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Antinuclear antibodies (ANA) patterns in paraneoplastic cerebellar degeneration during the course of disease and treatment protocols – A case report

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ABSTRACT

Paraneoplastic cerebellar degeneration (PCD) is a rare disease that is triggered by an abnormal immune response to a malignant tumor by cross-reaction of antibodies. The low prevalence of this condition has not allowed for large-scale randomized controlled trials. Suspecting a paraneoplastic syndrome followed by rapid diagnosis is crucial before the symptoms irreversibely progress. Indirect immunofluorescence (IIF) with HEp-2 cells is currently the most widely used screening technique for the detection of a wide range of nuclear and cytoplasmatic autoantibodies. Here, we present a case of a female Caucasian patient, 61 years of age, who started having sudden symptoms of PCD starting April 2016 that progressed through the course of 10 months before the final diagnosis. Assuming that antinuclear antibodies (ANA) testing could give rise to suspicion of an underlying malignancy but also to an underlying autoimmune etiology of PCD, we followed the ANA patterns of the patient during the course of disease and treatment protocols. A total of four ANA follow ups were done on serum dilution 1:100 and all showed weak positive results on hepatic cells and a mix of similar patterns that, through the course of time, differed slightly on HEp-2 cells. Finding positive antinuclear or anticytoplasmic auto-antibodies might guide toward an extensive and useless search for a systemic autoimmune disease ignoring the possibility of searching for paraneoplastic-specific antibodies. An unspecified mix of patterns should not be ignored and might, through further research, show to be more valuable in the ANA screening than is the case now. Weak positive results should not mislead into thinking that there is no overall effect on health, since quite the opposite was the case here. In our example, neither the tumor response to treatment, neurological presentation nor the immunological treatment had a strong effect on the ANA patterns which remained almost identical throughout the course of disease and treatment. Ultrastructural and molecular events in the pathogenesis of the disease could have caused certain minor changes in the pattern but are not of clinical value at the moment and further research is needed.

1. Introduction

Paraneoplastic cerebellar degeneration (PCD) is a rare condition that belongs to a group of paraneoplastic syndromes that manifests symptoms neurologically [1]. It is triggered by an abnormal immune response to a malignant tumor, usually occult and undetected, that affects parts of the nervous system by cross-reaction of antibodies [5,7]. It affects 1–3% of all cancer patients [11]. It's most common variant is the ataxic syndrome associated with Anti-Yo antibody, or Purkinje cell cytoplasmic antibody type 1 (PCA1) [1].

The typical presentation involves the subacute development of pan-cerebellar deficits with a clinical plateau within 6 months. These neurologic symptoms most often present themselves prior to the diagnosis of the tumor and respond poorly to treatment [1]. The low prevalence of this condition has not allowed for large-scale randomized controlled trials. Suspecting a paraneoplastic syndrome and a rapid diagnosis is crucial before the symptoms irreversibely progress. The oncologic outcome of patients with antibody-associated paraneoplastic syndromes does not significantly differ from patients who do not have the antibodies or paraneoplastic syndrome, therefore, early suspicion will help in oncological treatment as well.

Indirect immunofluorescence (IIF) with HEp-2 cells is currently the most widely used screening technique for the detection of a wide range of nuclear and cytoplasmic autoantibodies [13]. The target antigens of

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antinuclear antibodies (ANA) exist in a whole cell, containing the nucleus and cytoplasm, and they are positive in many diseases [4].

Assuming that ANA testing could give rise to suspicion of an underlying malignancy but also to an underlying autoimmune aetiology of PCD, we followed the ANA patterns of the patient during the time course of disease and treatment protocols.

2. Case description

A female caucaisan patient, 61 years of age, married, a mother of three, started having sudden vertigo symptoms with nausea in April 2016 with nystagmus detected and complaints on dryness of mouth. Family history included diabetes and the patient had penicillin and alimentary allergies. The neurologist diagnosed her with persistent vertigo syndrome, vertebrobasilar insufficiency syndrome and obstruction of the labyrinth and treated successively with betahistine dihydrochloride, nicergoline and cinnarizine without success. Differential diagnosis included Sjogrens syndrome and immunology tests were done showing negative ENA profile (extractable nuclear antigen) and anti dsDNA (anti-double stranded DNA) and positive ANA 1/100.

