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PREVALENCE OF ANCA IN PATIENTS WITH MULTI SYSTEM DYSFUNCTION AND LUNG PARENCHYMAL DISORDERS IN INTENSIVE CARE SETTINGS

Col. Dr. Vishal Marwaha	Principal, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.
Dr. Pranav Raman Chickermane	Senior Resident, Department of Clinical Immunology and Rheumatology, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.
Dr. Neeraj Sidharthan*	Associate Professor, Department of Clinical Hematology, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India. *Corresponding Author
Dr. Mithun C.B.	Associate Professor, Department of Clinical Immunology and Rheumatology, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.
Dr. Bharat Pillai	House surgeon, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.
Dr. Arun Tiwari	Senior Resident, Department of Clinical Immunology and Rheumatology, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.
Dr. Vaishnavi Sujathen	House surgeon, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.
Dr. Belinda Philip	House surgeon, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.
Movva Hamsini	III MBBS (Part-I) student, Amrita School of Medicine, Amrita Institute of Medical Sciences, Amrita Vishwa Vidyapeetham, Kochi-682041, India.

ABSTRACT

BACKGROUND- ANCA (Anti-neutrophil cytoplasmic antibody (ANCA)- associated vasculitides (AAV) present a diagnostic challenge to clinicians. They can be misdiagnosed if over reliance is placed on ANCA testing. This study has been done to assess the ANCA positivity in patients with multisystem dysfunction and lung parenchymal disorders, which often mimic AAV.

METHODS- This prospective study was conducted from June 2007 to June 2010. A total of 100 patients- 50 each with multisystem dysfunction (MSD) and parenchymal lung diseases were included in the study. ANCA testing was done in all cases by indirect immunofluorescence (IIF) and enzyme-linked immune sorbent assay (ELISA), i.e., anti-proteinase 3 (anti-PR3) and anti-myeloperoxidase (anti- MPO).

RESULTS- Of the 100 patients studied, two were diagnosed to have granulomatosis with polyangiitis (GPA). ANCA by IIF and anti-PR3 by ELISA were positive in one out of the 2 cases with GPA. Among the non- GPA cases (n= 98), 8 were false positive by IIF with all of them showing an atypical pattern. None of the 98 non-GPA cases was positive for anti-PR3 by ELISA while 4/98 of these cases were falsely positive for anti-MPO by ELISA.

CONCLUSION- We found that cytoplasmic and perinuclear pattern on ANCA by IIF showed high specificity for AAV. False positive cases on ANCA by IIF showed an atypical pattern. ANCA (anti-PR3) by ELISA is very specific for vasculitis. ANCA (anti-MPO) by ELISA was found to be less specific for vasculitis. In the patient of GPA with positive anti-PR3, the anti-PR3 levels corroborated with disease activity. Tissue diagnosis still remains the gold standard.

KEYWORDS : ANCA, indirect immunoflorescence (IIF), enzyme-linked immune sorbent assay (ELISA), ANCA- associated vasculitides (AAV)

INTRODUCTION:

Anti-neutrophil cytoplasmic antibodies (ANCAs) such as those directed towards proteinase 3 (PR3) and myeloperoxidase (MPO), are associated with a distinct form of small vessel vasculitis , known as ANCA-associated vasculitis (AAV), a term that encompasses granulomatosis with polyangiitis (GPA) formerly known as Wegener's granulomatosis (WG), microscopic polyangiitis (MPA), and eosinophilic granulomatosis with polyangiitis (EGPA), formerly known as Churg Strauss syndrome (CSS) [1]. The AAVs are an important cause of morbidity and mortality. They present a challenge to clinicians, given their rarity. The two commonly used methods of testing for ANCA are indirect immunofluorescence (IIF) and enzyme-linked immune sorbent assay (ELISA), the latter detecting anti-proteinase 3 (anti-PR3) and anti-myeloperoxidase (anti- MPO) antibodies. These tests when used in appropriate clinical settings provide support for the diagnosis of an AAV [2]. On the other hand, the predictive value of these tests is significantly reduced when applied in inappropriate clinical settings. False positive ANCA has been reported in many conditions which include rheumatic autoimmune diseases, inflammatory bowel disease, infections and malignancies [3]. A number of conditions such as parenchymal lung disorders and those involving multi-system dysfunction (MSD) may mimic AAVs. Hence, it is important to determine the frequency and pattern of ANCA in these conditions so as to avoid misdiagnosis and inappropriate therapy [4]. As there is paucity of data in this regards, we conducted this study to evaluate the frequency of ANCA by indirect immunofluorescence and ELISA in these conditions.

