

Clinical Outcomes of Patients with Dual Positivity for Proteinase 3 and Myeloperoxidase Specific Antineutrophil Cytoplasmic Antibodies

Jeremy Chou¹, Katrina Randall^{1,2} and Paul Gatenby^{1,2*}

¹Australian National University Medical School Peter Baume Building 42, Linnaeus Way, Canberra ACT 0200

²Immunology Department, The Canberra Hospital, Canberra, Australia

*Corresponding author: Paul Gatenby, Immunology Department, The Canberra Hospital, PO Box 11 Woden ACT 2606, Australia, Tel: 61-2-6244-4955; Fax: 61-2-6244 4956; E-mail: paul.gatenby@act.gov.au

Received date: May 18, 2015; Accepted date: June 27, 2015; Published date: June 29, 2015

Copyright: © 2015 Chou J, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Objective: Granulomatous polyangiitis patients generally have ANCA targeting Proteinase 3 (PR3), while microscopic polyangiitis and eosinophilic granulomatous polyangiitis patients generally have ANCA targeting myeloperoxidase (MPO). Patients positive for both PR3-ANCA and MPO-ANCA are uncommon and any diagnostic value of this situation has not been well described. The aim of this study was to determine whether there were any patterns of clinical presentations or outcomes of patients who tested positive for both PR3-ANCA and MPO-ANCA.

Methods: A retrospective clinical audit was carried out. A list of patients who had tested positive for both PR3-ANCA and MPO-ANCA was obtained from ACT Pathology from 2003-2013. Medical records were then used to determine their clinical outcomes.

Results: 3 of the 15 study patients were found to have ANCA-associated vasculitis (AAV), two with MPA and the other EGPA along with a non-AAV autoimmune disease. 1 patient had drug-induced vasculitis and 4 had a non-AAV autoimmune disease. An additional three patients had a non-AAV autoimmune disease, but one concurrently had a malignancy while the other two had recurrent infections. Two patients had a malignancy, another one only had recurrent infections, and the remaining patient was lost to follow up with an undiagnosed inflammatory condition.

Conclusion: Pathology results showing dual positivity for PR3-ANCA and MPO-ANCA in itself does not appear to indicate any particular pattern of clinical outcome and so cannot be used to draw any clinical conclusions at the time of presentation. The 3 cases of AAV demonstrated MPO-predominance which fitted with their final diagnosis.

Keywords: Antineutrophil cytoplasmic antibody; ANCA; Dual-positivity; Clinical outcome; Myeloperoxidase; MPO; Proteinase 3; PR3

Introduction

Testing serum by immunofluorescence for anti-neutrophil cytoplasmic autoantibodies (ANCA) testing is a standard diagnostic procedure routinely used in patients with suspected vasculitis. This is usually accompanied by ELISAs for ANCAs targeting Proteinase 3 (PR3) and myeloperoxidase (MPO) [1]. ANCAs specific for certain antigens have been for some time helpful in differentiating various types of ANCA associated vasculitides (AAV), namely granulomatosis with polyangitis (GPA, formerly called Wegener's granulomatosis), microscopic polyangitis (MPA), and eosinophilic granulomatosis with polyangitis (EGPA, formerly called Churg-Strauss syndrome). GPA patients often test positive for PR3-ANCA, while MPA and to a lesser extent EGPA patients often test positive for MPO-ANCA [2]. Proper diagnosis and classification of AAV and other conditions which could produce positive PR3-ANCA, MPO-ANCA, or both have important implications in the proper treatment and prognosis of these patients [2-4].

Dual positivity for both PR3-ANCA and MPO-ANCA is uncommon, and its associations with clinical presentations and

outcomes have not been extensively studied nor well described. Available literature on the clinical characteristics of patients with dual positivity for PR3-ANCA and MPO-ANCA have thus far been mostly in studies evaluating AAV diagnostic tools, patients with infective endocarditis and other chronic infections such as chronic hepatitis C and tuberculosis [5-11].

The occurrence in infection raises concerns regarding correctly diagnosing such patients to avoid inappropriately prescribing immunosuppressive medications. However no correlation was found between the dual ANCA positivity and the clinical severity of such patients, nor were any correlations with the later development of systemic vasculitis processes reported [6,7,9].

PR3-ANCA and MPO-ANCA dual positivity have also been reported in case studies involving pachymeningitis, mixed connective tissue disease, and tubulointerstitial nephritis, but again no general conclusion can be drawn from these case reports [8,10,11].