Her symptoms gradually progressed through the course of 6 months affecting her quality of life and work. In late October 2016 a lymph node in the left axilla was palpated without a palpable tumor of the breasts. Ultrasonography diagnostics reveiled a 2 cm small tumor in the left breast. Pathohistology showed it to be Invasive mammar carcinoma of the breast, Gr II, Immunochystochemistry: Estrogen -, Progesterone -, Her2 3+, excessive inflammatory infiltrate with granulomatosis reaction). MRI of the head in November excluded metastasis of the brain.

The oncologist indicated neoadjuvant therapy prior to partial mastectomy and radiotherapy. The patient started with chemotherapy and trastuzumab with pertuzumab. She rapidly neurologically deteriorated during cancer treatment with onset of ataxia and dysarthria by the second cycle of chemotherapy. By January the patient was unable to walk without assistance, constantly complaining that she did not handle well chemotherapy and further reductions were made in the dosage and excluding of pertuzumab, and finally trastuzumab. A repeated MRI of the head in February revealed a fresh lesion in the left cerebellum of 5 mm. It was interpreted as an ischaemic lesion or CVI of the cerebellum.

After further neurologic deterioration the patient became wheelchair bound and paraneoplastic syndrome was suspected, anti-Yo antibodies were done in February by Euroimmun immunoblot and showed ++ (30). Plans for neoadjuvant chemotherapy were abandonded and emergency mastectomy was done. Postoperative pathohystology result reveiled no residual tumor of the left breast which corresponds to a complete neoadjuvant treatment response.

Ten days after surgery, the patient continued to deteriorate neurologically. A neuroimmunologist specialized in paraneoplastic syndromes was contacted and the patient continued treatment for the condition in Turkey. During phsyical examination in February there was mild dysarthria, mild ataxia in arms, moderate to severe ataxia in legs, walked a few steps with assistance of others. Repeat indirect immunofluorescent examination showed cytoplasmic Purkinje cell staining in monkey cerebellar sections, together with ++ positivity on immunoblot examinations. ANA was weekly positive at 1/100, whereas ENA profile testing, anti dsDNA, anti ganglioside panel IgG and IgM, anti-glutamic acid decarboxylase (anti-GAD), anticardiolipin antibodies and β2 glicoprotein antibodies were all negative.

In three weeks, treatment protocols included seven days of pulse therapy without significant benefit, 5 plasma exchanges in 10 days with slight improvement in limb ataxia and unchanged gait ataxia, and cyclophosphamide 1000 mg, after which azathioprine was continued. One month after plasmapheresis, intravenous immunoglobulin (IVIG) 2 g/kg of total body weight was applied with the suggestion to continue IVIG maintenance dosage of 0,4 g/kg monthly and yearly repeat of booster IVIG dose 2 g/kg.

PET/CT scan in March was without abnormalities. Oncologist treatment protocol was completed with radiotherapy in April (partial dosage because of patients refusal to continue radiotherapy after 32Gy) and with adjuvant 2 cycles of trastuzumab (patient denied to continue adjuvant treatment).

The patient is currently solely on azathioprine, halting IVIG monthly maintenance of 0,4 g/kg after 13 months, currently going through occupational physical therapy and is on Quetiapine because of personality changes and dysphoric mood changes. There is no memory loss or cognitive loss. The patient is still wheelchair bound with dysarthria and ataxia. Further treatment depends on financial and organisational means.

Following the final diagnosis of PCD, with the consent of the patient, serum for ANA (Euroimmun) testing was obtained in multiple occasions corresponding to different treatment protocols through the course of the disease. A total of four ANA follow ups were done, one after final diagnosis, one after surgery, the third after pulse therapy, plasmapheresis, cyclophosphamide and azathioprine and the final after initiation of IVIG. On serum dilution 1:100 all showed weak positive results on hepatic cells and a mix of similar patterns that differed slightly on HEp-2 Cells.

