Multispecific Anti Neutrophil Cytoplasm Antibodies: A case report

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Anticorps Anti Cytoplasme des Polynucléaires Neutrophiles à spécificités multiples : À propos d'un cas

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RÉSUMÉ

But : Rapporter le cas de la présence d'ANCA d'aspect atypique en IFI.

Observation : Patiente de 50 ans présentant une fibrose pulmonaire dont le bilan immunologique a objectivé la présence d'ANCA d'aspect atypique en immunofluorescence indirecte (IFI) sur polynucléaires neutrophiles (PNN) fixés à l'éthanol. Le typage des ANCA par technique ELISA multiantigénique a montré une multispécificité à l'égard de différentes protéines du PNN (Protéinase 3, Myéloperoxydase, BPI, lysozyme, élastase et cathepsine G) pouvant être rattachée à l'hypergammaglobulinémie polyclonale présente chez cette malade.

Conclusion : Les recommandations internationales sur la stratégie de détection des ANCA préconisent de rechercher uniquement les spécificités majeures anti Myéloperoxydase et anti Protéinase 3 qui constituent de bons marqueurs pour les vascularités systémiques touchant les vaisseaux de petit calibre. Les techniques ELISA multiantigéniques peuvent être néanmoins utiles pour rechercher aussi les cibles dites mineures des ANCA et expliquer ainsi certains aspects atypiques observés lors de leur recherche en IFI mais leurs résultats doivent être interprétés avec précaution.

Mots-clés

Anticorps Anti Cytoplasme des Polynucléaires Neutrophiles. Immuno Fluorescence Indirecte. Enzyme-Linked ImmunoSorbent Assay. Spécificité

SUMMARY

Aim : To report the presence af ANCA with an unusual polyreactivity.

Case report : 50 year-old woman with pulmonary fibrosis whose immunological investigations showed the presence of ANCA with an unusual polyreactivity against several neutrophil proteins (PR3, MPO, BPI, lysozyme, elastase and cathepsine G) which could be related to a polyclonal hypergammaglobulinemia occurring in this patient.

Conclusion : The international consensus on the testing of ANCA recommends seeking major specificities like MPO and PR3 which are good markers of ANCA-associated vasculitides. The use of multiantigenic ELISA can be helpful to detect various target antigens at the same time and may thus explain some atypical fluorescent patterns observed when searching for ANCA by Indirect immunofluorescence, these results, however, must be interpreted with caution.

Key-words

Anti Neutrophil Cytoplasm Antibodies. Indirect Immuno Fluorescence Enzym Linked ImmunoSorbent Assay. Specificity

Anti neutrophil cytoplasm antibodies (ANCA) are autoantibodies which target components in granules of neutrophils and monocytes and are classically described in primary systemic vasculitides [1].

Indirect Immunofluorescence (IIF) on ethanol fixed human neutrophils is the standard method of ANCA detection. Two staining patterns are usually distinguishable on IIF: the perinuclear pattern (pANCA) associated with antibodies to myeloperoxydase (MPO) and the cytoplasmic pattern (cANCA) associated with antibodies to serine proteinase 3 (PR3). MPO and PR3 are considered the main ANCA target antigens. They are sought by enzyme linked immunoassay (ELISA)[2].

ANCA against MPO are detected in Churg and Strauss syndrome and microscopic polyangiitis; whereas ANCA with PR3 specificity are found in patients suffering from Wegener's granulomatosis [1,3]. However, the distinction of antigenic specificities according to fluorescent patterns is not that obvious. Indeed, several papers [4-6] showed that pANCA or cANCA patterns were also linked to other granule antigens such as lysozyme, lactoferrin, cathepsin G, BPI (Bactericidal permeability increasing protein). In addition, other fluorescence patterns described as "atypical" are often seen when searching ANCA by IIF [6,7]. Clinical significance of these minor target antigens still remains to be established.

We here describe the case of a patient with pulmonary fibrosis whose immunological investigations have shown multispecific ANCA directed against several neutrophil antigens.