MATERIALS AND METHODS:

This two centre study was conducted at Command Hospital (Central Command), Lucknow and Army hospital (R&R), Delhi Cantt. from Jun 2007 to Jun 2010.After obtaining clearance from the hospitals' ethics committee and informed consent from the patients, 100 patients with MSD (n=50) and parenchymal lung diseases (n=50) based on clinical

and radiological features admitted to the intensive care unit and critical care medical wards of the above hospitals were enrolled on the study. A detailed history and clinical examination was done as per protocol and involvement of systems ascertained. Baseline investigations included hemogram, C-reactive protein, liver and renal function tests, fasting and post-prandial blood sugar, anti-nuclear antibodies (ANA) by indirect immunofluorescence, urinalysis, chest radiograph and electro-cardiogram in all the cases. Serum C3, C4 and anti-ds-DNA testing was done if SLE was a distinct possibility. CD4, CD8 levels were done in HIV positive patients. Radiographs of the paranasal sinuses, ultrasound abdomen and 2D ECHO were done, wherever indicated. Renal biopsy was done in patients with active urinary sediments or with suspicion of nephritis. Relevant cultures were done(blood, urine, as indicated). HIV-1 & 2, HBsAg, Anti-HCV testing and Anti-cardiolipin antibody (IgG& IgM), lupus anticoagulant, RF, cryoglobulins were done in suspected cases of secondary vasculitis or antiphospholipid syndrome. Pre-test counselling was done for HIV testing. ANCA testing was done in all cases by IIF and ELISA (proteinase-3 and myeloperoxidase). In patients diagnosed to have systemic vasculitis, ANCA was tested by ELISA (proteinase-3 and myeloperoxidase) every 3months. The data was collected as per the protocol and filled every 3 months in cases of systemic vasculitis. Patients had the right to leave at any time during the study.

RESULTS:

A total of 100 patients with multi-system dysfunction (MSD) or lung parenchymal disorders were included in the study. There were 50 patients in the MSD group and 50 in parenchymal lung disorder group. The MSD group consisted of 29 (58%) males and 21 (42%) females with a mean age of 41.32 years (range- 19 to 70 years). One patient was below 20 years of age. Twenty-four patients were in age group 21 to 40 years with many of them diagnosed to have systemic lupus erythematosus. The age group 41-60 years comprised of 21 patients with 4 patients age above 60 years of age. Diabetes and malignant diseases were the primary diagnoses in majority of patients in these age groups. In the MSD group, infections accounted for 32 (64%) cases and connective tissue disorders comprised 18 (36%) cases. Septicaemia was the common denominator in 29 (58%) cases (Table-1).

In the parenchymal lung disorder group, the sex distribution was same as the MSD group with a mean age of 48.02 years (19 to 78 years). In this group, 2 patients were below 20 years of age, 14 in the age group 21-40 years and 24 in the age group between 40 and 60 years. Ten patients were above 60 years of age. Pulmonary tuberculosis was the most common diagnosis in the age group between 20-60 years and carcinoma lung in those above 60 years of age. In the parenchymal lung disorder group, the common diagnoses included pneumonia- 20 cases (40%), pulmonary tuberculosis- 14 (28%), disseminated tuberculosis- 5 (10%) and carcinoma lung -5 (10%) (Table-2).

It is pertinent to note that a higher proportion of male patients in our study may be attributed to the fact that most of the patients were armed forces personnel referred for subspecialty consultation. In the MSD group,

In all, 2 patients were diagnosed to have granulomatosis with polyangiitis (GPA). One of these GPA cases was positive for ANCA by both IIF and ELISA (anti-PR3). This patient had anti-PR3 titre of 18 units which later decreased to 16 units after 1 month and serially decreased on subsequent testing to reach a level of 3 units post treatment. He did not relapse and his anti-PR3 level continued to be within normal limits during the follow up period. This patient had involvement of the upper and lower respiratory tracts and the kidneys. The other patient with limited GPA (no renal involvement) tested negative for ANCA by both IIF and ELISA (Table-3).