During the time of disease diagnosis, with present high levels of Anti-Yo antibodies, IIF on HEp-2 cells showed ANA patterns classified by ICAP (International consensus on ANA patterns) as following: CENP-F like/AC-14, dense fine speckled nuclear/AC-2 and dense fine speckled cytoplasmic/AC-19 (Fig. 1). Almost a month later with the serum after mastectomy and prior to PCD treatment with the peak in anti-Yo levels (39++), the ANA results had a finer speckled nuclear pattern (AC-4) and occasional HEp-2 cells with PCNA like pattern (AC-13) (Fig. 2). The third serum sample was after initiating treatment for PCD, almost two months later, the anti-Yo levels dropped to the lowest point of + (8) on immunoblot, the ANA results were with the same patterns, with a note that the speckled nuclear patterns had distinctive nucleolar staining in terms of SSB or Scl antigen association (Fig. 3). The final ANA results were a month later in May, with a note that cytoplasmic patterns were more fluorescent than nuclear, the nucleoli weren't that accentuated anymore and the CENP-F-like pattern was not as fluorescent as on the first ANA testing (Fig. 4).

3. Discussion

Reviewing previous literature regarding paraneoplastic syndromes, anti-Yo antibodies and immunological testing we found several findings that may corroborate ours. It has previously been recognized that a patient with an auto-immune disease could also present multiple systemic auto-antibodies [8]. PCD reflects a breakdown in self-tolerance associated with cancer. Inner changes in tumor cells can lead to some new

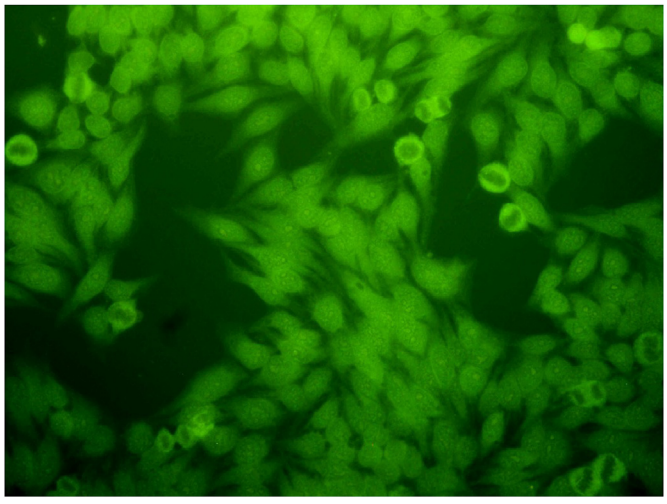

Fig. 1. CENP-F like, dense fine speckled nuclear and dense fine speckled cytoplasmic.

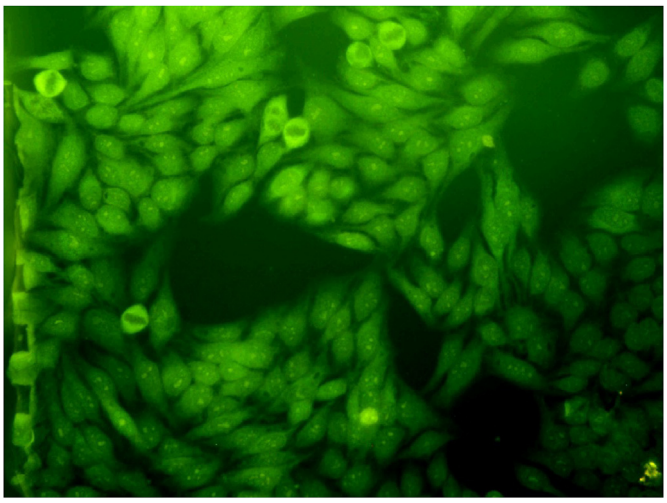

Fig. 2. Finer speckled nuclear pattern and occasional HEp-2 cells with PCNA like pattern.

