (53-73)	44 (36-57)	**<0.001**	**<0.001**	0.19	**<0.001**
GPA	208 (79)	97 (24)	88 (91)	**<0.001**	**<0.001**	0.91	**<0.001**
PR3-ANCA (ANCA-pos.)	191/248 (77)	38/409 (9)	47/56 (84) ‡	**<0.001**	**<0.001**	>0.99	**<0.001**
Creatinine >125 µmol/l	94 (36)	282 (69)	34 (35)	**<0.001**	**<0.001**	>0.99	**<0.001**
Induction				**<0.001**	**<0.001**	**<0.001**	**<0.001**
Oral CYC	156 (71)	67 (16)	40 (42)				
Pulsed CYC	3 (1)	248 (60)	32 (33)				
Other	62 (28)	96 (23)	24 (25)				
Disease activity	17 (11-23)	20 (15-23)	17 (12-22)	**0.001**	**0.001**	>0.99	0.20
Systemic	216 (82)	373 (91)	62 (64)	**<0.001**	0.06	**0.05**	**<0.001**
Cutaneous	46 (17)	49 (12)	33 (34)	**<0.001**	>0.99	0.11	**<0.001**
Mucosa/eyes	64 (24)	47 (11)	27 (28)	**<0.001**	**0.001**	>0.99	**0.01**
Otolaryngeal	179 (68)	150 (36)	71 (73)	**<0.001**	**<0.001**	>0.99	**<0.001**
Chest	121 (46)	294 (72)	67 (69)	**<0.001**	**<0.001**	**0.006**	>0.99
Cardiovascular	7 (3)	13 (3)	2 (2)	>0.99			
Abdominal	4 (2)	66 (16)	6 (6)	**<0.001**	**<0.001**	>0.99	0.71
Renal	161 (61)	387 (94)	60 (62)	**<0.001**	**<0.001**	>0.99	**<0.001**
Neurological	61 (23)	76 (18)	14 (14)	>0.99			

Variables shown as N (%) or median (IQR). BR Brazil; CN China; CYC cyclophosphamide; GPA granulomatosis with polyangiitis; NL Netherlands. † Bonferroni corrected. ‡21 patients PR3-ANCA positive ELISA; 26 patients c-ANCA on IIF, no ELISA.

In multivariable logistic regression, country remained significantly associated with systemic, chest, abdominal and renal involvement of AAV independent of ANCA-specificity. Presence of PR3-ANCA was the main predictor of mucosa/eye and ENT involvement. Results are shown in **Table 2**.

Table 2. Multivariable logistic regression of organ manifestations per country

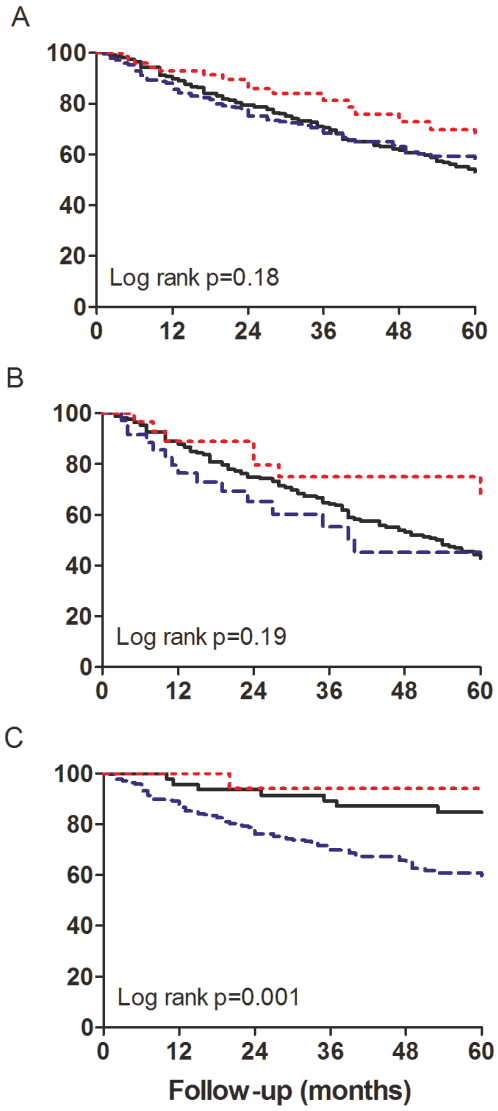
Variable		OR (95% CI)	P-value†
Systemic	China (vs Netherlands)	3.8 (2.1 to 6.9)	<0.001*
	Brazil (vs Netherlands)	0.3 (0.1 to 0.5)	
	PR3-ANCA (vs MPO-ANCA)	3.3 (1.8 to 6.2)	0.009*
Skin	China (vs Netherlands)	0.8 (0.5 to 1.5)	0.14
	Brazil (vs Netherlands)	2.8 (1.5 to 5.2)	
	PR3-ANCA (vs MPO-ANCA)	1.6 (0.9 to 2.8)	>0.99
Eye/mucosa	China (vs Netherlands)	1.1 (0.6 to 1.9)	>0.99
	Brazil (vs Netherlands)	1.1 (0.6 to 2.2)	
	PR3-ANCA (vs MPO-ANCA)	4.4 (2.4 to 7.8)	<0.001*
Otolaryngeal	China (vs Netherlands)	0.8 (0.5 to 1.2)	>0.99
	Brazil (vs Netherlands)	1.0 (0.5 to 2.0)	
	PR3-ANCA (vs MPO-ANCA)	5.3 (3.4 to 8.4)	<0.001*
Chest	China (vs Netherlands)	3.6 (2.3 to 5.7)	<0.001*
	Brazil (vs Netherlands)	2.7 (1.4 to 5.1)	
	PR3-ANCA (vs MPO-ANCA)	1.5 (0.9 to 2.3)	>0.99
Abdominal	China (vs Netherlands)	17.4 (5.5 to 55.4)	<0.001*
	Brazil (vs Netherlands)	7.1 (1.9 to 26.1)	
	PR3-ANCA (vs MPO-ANCA)	1.8 (0.9 to 3.8)	>0.99
Renal	China (vs Netherlands)	7.7 (4.2 to 14.1)	<0.001*
	Brazil (vs Netherlands)	1.1 (0.6 to 2.0)	
	PR3-ANCA (vs MPO-ANCA)	0.7 (0.4 to 1.3)	>0.99