As in other reports we have examined the initial and final diagnosis of a group of patients with dual positivity for PR3-ANCA and MPO-ANCA. The aim of this study is to determine whether there are any particular patterns of clinical presentations or outcomes in patients who tested positive for both PR3-ANCA and MPO-ANCA.

Methods

Study and patients

This study required a low risk ethics approval which was granted by the ACT Health Human Research Ethics Committee. A retrospective clinical audit was carried out. To generate a list of patients who had tested positive for both PR3-ANCA and MPO-ANCA on the same date, a data extraction was conducted through ACT Pathology from 2003-2013. Any repeated patient entries were subsequently deleted. We then reviewed all documents of their medical and pathology records available till July 1st 2014. A medical history was established for each patient with information regarding their diagnosis and medical management, with particular note of when ANCA tests were conducted and their respective results. Clinical outcomes for the patients were then determined using the available information. Particular attention was paid to the circumstances that lead to ANCA test ordering. Any significant background conditions and co-morbidities were included as well.

Patients with unclear final diagnoses were either followed up with their last known doctor, or, when enough information was available, given a diagnosis using current diagnostic algorithm and tools. For

this study, the Watt's Algorithm for classifying AAVs was used to diagnose one of the patients with MPA as no medical record documents available provided any explicit outcomes for the patient [12,13]. We then assigned the patient outcomes into several major categories of ANCA related outcomes to look for any particular associations or patterns of clinical presentations.

ANCA determination

At ACT Pathology ANCA requests are screened by indirect immunofluorescence (Euroimmun, Lubeck, Germany) and PR-3 and MPO ELISA are measured using commercial kits (Organtec, Mainz, Germany).

Results

The data extraction yielded a list of 15 patients for this study. The full outcome description for each patient is summarized in Table 1. The clinical details for each patient can be found in the online appendix. Table 2 summaries the major outcome categories of each patient, and the PR3-ANCA and MPO-ANCA levels of their first ANCA test. No patients were positive for hepatitis C and TB was excluded in those in whom it was clinically appropriate.

Patient code	Outcome Summary Description	Note
DA01	Recurrent urosepsis on a background history of Type II Diabetes, chronic renal impairment, hypertension, and CVA. Cause of death not available.	deceased
DA02	Right optic perineuritis	
DA03	Bronchiectasis, epilepsy, Hashimoto, thyroiditis, and recurrent aspirational pneumonia on a background of Down syndrome. Cause of death not available.	deceased
DA04	Microscopic polyangitis with pulmonary haemorrhage and renal failure.	
DA05	Non-AAV Autoimmune disease with chronic Pyrexia due to atypical pneumonia (M. Pneumonia)	
DA06	Died of hospital acquired pneumonia on a background of immunosuppression due to T-cell lymphoma	deceased
DA07	PTU induced Vasculitis	
DA08	Hodgkin's disease, Stage 3 CKD, Heart failure, and Kidney Cysts (unknown cause) with a 20+ year history of psoriatic Arthritis.	
DA09	Type II diabetes with possible primary hyperaldosteronism on a background history of severe HT, aortic dissection treated with aortic graft stroke, and multiple ischaemic attacks.	
DA10	Churg-Strauss Syndrome with a history of psoriatic Arthritis	
DA11	Non-AAV Autoimmune disease with chronic Pyrexia due to atypical pneumonia (M. Pneumonia)	
DA12	CMML-1 with Jak 2 mutation, polycythaemia rubra vera, and thrombocytopenia. Cause of death not available.	deceased
DA13	Microscopic polyangitis. Admitted for acute renal failure and died next day from acute pulmonary haemorrhage	deceased
DA14	IgA mesangial Proliferative GN	
DA15	Sarcoidosis/ACE causing recurrent sinusitis with staph aureus infections and flares lupus pernio on left elbow	

Table 1: Outcome summaries for patients with dual positivity for PR3-ANCA and MPO-ANCA.