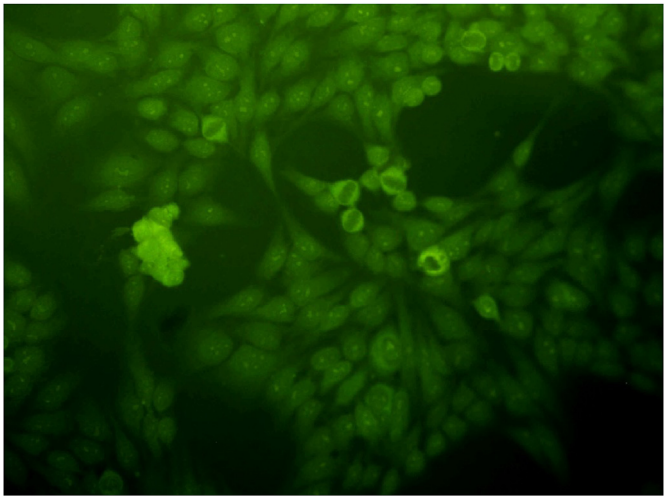

Fig. 3. Same patterns, with a note that the speckled nuclear patterns had distinctive nucleolar staining in terms of SSB or Scl antigen association.

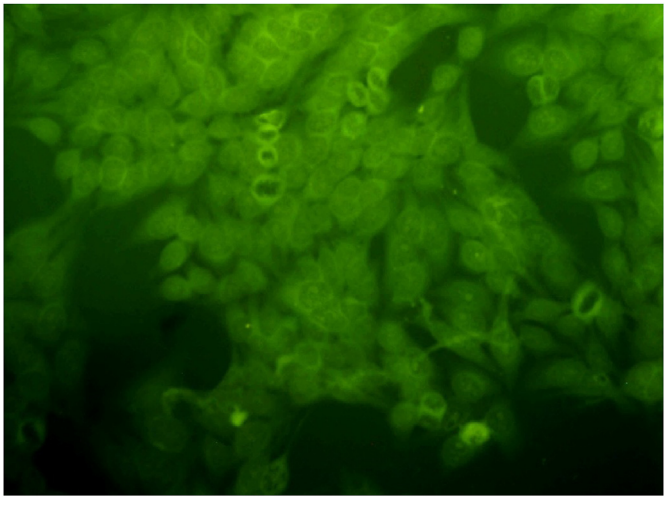

Fig. 4. Cytoplasmic patterns are more fluorescent than nuclear, the nucleoli aren't that accentuated anymore and the CENP-F-like pattern isnot as fluorescent as on the first ANA testing.

antigens, but many antigens are not unique to tumor cells and may be present in healthy cells as well. In PCD, this leads to an antigen-driven autoimmune cross-reaction, stimulated by similar neuronal antigens ectopically expressed in cancer cells.

It is important to note that only 30–50% of clinical defined cases of paraneoplastic neuropathies have antineuronal antibodies, which means they are also a clinically and immunologically heterogeneous disease. In a study by Tschernatsch et al. [2], cases of antinuclear antibody positive patients without antineuronal antibodies have been found in 25% cases, where ANA and antineuronal antibody positive patients were found in 22% cases.

There is an increased incidence of antinuclear antibodies in malignant conditions. The most important non-autoimmune cause of rare ANA patterns is carcinoma, particularly in patients with rare cell-cycle related ANAs such as CENP-F. Paraneoplastic syndromes are more frequent in patients with cancer-associated positive ANAs [6]

In a study by Moll et al. [11] systemic auto-antibodies were found in 52% of patients with paraneoplastic neurological syndromes compared with only 16% in the control group with cancer only and 15% in the group of healthy controls. Also, ANA positive paraneoplastic neuropathy is more frequently associated with breast cancer and in female patients, and may define a certain subgroup of paraneoplastic neuropathies with different clinical and immunological features. In a study by Solans-Laqué et al. [9] antinuclear antibodies were frequently detected among patients with solid neoplasms of the breast (28.2%), colon (27.9%) and lung (26.6%), or with lymphoproliferative disorders (31.8%).