*Country and ANCA specificity were entered simultaneously as predictors of organ involvement. * Statistically significant. † Bonferroni corrected.*

Relapse-free survival

Using a log rank test, relapse-free survival did not differ between countries (Bonferroni-corrected P>0.99). Chinese MPO-ANCA positive patients had a higher risk of relapse, but only before Bonferroni correction (**Figure 1**). Relapse free survival was not affected by induction therapy (corrected P>0.99). In multivariable Cox regression, after correction for ANCA-specificity, otolaryngeal and chest involvement, as well as serum creatinine >125 µmol/l at diagnosis, Chinese patients (HR 1.9, 95% CI 1.3 to 2.8), but not Brazilian patients (HR 0.5, 95% CI 0.2 to 1.1), had an increased risk of relapse compared to Dutch patients (Bonferroni-corrected P=0.03).

Figure 1. 60-month relapse free survival per country stratified by ANCA type



Relapse-free survival (%) for Brazilian (red line), Chinese (blue line) and Dutch (black line) patients. A: overall, B: PR3-ANCA positive, C: MPO-ANCA positive. P-values shown are unadjusted.

Mortality

In log rank analysis, Chinese patients had a significantly higher 5-year mortality rate than Brazilian and Dutch patients (Bonferroni-corrected P<0.001). This was true for both MPO-ANCA and PR3-ANCA positive patients (**S1 Figure**). In multivariable Cox regression with adjustment for age, serum creatinine >125 μmol/l at diagnosis, pulmonary involvement and induction treatment, patients from China still had a higher risk of mortality (Bonferroni-corrected P<0.001) compared to Dutch patients (HR 15.5, 95% CI 6.7 to 36.0). Brazilian patients also had an increased risk of mortality (HR 3.5, 95% CI 1.1 to 11.6), although this difference was no longer statistically significant after correction for multiple comparisons.

DISCUSSION

In this study, we found differences in distribution of ANCA-specificity, clinical characteristics and clinical outcome between Brazil, China and The Netherlands. Except for eye/mucosa and ENT involvement, both of which were mainly associated with PR3-ANCA positivity, inter-regional differences in clinical manifestations persisted after correction for ANCA specificity.

After correction for ANCA specificity, otolaryngeal involvement, chest involvement and serum creatinine >125 μmol/l at diagnosis, relapse risk was significantly higher for Chinese patients compared to Dutch and Brazilian patients. Based on a lower frequency of PR3-ANCA positivity and patients with otolaryngeal involvement [13], as well as a higher frequency of patients with elevated creatinine levels at diagnosis [2], Chinese patients were expected to have a lower risk of relapse compared to Dutch and Brazilian patients. However, despite these disease characteristics, they had a similar risk of relapse. This indicates an additional risk factor for relapse in Chinese patients that is not, or to a lesser extent, present in Dutch and Brazilian patients. As a relatively large number of Chinese patients used IV cyclophosphamide, which has been associated with an increased risk of relapse compared to oral cyclophosphamide [15], we hypothesized that the more frequent use of IV cyclophosphamide in China might explain the higher-than-expected risk of relapse for this group. However, the type of induction therapy was not associated with relapse-free survival in this study, possibly due to the Chinese treatment protocol dictating longer duration of IV cyclophosphamide (6-9 months) compared to oral cyclophosphamide (3-4 months) induction therapy [8].

Chinese patients had a higher risk of mortality regardless of ANCA type, even after correction for factors associated with mortality such as age, elevated serum creatinine at diagnosis, pulmonary involvement and type of induction treatment. This again indicates that AAV in Chinese patients behaves differently during follow-up than in comparable Brazilian and Dutch patients. One explanation might be a worse renal function in Chinese AAV patients, most likely because the Chinese cohort being derived from a tertiary Nephrology referral center. Alternatively, unmeasured differences in treatment or follow-up could be an explanation, although reported adherence to the treatment protocol is strong in all countries.