CASE REPORT

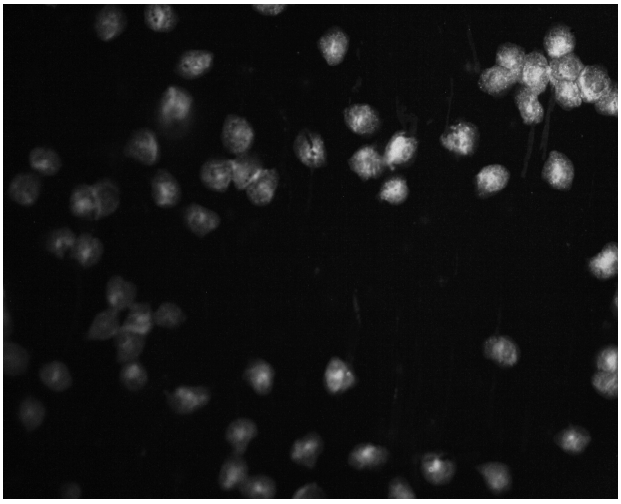
A fifty years old woman, coming from a rural region of eastern North of Tunisia, was admitted to a pneumology department in December 2008 for a non productive cough from which she had suffered for almost four years. She has noticed dyspnea, fatigue and weight loss during the last three months. She did not have haemoptysis. She also complained of arthralgias of the lower limbs and the wrists without morning stiffness. She had not been under medication. On admission, the patient was apyretic and had no dyspnea . Crackle sounds were audible in lung fields. On chest X ray, bilateral interstitial opacities were present. Diffuse interstitial fibrosis was found on thoracic CT scan. A restrictive ventilatory syndrome was demonstrated on spirometry

Bronchial endoscopy with biopsy showed unspecific inflammatory patterns. Bronchoalveolar lavage fluid analysis indicated a hypercellularity with neutrophilia.

Routine laboratory tests showed hypochromic microcytic anaemia, a polyclonal hypergammaglobulinemia (24g/l). ESR was 120mm/h; C-reactive protein was 60 mg/l.

Serological investigations were performed to search for an underlying autoimmune disease and results were as follows: Antinuclear antibodies (ANA) negative (IIF on HEp2 cells), rheumatoid factor: 45 IU / ml (nv < 30 IU/ ml)] and anti CCP antibodies [Binding site ™] were negative. ANCA positive (atypical pattern) by IIF on ethanol fixed neutrophil (Euroimmun™) (figure 1).

Figure 1 : Atypical pattern (IIF on ethanol fixed neutrophils)



An ELISA (Orgentec ANCA Combi™) to determine ANCA specificity gave the following results: **BPI** ++++; **MPO** ++; **PR3** +; **Lysozyme** ++; **Elastase** +; **Cathepsine G** +; **Lactoferrine** –.

Precipitins against poultry antigens were negative. A deep muscle biopsy disclosed no abnormalities and vasculitis was ruled out even if ANCA were positive.

Diagnosis was usual interstitial pneumonia (UIP) and the patient was put under steroids since January 2009.

A later control of ANCA (May 2009) with the same assay (Orgentec ANCA Combi™) was as follows: anti MPO+ anti BPI+++, anti lysozyme ++, Anti PR3, anti elastase, anti cathepsin G and anti lactoferrin antibodies were negative.

However, our patient had no clinical improvement and steroids were stopped (march 2010). A CT scan revealed a worsening of the lesions. A last control (April 2010) of ANCA revealed reactivity against MPO+++ and BPI ++++

DISCUSSION

ANCA, mainly those targeting MPO and PR3 have proven to be valuable serologic markers in primary small vessels vasculitides. ANCA also occur in infectious diseases, chronic inflammatory bowel diseases, connective tissue diseases and, in these cases, antigenic reactivities are usually against targets other than MPO and PR3 [4].

In this case report, multispecific ANCA, with atypical fluorescent pattern, have been found in a patient suffering from an interstitial pulmonary disease with no evidence of systemic vasculitis.

Atypical fluorescence patterns may have various explanations: they can be related to neutrophil preparations which are extremely sensitive to the fixatives and to the fluorescent conjugate [8]. In addition, IIF assay is prone to false positive

results because of other occuring autoantibodies like ANA and anticytoplasmic antibodies (cytoskelatel) [6]. Nevertheless, these possible interferences can be assessed by using IIF on HEp 2 cells for ANA detection, or by using IIF on formalin fixed neutrophils when detecting ANCA.

Formalin fixed neutrophils may help to distinguish between perinuclear patterns related to ANA and a "true" pANCA pattern related to ANCA directed against neutrophil granule antigens. For these reasons, there are commercial kits including ethanol fixed, formalin fixed neutrophils and HEp2 cells on the same slides. In our observation, however, atypical fluorescence patterns could be explained by the unusual polyreactivity of ANCA. In fact, we were able to demonstrate by multiantigenic ELISA that ANCA occurring in our patient were multispecific, targeting the main proteins MPO and PR3 but also other minor antigens. Dual specificity for both MPO and PR3 was rarely reported in the literature and the authors indicated that it was due to a non-specific binding in ELISA [6].