In breast cancer, tumor size and ANA seropositivity have been described to be inversely interrelated [10] This was the case with our patient where a small tumor was detected, which corresponds with the pathogenesis of the disease, where the immune response to tumor is strong and the tumors often present as either small or even occult and undetectable, mentioned by Jarius et al. [5].

Regarding the ANA patterns recognized in previous studies, no common patterns were detected. Instead, a variety of different patterns are mentioned including SSA/Ro52, Histone, PCNA, CENP-F etc. While some patterns are classically considered specific for antibodies to one specific antigen, most patterns can be attributed to antibodies to one of several antigens. Anti-cytoplasmic antibodies should not be ignored, as it may indicate the presence of antibodies to ENA in the absence of nuclear staining. A simultaneous reactivity of both cytoplasmic and nuclear or nucleolar antigens was also reported. Cytoplasmic and nucleolar staining can indicate a presence of antibodies to ribosomal ribonucleoprotein, since the nucleolus is an active site of synthesis of ribosomal RNA and that nucleolar ribonucleoproteins are precursors of cytoplasmic ribosomal ribonucleoprotein [12].

To date, around 30 different autoantibodies targeting brain antigens have been reported in patients with anticytoplasmic antibodies, many of which are of paraneoplastic origin. The synthesis of natural autoantibodies is based mainly on the activity of the of B $CD5^{+}$ limphocytes subpopulation. As mentioned earlier, the most common variant of PCD is anti-Yo, or Purkinje cell (PC) cytoplasmic antibody type 1 positive (PCA1). Anti-Yo binds with clusters of free and membrane bound ribosomes in the endoplasmic reticulum, on the trans-face of the Golgi apparatus or with nuclear antigens of Purkinje cells. These are all ultrastructural localizations of antibody-binding sites in the cell. Most of these antibodies target antigens involved in the mGluR1/calcium pathway which is essential for the proper functioning and survival of the Purkinje cell. A staining of the cytoplasm was observed amongst multiple other large cytoplasm-rich neurons in the brain, brainstem, retina anterior horns, sensory and sympathetic ganglia, myenteric plexus, stellar neurons and Schwann cells. Regarding cells outside the CNS, staining of the adrenal medulla and of epithelial cells of the renal glomerulus has been observed [3].

4. Limitations

As with any case report, the primary limitation of this paper is that a tendency of overestimation of specificity due to selection and publication bias may be present. We used a standard serum dilution for ANA of 1:100. However it is important to mention that low ANA titres may be present in healthy persons affecting overall sensitivity. Titres up to 1:80 occur in 13% of healthy individuals, 1:160 in 5% and 1:320 in 3% respectively. Unfortunately, after detecting ANA patterns in our case, our laboratory did not have the technical means to further determine the suspected antigens using other, more objective and quantitative methods such as ELISA.

5. Conclusion

The discovery of antinuclear or anticytoplasmic auto-antibodies might mislead the physician toward an extensive and useless search for connective or systemic tissue if paraneoplastic-specific antibodies are not searched for. An unspecific mix of patterns should not be ignored and might, through further research, show to be more valuable in the ANA screening than is the case now, where focus on only very specific patterns is associated with specific antigens and diseases. Also, weak positive results should not mislead into thinking that there is no overall effect on health, since quite the opposite was apparent with our patient. In our case, neither the tumor response to treatment, neurological presentation nor the immunological treatment had a strong effect on the ANA patterns which remained almost identical throughout the course of disease and treatment. Ultrastructural and molecular events in the pathogenesis of the disease could have caused certain minor changes in the pattern but are not of clinical value at the moment and further research is needed.

Credit author statement

Conceptualization was done by Amira Ćerimagić. Methodology was developed by Amira Ćerimagić and Nejra Džananović Investigation was done by Amira Ćerimagić and Nejra Džananović Resources was encouraged by Nejra Džananović Writing of original draft was done by Amira Ćerimagić Writing – review and editing done by Nejra Džananović Visualization was done both by Amira Ćerimagić and Nejra Džananović Supervision was done by Nejra Džananović Project administration was done by Amira Ćerimagić.

Declaration of competing interest

The authors declare that they have no competing interests.

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