Among the non-GPA cases, 8/98 cases were falsely positive for ANCA by IIF. All the 8 patients showed an atypical pattern on IIF and none of them was positive for ANCA by ELISA. While all the 98 non-GPA cases were negative for PR3-ANCA by ELISA, 4 of them were falsely positive for MPO-ANCA by ELISA. ANCA pattern in non-GPA cases is given in table -4.

The false positive ANCA cases & their features are shown in Table as per Table 6 & Table 7.

DISCUSSION:

In current clinical practice, the diagnosis of AAVs relies on the presence of ANCA and histological examination of the involved organ [5]. ANCA are the serological hallmark of active AAVs and are found in approximately 90% of adult patients with active GPA, 75% with MPA, and 30% with EGPA [6,7,8]. A negative test does not necessarily rule out this group of vasculitides. On the other hand, multiple conditions, including connective tissue disorders, infections and malignancies, some of which may clinically mimic AAVs can be associated with false positive ANCA tests. This highlights the importance of ordering ANCA testing in appropriate clinical settings where the pre-test probability for AAVs is high, such as glomerulomephritis, especially rapidly progressive glomerulonephritis, pulmonary hemorrhage, multiple lung nodules, long-standing sinusitis and otitis, chronic destructive disease of the upper airways, sub-glottic tracheal stenosis, mononeuritis multiplex, retro-orbital mass and scleritis [9].

Indirect immunofluorescence (IIF), the method by which ANCA were first identified, uses ethanol-fixed neutrophils as the substrate. In the presence of ANCA, one of the two main immunofluorescence (IF) patterns will be observed: predominant cytoplasmic fluorescence with central interlobular accentuation (c-ANCA) or perinuclear fluorescence, frequently with nuclear extension (p-ANCA) [10]. Patterns lacking the typical fluorescent features of c- or p-ANCA may also occur and are referred to as atypical ANCA. In patients with AAVs, c-ANCA and p-ANCA patterns are generally associated with antibody specificity for PR3 and MPO, respectively, although occasionally the reverse is seen [10].

The international consensus statement on testing and reporting of ANCA in 1999 recommended the use of IIF as the initial screening method to detect the presence of ANCAs. Samples which tested positive by IIF were then tested by immunoassays to detect ANCAs specific for PR3 and MPO [10]. In 2016, a large multicentric study by the European Vasculitis Study Group (EUVAS), the diagnostic performance of antigen-specific immunoassays was confirmed to equal or even exceed the diagnostic performance of IIF [11]. This formed the basis of the revised international consensus statement on the testing of ANCAs which was published in 2017. According to the newer consensus statement, high quality antigen-specific assays for PR3-ANCAs and MPO-ANCAs should be used as the primary screening method for ANCA. If the results for both PR3-ANCAs and MPO-ANCAs are negative and there is still a strong suspicion of small- vessel vasculitis, then use of other immunoassays and/or IIF, or referral to an experienced laboratory is recommended [9].

Four out of the 48 (8.33%) non-vasculitic MSD cases and 4 out of the 50 (8%) patients with lung parenchymal disorders in our study were positive (false positive) for ANCA by IIF. All of the false positive IIF specimens displayed an atypical IIF pattern and were negative for both PR3-ANCA and MPO-ANCA by ELISA (Tables 5 and 6). Vassilopoulos et al, in their study found false positive ANCA by IIF alone in 15 out of 96 (16%) patients with non-vasculitic MOD (multi organ dysfunction) and 5 out of 29 (17%) of patients with various pulmonary disorders. The majority of the positive IIF specimens from each group showed an atypical IIF pattern (73% and 80%, respectively) [4]. Four (2 from the MSD and 2 from lung parenchymal disorders group) of the 98 non-GPA cases in our study were positive for ANCA by ELISA (MPO-ANCA positive). All of them were negative for ANCA by IIF. In the study by Vassilopoulos et al, only one specimen from patients with non-vasculitic disorders was positive for MPO-ANCA [4].