In line with previous studies, PR3-ANCA positivity, as well as eye/mucosa and ENT involvement, were less common in China compared to Brazil and the Netherlands [1]. The high frequency of Chinese patients with kidney involvement and elevated serum creatinine in this study is most likely due to the participating center in China being a tertiary nephrology referral center. Most other differences in disease characteristics between patients from China versus the other two countries can be explained by a lower frequency of PR3-ANCA positive patients in China. An exception is the higher frequency of abdominal pain/bloody diarrhea in China compared to the Netherlands.

Striking differences between Brazil and the other countries were a higher frequency of skin manifestations (ulcers and purpura) compared to Chinese patients and a lower frequency of systemic AAV manifestations (malaise) compared to both other countries. We did not find a clear explanation for these differences in the present study. The difference might result from genetic or environmental factors. Alternatively, the differences might be due to the Rheumatology department being the main source of patients from the Brazilian cohort, while Dutch and Chinese patients were partly or fully included from Nephrology departments.

Dutch patients have a relatively low frequency of pulmonary nodules. A possible explanation might be less frequent pulmonary imaging in the Netherlands, considering that imaging may still show pulmonary abnormalities in asymptomatic patients. Unfortunately, data on whether or not imaging was performed was not collected in the database.

A major strength of this study is the availability of long-term follow-up data, allowing for comparison of treatment outcomes between countries. Also, Chinese and Dutch patients were both recruited from one center, each using their own standardized treatment protocol. This results in less variation in treatment of patients from the respective countries. Another strength is the strict correction for multiple comparisons, which increases confidence in the relevance of statistically significant findings in this study.

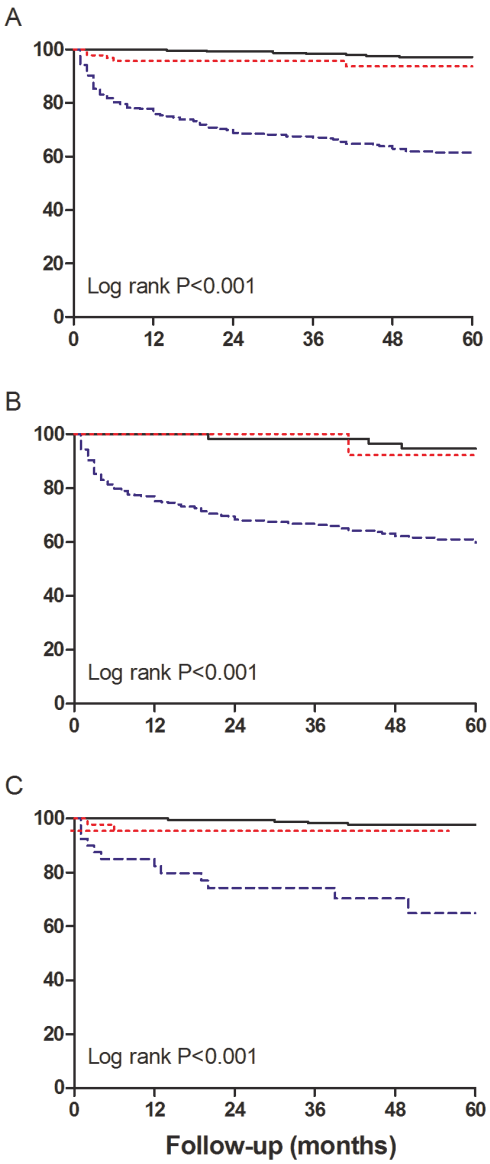
The study also has several limitations. First, data for each study has been collected in different clinical centers. This results in different treatments per country, as well as possible differences in disease assessment, although the same criteria were used to classify patients and the same BVAS version was used to score disease manifestations. Second, differences in distribution of recruiting specialties could have resulted in over- or underrepresentation of patients with certain organ manifestations in a country. For example, the frequency of renal involvement may be overestimated in Chinese patients of this study, because the recruiting center was a nephrology specialty referral hospital. Lastly, exact serum creatinine levels at diagnosis were not available for all cohorts. Also, insufficient data about renal function and dialysis dependence over time was available to include them in the study. These data would have been especially relevant in relation to overall survival in the different cohorts.

In conclusion, differences in mucosa/eye and ENT involvement between Chinese patients versus Dutch and Brazilian patients could be explained by the lower frequency of PR3-ANCA in China. Other differences in organ manifestations between countries could

not be explained by differences in ANCA-specificity. Chinese patients have a similar risk of relapse to Dutch and Brazilian patients despite a theoretically lower risk of relapse based on disease characteristics and ANCA-specificity, as well as a higher risk of mortality, suggesting the presence of additional risk factors for relapse and mortality in the Chinese population.

SUPPORTING INFORMATION

S1 Figure. 60-month overall survival per country stratified by ANCA type



Overall survival (%) for Brazilian (red line), Chinese (blue line) and Dutch (black line) patients. A: overall, B: MPO-ANCA positive, C: PR3-ANCA positive. P-values shown are unadjusted.

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