Three (20%) patients were found to have an AAV, two with MPA, and the third with EGPA along with a non-AAV autoimmune disease of psoriatic arthritis. No patients in this study were found to have GPA. One (7%) patients had drug induced vasculitis due to propylthiouracil. Four (27%) patients had only a non-AAV autoimmune disease as an outcome, having either optic perineuritis,

Hashimoto thyroiditis, IgA mesangial proliferative glomerulonephritis, or an unclarified non-AAV autoimmune process. An additional three (20%) patients had a non-AAV autoimmune disease, but one concurrently had a malignancy and the other two recurrent infections. One was diagnosed with Hodgkin's disease on a background of arthritis, another had sarcoidosis with recurrent

sinusitis due to *S. aureus*, and the third was described as a pulmonary non-AAV autoimmune process along with recurrent infections by *M. pneumoniae*. This patient had been seen in our department where extensive investigations including lung biopsy did not support an initial suspicion of GPA. Two (13%) patients had a malignancy one

with T-cell lymphoma and another with chronic myelomonocytic leukaemia-1 (CMML-1). One (7%) patient had recurrent infections in the form of urosepsis as their only outcome. The remaining patient was lost to follow up with an undiagnosed inflammatory condition associated with their PR3-ANCA and MPO-ANCA dual positivity.

Patient code	GPA	MPA	EGPA	drug Induced	Non-Aav Autoimmune	Recurrent Infection	Neoplasm/ Malignancy	deceased	Initial Reading(U/ml)	
									PR3-ANCA	MPO-ANCA
DA01	-	-	-	-	-	+	-	+	14	6
DA02	-	-	-	-	+	-	-	-	19	51
DA03	-	-	-	-	+	-	-	+	10	48
DA04	-	+	-	-	-	-	-	-	7	85
DA05	-	-	-	-	+	+	-	-	37	9
DA06	-	-	-	-	-	-	+	+	10	7
DA07	-	-	-	+	-	-	-	-	383	28
DA08	-	-	-	-	+	-	+	-	8	11
DA09	-	-	-	-	-	-	-	-	6	8
DA10	-	-	+	-	+	-	-	-	10	87
DA11	-	-	-	-	+	-	-	-	73	40
DA12	-	-	-	-	-	-	+	+	18	7
DA13	-	+	-	-	-	-	-	+	6	35
DA14	-	-	-	-	+	-	-	-	14	26
DA15	-	-	-	-	+	+	-	-	10	26

Table 2: Outcome and results for first PR-3 and MPO-ANCA test.

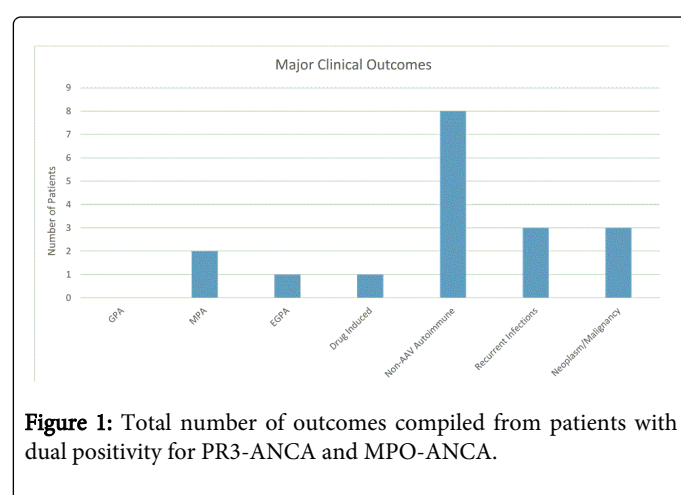


Figure 1: Total number of outcomes compiled from patients with dual positivity for PR3-ANCA and MPO-ANCA.

Of the 15 patients in the study, 5 have died. One due to MPA related complications, two due to their malignancies, and the remaining two with unclear causes of death. 6/15 (40%) and 9/15 (60%) patients displayed a PR3-ANCA or MPO-ANCA predominance over the other respectively. MPO-ANCA predominance was associated with MPA and EGPA, but in this small series PR-3 ANCA

was not associated with GPA. Figure 1 shows the total number of each outcome category compiled from all patients.

Discussion

AAV are not the only conditions that can lead to dual positivity for PR3-ANCA and MPO-ANCA. However in this small series there were no cases of infective endocarditis, pachymeningitis, mixed connective tissue disease, or tubulointerstitial nephritis all described in previous case studies of dual PR3-/MPO-ANCA positive patients. This study reinforces previously published literature in that patients testing positive for both PR3-ANCA and MPO-ANCA can eventuate in a wide range of final diagnoses.

Of the fifteen patients, only three were found to have AAV. The ANCA expression for these AAV patients showed a low PR3-ANCA and high MPO-ANCA pattern of expression, which is consistent with the usual specificity of ANCA expression for their respective type of AAV (PR3-ANCA for GPA, and MPO-ANCA for both MPA and EGPA) and the dominant ANCA was used to make the diagnosis.