None of our non-vasculitic patients was positive for ANCA by both IIF and ELISA. This is significant because ANCA positivity by both IIF and ELISA is very specific for diagnosis of systemic vasculitis.

In our study, the 4 cases from the MSD group who were positive for ANCA by IIF included those diagnosed with enteric fever, HIV and acute renal failure (with septicaemia being a common denominator in these 3 cases) and SLE with lupus nephritis in the fourth. One case with SLE with neuro-psychiatric involvement and another with prostate malignancy with septicaemia were positive for MPO-ANCA by ELISA.

Out of the 4 cases in the lung parenchymal disorder group who were positive for ANCA by IIF, 3 were diagnosed with infections (tuberculosis, pneumonia and lung abscess in one patient each) and the fourth with lung carcinoma. One patient with lung cancer and another with pulmonary tuberculosis were positive for MPO-ANCA by ELISA.

In the study by Vassilopoulos et al, the diagnoses in patients in the non-vasculitic MSD group who were positive for ANCA by IIF included systemic infections including sepsis, liver failure, HELLP syndrome, haemolytic-uremic syndrome, microangiopathic haemolytic anemia due to scleroderma, SLE with pulmonary hemorrhage, relapsing polychondritis and radiocontrast-induced severe anaphylactic reaction. In the group of patients with pulmonary diseases, ANCA positivity by IIF was seen in 3 patients with interstitial lung disease (idiopathic pulmonary fibrosis) and 2 patients with primary carcinoma of the lung [4].

It will be pertinent to mention that De clerk et al have reported a 39 year old patient who was treated for GPA on basis of clinical profile and positive ANCA report. Later, sputum culture was positive for tuberculosis [12]. Vahid et al have reported a female patient with pulmonary disease who was positive for ANCA by ELISA. She was initially diagnosed and treated as GPA. However, as she did not improve, she was investigated further and subsequently a lung biopsy performed led to a diagnosis of pulmonary aspergillosis [13].These cases highlight the importance of tissue diagnosis and sputum microscopy and culture to avoid misdiagnosis of AAV in patients with infections and treating them inappropriately with immuno suppressants.

In our study, none of the non-GPA patients was positive for PR3-ANCA, indicating that it is extremely specific for GPA. As regards sensitivity, one out of the 2 cases of GPA was positive for PR3-ANCA. This patient had involvement of the upper and lower airways and kidneys. PR3-ANCA positivity correlates with renal involvement in GPA. The other patient with GPA who tested negative for ANCA by IIF and ELISA had limited GPA (no renal involvement). It is well known that such patients can be PR3-ANCA negative. Thus, in the presence of only 2 cases of GPA, no meaningful data analysis was possible.

CONCLUSION:

In the presence of only 2 cases of GPA in our study, it is difficult to ascertain specificity and sensitivity of ANCA testing in GPA as the number of cases was too small. PR3-ANCA by ELISA is very specific for AAV. MPO-ANCA by ELISA is less specific for AAV compared to PR3-ANCA. In the patient with GPA, repeated PR3-ANCA by ELISA corroborated with clinical activity. Tissue diagnosis still remains the gold standard to diagnose systemic vasculitis. Interpretation ofANCA must be done in the light of clinical picture and ANCA testing is corroborative in diagnosing AAV.

Table-1: PATIENT DATA- MULTI SYSTEM DYSFUNCTION (n=50)	
1. INFECTIVE CONDITIONS	**32**
a) Complicated malaria	01
b) Diabetes mellitus	
• Diabetes mellitus with UTI with septicaemia	04
• Diabetes mellitus with diabetic foot with septicaemia	02
• Diabetic ketoacidosis with septicaemia	01
c) HIV Infection	
HIV infection with septicaemia	04
• HIV infection with cerebral toxoplasmosis with MSOF	01
• D) CNS Infection	
• Viral meningoencephalitis with MSOF and ARDS	01
• Acute pyogenic meningitis with septicaemia	01
e) Enteric fever with septicaemia with MSOF	03
f) BPH with UTI with septicaemia with MSOF	01
g) Disseminated TB	02
h) Neoplastic	
• Carcinoma prostate with spinal metastasis with septicaemia	01
• Multiple myeloma with septicaemia	01
I) Hepatic	
• Cirrhosis liver with SBP with septicaemia	02
• Pyogenic liver abscess with septicaemia	01
j) Infective endocarditis	02
k)Acute renal failure with septicemia, MSOF	02
l) Chronic kidney disease with septicaemia	02
2. CONNECTIVE TISSUE DISORDERS	**18**
a) SLE with lupus nephritis	08
b) SLE with secondary APS	03
c) SLE with neuropsychiatric involvement	01
d) SLE with skin and articular involvement	01
e) SLE- myositis overlap	01
f) Adult onset Still's disease	02
g) Granulomatosis with polyangiitis	02