Multiantigenic ELISA for ANCA typing are of recent appearance and their use remains probably not very widespread. In fact, the usual recommendations are to seek major specificities like MPO or PR3 according to pANCA or cANCA fluorescence patterns and most laboratories use monospecific ELISA for this purpose [2]. So clinical and etiopathogenic significance of ANCA directed against minor neutrophil antigens is not yet clear.

Several papers have also described multispecific ANCA with a multiple combination of target antigen reactivities [5, 9] which could be involved in a later development of vasculitis or simply represent markers of an inflammatory process [5]. Antigenic specificities like BPI, found in cystic fibrosis seem to be the result of immune reactions which occur during long standing infectious diseases [10]. Others, as anti elastase, were described in patients with cocaine-induced midline destructive lesions [11]. Some authors suggest that ANCA, in particular MPO antibodies occurring in pulmonary fibrosis, increase the risk of development of a microscopic polyangiitis [12, 13].

Finally, it is conceivable, in our case report, that these multispecific ANCA are only transient inflammatory markers which are related to a polyclonal immune reaction and a chronic neutrophil activation underlying pulmonary fibrosis. According to this idea, several weak antigen reactivities like PR3, elastase and cathepsine G turned to negative in a few months.

CONCLUSION

In ANCA determination, a positive IIF result must be followed by a monospecific solide phase assay for PR3 or MPO antibodies.

Nevertheless, multiantigenic ELISA which allows the detection of several specificities at the same time may be useful to explain atypical patterns. Moreover it may help to establish the diagnostic value of ANCA directed against minor antigen targets. Interpretation, however, must be considered with caution, in particular in front of weak reactivities in ELISA technique. In addition, it is important to recall that ANCA determination should be dictated by clinical data strongly suggesting a systemic vasculitis [1].

References

1. Bosch X, Guilbert A , Font J. Antineutrophil cytoplasmic antibodies. Lancet 2006 ; 368 :404-418
2. Savige J, Gillis D, Benson E et al. International consensus statement on testing and reporting of anti neutrophil cytoplasmic antibodies (ANCA). Amj of Clin Pathol 1999; 111:507-513
3. Savige J, Davies D, Falk RJ et al. Antineutrophil cytoplasmic antibodies and associated diseases: a review of the clinical and laboratory features. Kidney I. 2000; 57:846-862
4. Sghiri R, Meddeb H J, Bouguila J, Jarray M, Bahri F, Nouira R et al. Anti neutrophil cytoplasmic antibodies and associated diseases. Pathologie Biologie. 2009; 57:398-402
5. Talor M V, Stone JH, Stebbing, J,Barin J, Rose N R, Burek C L. Antibodies to selected minor target antigens in patients with antineutrophil cytoplasmic antibodies (ANCA).Clin. Exp Immunol 2007; 150:42-48
6. Savige JA, Paspaliaris B, Silvestrini R, Davies D, Nikoloutsopoulos T, Sturgess A et al. A Review of immunofluorescent patterns associated with antineutrophil cytolasmic antibodies (ANCA) and their differentiation from other antibodies. J Clinic Pathol 1998; 51:568-575
7. Wong RCW, Field K. MPO-ANCA may produce a combination of pANCA and atypical cytoplasmic ANCA indirect immunofluorescent patterns on certain ethanol fixed neutrophil substrates. J Clinic Pathol 2001; 54: 335
8. Pollock W, Clarke K, Gallagher K, et al. Immunofluorescent patterns produced by antineutrophil cytoplasmic antibodies (ANCA) vary depending on neutrophil substrate and conjugate. J Clinic Pathol 2002; 55:680-683
9. Beauvillain C, Delneste Y, Renier G, et al. Antineutrophil cytoplasmic autoantibodies: how should the biologist manage them ? Clinical Revew of allergy and immunology 2008;35:47-58
10. Schultz H. From infection to autoimmunity: A new model for induction of ANCA against the bactericidal/permeability increasing protein (BPI). Autoimm Rev 2007; 6: 223–227
11. Wiesner O, Russell KA, Lee AS et al. ANCA reacting with human neutrophil elastase as a diagnostic marker for cocaine-induced midline destructive lesions but not autoimmune vasculitis. Arthritis Rheum 2004; 50:2954-2965
12. Foulon G, Delaval P, Valeyre D, Wallaert B, DebrayMP, Brauner M et al. ANCA-associated lung fibrosis: Analysis of 17 patients. Resp Medi 2008; 102: 1392-1398
13. Hervier B, Pagnoux C, Agard C, et al. Pulmonary fibrosis associated with ANCA-positive vasculitides. Retrospective study of 12 cases and review of the literature .Ann of Rheum Diseases 2009; 68:404-407