Comparison of PR3-ANCA and/or MPO-ANCA positive patients with either AAV or prolonged infections in a previous study found that, though there were no statistical differences in titres, patients with infections were more likely to express both PR3-ANCA and MPO-

ANCA, with a high PR3-ANCA and low MPO-ANCA pattern of expression [7]. Similar findings were obtained in our study; two of the three patients with recurrent infections did have high PR3-ANCA and low MPO-ANCA expression. However the third patient had a much higher MPO-ANCA levels than PR3-ANCA. It is uncertain if any of their concurrent disease processes contributed to their difference in the type of ANCA that was more highly expressed. However these results demonstrate the limited capacity of dual PR3-/MPO-ANCA positivity alone in differentiating between infections and AAV.

Propylthiouracil is the most common cause of drug induced ANCA positive vasculitis. The majority of patients reported with drug induced vasculitis express very high levels of MPO-ANCA [14,15], although dual positivity is seen in many cases. In fact, a characteristic of PTU-induced vasculitis is reactivity of antibodies against a number of cytoplasmic granules [16]. The single case of drug induced vasculitis in this study, due to propylthiouracil, similar to those described in large series [16] had very high levels of PR3-ANCA compared to MPO-ANCA. However, there have been other reported cases of drug induced vasculitis with high PR3-ANCA and low MPO-ANCA [16].

AAV and its treatment have been associated with an increased risk of malignancies, in particular urinary tract cancer, leukaemia, and non-melanoma skin cancer [17,18]. However the malignancies found in the patients in this study did not appear to be due to AAV or its treatment. Malignancies in all three patients in this study were of blood cell origins, namely T-cell lymphoma, Hodgkin's disease, or CMML-1. However other than one having concurrent arthritis, none had any signs of AAV or any other systemic autoimmune processes. The PR3-ANCA and MPO-ANCA levels in patients with malignancy outcomes were amongst the lowest of all 15 patients. However, no clear associations can be made between the levels of dual PR3-/MPO-ANCA positivity and malignancy development without further studies involving larger sample sizes.

The majority of patients in this study with other non-AAV autoimmune diseases had higher levels of MPO-ANCA compared to PR3-ANCA. This may suggest that dual PR3-/MPO-ANCA positive patients with a higher MPO-ANCA level relative to PR3-ANCA, who are not found to have clear signs of AAV, are more likely to have an ongoing autoimmune disease than the other outcomes evaluated in this study. However, there were still two patients with higher PR3-ANCA levels, and the levels of PR3-ANCA and MPO-ANCA in these patients do not appear to indicate any correlation with the type of autoimmune disease present, nor the disease severity, and as such would be of limited diagnostic value.

Limitations of this study include the limited number of dual PR3-ANCA and MPO-ANCA positive patients available for this study due to their rare occurrence. This limits the capacity for comparison with other patients with dual PR3-ANCA and MPO-ANCA positivity. There were also limitations on the amount of information that could be found with a number of patients due to difficulties with obtaining further information.

The clinical implications of these findings include a number of points. Firstly, the diagnosis of AASV needs to be pursued by more than just a positive ANCA. Other evidence needs to be obtained although a predominant MPO-ANCA may prove a safer indicator of MPA or EGPA than a predominant PR3-ANCA is of GPA. Secondly, there is always a differential diagnosis with infection in particular being of major importance. These autoantibodies, like most are rarely

so specific and sensitive that diagnoses can be made without reference to other clinical and laboratory features.

In conclusion, these findings indicate that pathology results showing PR3-ANCA and MPO-ANCA dual positivity in itself does not indicate any particular pattern of clinical outcome. As a result, such findings cannot be used in practice to draw any conclusions at the time of presentation, and further investigations and evaluation of clinical findings are warranted to make a proper diagnosis. Additional studies with larger number of patients will need to be done to clarify whether the predominance of either PR3-ANCA or MPO-ANCA in patients testing positive for both ANCAs can provide any diagnostic or prognostic information.

Acknowledgements

Monica Brady from ACT Pathology assisted with data extraction and Gloria Spyropoulos from The Canberra Hospital collated medical records.