UTI- urinary tract infection, HIV- Human immunodeficiency virus, CNS- central nervous system, MSOF- Multi-system organ failure, ARDS- Acute respiratory distress syndrome, BPH- Benign prostatic hyperplasia, TB- tuberculosis, SBP- subacute bacterial peritonitis, SLE- systemic lupus erythematosus, APS- Anti-phospholipid syndrome.

TABLE-2: PATIENT DATA- PARENCHYMAL LUNG DISORDER (n=50)		
1)	**Pneumonia**	**20**
a)	Lobar pneumonia	09
b)	CVA with pneumonia	04
c)	Bilateral pneumonia	04
d)	COPD with right lower lobe pneumonia	02
e)	Diabetes mellitus with fungal pneumonia	01
2)	**Pulmonary tuberculosis**	**14**
3)	**Disseminated tuberculosis** (Pulmonary +extra-pulmonary)	**05**
4)	**Carcinoma lung**	**05**
a)	Carcinoma lung	03
b)	Carcinoma lung with brain metastasis	02
5)	**Lung abscess**	**03**
6)	**Interstitial lung disease**	**02**
7)	**Diffuse alveolar haemorrhage**	**01**

Table-3-PATIENTS DIAGNOSED WITH GPA (ANCA PATTERN)

a) No of cases:	2
b) IIF positive:	1 (50%)
c) IIF negative:	1 (50%)
d) ANCA positive (PR3):	1 (50%)
e) ANCA negative:	1 (50%)
f) ANCA positive (MPO):	0
g) ANCA negative (MPO):	2 (100%)

Table 4- ANCA TESTING IN NON-GPA PATIENTS (n=98)

a) IIF positive	8/98 (Atypical pattern)
b) IIF negative	90/98
c) ANCA positive (PR3)	0/98 (none)
d) ANCA negative (PR3)	98/98
e) ANCA positive (MPO)	4/98 (false positive)
f) ANCA negative (MPO)	94/98

Sl. No	Age (yrs)	Sex	ANCA (IIF)	ANCA (ELISA)	Diagnosis
1	41	M	Positive	Negative	HIV infection with septicaemia
2	70	M	Positive	Negative	Enteric fever with septicaemia
3	38	M	Positive	Negative	ARF with septicaemia
4	30	F	Positive	Negative	SLE with lupus nephritis
5	59	M	Negative	Positive (MPO- NCA)	Ca prostate with spinal metastasis with septicaemia
6	27	M	Negative	Positive (MPO- NCA)	SLE with neuropsychiatric involvement

6/48 were false positive ANCA
4/48 were ANCA positive by IIF (atypical pattern)
2/48 were ANCA (MPO) positive by ELISA

Table-6 : false Positive Cases (parenchymal Lung Disease Group)

Sl. No	Age (yrs)	Sex	ANCA (IIF)	ANCA (ELISA)	Diagnosis
1	49	M	Positive	Negative	Pulmonary tuberculosis
2	78	M	Positive	Negative	Pneumonia (Right lower lobe)
3	72	M	Positive	Negative	Carcinoma lung
4	51	F	Positive	Negative	Lung abscess
5	76	M	Negative	Positive (MPO-ANCA)	Carcinoma lung with brain metastasis
6	48	F	Negative	Positive (MPO-ANCA)	Pulmonary TB

6/50 were false positive ANCA
4/50 were ANCA positive by IIF (atypical pattern)
2/50 were ANCA (MPO) positive by ELISA

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