References

1. Csernok E (2013) ANCA testing: the current stage and perspectives. *Clin Exp Nephrol* 17: 615-618.
2. Millet A, Pederzoli RM, Guillevin L, Witko SV, Mouthon L (2014) Republished: antineutrophil cytoplasmic antibody-associated vasculitides: is it time to split up the group? *Postgraduate medical journal* 90:290-296.
3. Lionaki S, Blyth ER, Hogan SL, Hu Y, Senior BA, et al. (2012) Classification of antineutrophil cytoplasmic autoantibody vasculitides: the role of antineutrophil cytoplasmic autoantibody specificity for myeloperoxidase or proteinase 3 in disease recognition and prognosis. *Arthritis and rheumatism* 64:3452-3462.
4. Flossmann O, Berden A, Groot Kd, Hagen C, Harper L, et al. (2011) Long-term patient survival in ANCA-associated vasculitis. *Ann Rheum Dis* 70: 488-494.
5. Hagen EC, Daha MR, Hermans J, Andrassy K, Csernok E, et al. (1998) Diagnostic value of standardized assays for anti-neutrophil cytoplasmic antibodies in idiopathic systemic vasculitis. EC/BCR Project for ANCA Assay Standardization. *Kidney international* 53: 743-753.
6. Veerappan I, Prabitha EN, Abraham A, Theodore S, Abraham G (2012) Double ANCA-positive vasculitis in a patient with infective endocarditis. *Indian J Nephrol* 22: 469-472.
7. Bonaci NB, Andrejevic S, Pavlovic M, Dimic Z, Ivanovic B, et al. (2010) Prolonged infections associated with antineutrophil cytoplasmic antibodies specific to proteinase 3 and myeloperoxidase: diagnostic and therapeutic challenge. *Clinical rheumatology* 29: 893-904.
8. Iguchi A, Wada Y, Kobayashi D, Sato H, Oyama T, et al. (2013) A case of MPO- and PR3-ANCA-positive hypertrophic cranial pachymeningitis with elevated serum IgG4. *Modern rheumatology/the Japan Rheumatism Association* 23: 151-155.
9. Tiliakos AM, Tiliakos NA (2008) Dual ANCA positivity in subacute bacterial endocarditis. *Journal of clinical rheumatology : practical reports on rheumatic & musculoskeletal diseases* 14: 38-40.
10. Murakami M, Shimane K, Takahashi H, Tomiyama J, Nagashima M (2013) ANCA-associated vasculitis with dual ANCA positivity in coexistence with mixed connective tissue disease. *Modern rheumatology / the Japan Rheumatism Association* 23: 156-161.
11. Ueda H, Ishimura E, Yunoki T, Tsuchida T, Matsumoto N, et al. (2005) Cimetidine-induced tubulointerstitial nephritis with both MPO-ANCA and PR3-ANCA. *Clin Exp Nephrol* 9: 332-334.
12. Watts R, Lane S, Hanslik T, Hauser T, Hellmich B, Koldingsnes W, et al. (2007) Development and validation of a consensus methodology for the classification of the ANCA-associated vasculitides and polyarteritis

-
- nodosa for epidemiological studies. *Annals of the rheumatic diseases* 66: 222-227.
13. Liu LJ, Chen M, Yu F, Zhao MH, Wang HY (2008) Evaluation of a new algorithm in classification of systemic vasculitis. *Rheumatology (Oxford)* 47: 708-712.
14. Gunton JE, Stiel J, Clifton BP, Wilmschurst E, McElduff A (2000) Prevalence of positive anti-neutrophil cytoplasmic antibody (ANCA) in patients receiving anti-thyroid medication. *European journal of endocrinology / European Federation of Endocrine Societies* 142: 587.
15. Choi HK, Merkel PA, Walker AM, Niles JL (2000) Drug-associated antineutrophil cytoplasmic antibody-positive vasculitis: prevalence among patients with high titers of antimyeloperoxidase antibodies. *Arthritis and rheumatism* 43: 405-413.
16. Liu HL, Gao MJ, Zheng YA, Liu GH (2013) Propylthiouracil induced antineutrophil cytoplasmic antibodies-associated vasculitis. *Chin Med J (Engl)* 126: 4814.
17. Mahr A, Heijl C, Guenno GL, Faurschou M (2013) ANCA-associated vasculitis and malignancy: current evidence for cause and consequence relationships. *Best practice & research Clinical rheumatology* 27: 45-56.
18. Pankhurst T, Savage CO, Gordon C, Harper L (2004) Malignancy is increased in ANCA-associated vasculitis. *Rheumatology (Oxford)* 43: 1532-1535.