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Case Report

Immune-Mediated Necrotizing Myopathy with Anti-Ku Antibodies and Simultaneous Papillary Thyroid Tumour: A Case Report

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Abstract

Objectives: Anti-Ku antibodies have been described in idiopathic inflammatory myopathies, where a high percentage met the criteria for immune-mediated necrotizing myopathy (IMNM). Furthermore, patients with anti-Ku or seronegative IMNM have a higher rate of malignancy. We report a patient with Rheumatoid Arthritis (RA) that developed IMNM with anti-Ku and a simultaneous diagnosis of papillary thyroid carcinoma. We aimed to elucidate the association between IMNM with anti-Ku and cancer in this patient.

Methods: We diagnosed IMNM with anti-Ku in a patient with RA. Anti-Ku positivity was confirmed by protein immunoprecipitation western-blot. To study Ku expression in the muscle and the tumour, we performed immunohistochemical staining of Ku70 in our patient's muscle and tumour biopsy as well as in two control muscle biopsies and three other papillary thyroid carcinoma biopsies.

Results: In the muscle, Ku70 was homogeneously expressed in some fibres in the healthy controls. However, in our patient, Ku70 presented a stronger expression in the subsarcolemmal region. In the tumour, Ku70 was heterogeneously expressed in the nuclei and homogeneously in the cytoplasm of the controls. Interestingly, our patient had strong homogeneous expression of Ku70 in the nuclei and little or no staining in the cytoplasm.

Conclusion: We report Ku expression and localization in both muscle and papillary thyroid carcinomas. Our patient showed differential Ku expression in both her muscle and tumour. Thus, anti-Ku antibodies could be a paraneoplastic phenomenon linking cancer to the development of IMNM.

Keywords: Myositis and Muscle Disease; Neoplasia, Autoantigens and Autoantibodies; Muscle; Histopathology

Introduction

Idiopathic inflammatory myopathies (IIM) are clinically and serologically heterogeneous autoimmune diseases characterized by inflammation in striated muscles. They present with muscle weakness, myalgia, elevated serum creatine kinase (CK) levels and myopathic pattern in the electromyogram (EMG). Symptoms develop over weeks or months, often first involving proximal muscles. Some IIM are associated with extra muscular manifestations including skin rashes, or interstitial lung disease (ILD) [1].There are five types among IIM: dermatomycosis's (DM), immune-mediated necrotizing myopathy (IMNM), sporadic inclusion-body myositis (IBM), overlap myositis and polymyositis1. Each IIM type has characteristic clinical, laboratory and histopathological findings. Thus, screening for autoantibodies and muscle biopsy are recommended for diagnosis. Accurate identification of the IIM is relevant to treatments, complications, outcomes and prognosis. Myositis-specific autoantibodies (MSA) allow classifying patients affected with similar phenotypic subsets. IMNM is characterized by progressive and often severe muscle weakness, very high serum CK levels, minimal extra-muscular manifestations, and muscle biopsy findings with prominent fibre necrosis and scarce inflammation [1,2]. Around two thirds of patients with IMNM have autoantibodies recognizing either SRP or HMGCR, the latter frequently seen in patients treated with statins1. However, differences between these two subtypes have been reported. For instance, patients with anti-SRP can have cardiac involvement and ILD, while patients with anti-HMGCR, as well as with seronegative IMNM, have an increased risk of malignancy [3]. Myositis-associated autoantibodies (MAA) are not specific to myositis and may be found in other autoimmune rheumatic diseases. MAA include anti-Pm-Scl, associated with systemic sclerosis (SSc) and myositis; anti-U1RNP and U3RNP associated with overlap syndromes often with myositis; and anti-Ku, usually found in systemic lupus erythematosus (SLE) and SSc but also in patients with overlap myositis with ILD [4]. The Ku protein is a heterodimer formed by the Ku70 and the Ku80 subunits that can bind to dsDNA and participates in its repair processes [5]. Interestingly, patients with anti-Ku are more likely to have a history of malignancy at the moment of SSc diagnosis compared with anti-Ku-negative patients [6]. There is an increased risk of malignancy in patients with IIM compared to the general population [7]. For instance, 32% of patients with DM have an associated cancer diagnosis. Most of cancer-associated myopathies appear to be paraneoplastic syndromes (PNS), which are complications of cancer caused by multiple mechanisms, one being immune-mediated. The most accepted hypothesis is that the immune response against the tumour is misdirected against healthy tissues due to cross-reactivity with tumoral antigens [7]. We report a 74-year-old woman who presented with severe myocarditis and IMNM. Screening for MSA and MAA revealed anti-Ku as the only autoantibody. Simultaneously, the patient was diagnosed with a papillary thyroid tumour. Thus, we investigated the presence of anti-Ku autoantibodies as a paraneoplastic phenomenon.

Case Description

A 74-year-old woman was admitted to our hospital with malaise, asthenia, muscular weakness, dysphagia and dyspnoea. The symptoms had begun 3 months before with increasing intensity and had been accompanied by unintentional 10kg weight loss in 4 months. The patient had a history of Rheumatoid Arthritis (RA) untreated due to intolerance to Methotrexate. The physical examination revealed proximal muscular weakness: 4/5 in shoulder girdle, 2/5 in pelvic girdle and 4/5 in neck flexors. Deep tendon reflexes were preserved. Sensory examination was normal. She showed oedema in lower extremities with bilateral positive fovea sign. Lung auscultation revealed bibasilar rales with left predominance. The initial blood tests are detailed in Supplementary Table S1. Electrocardiogram (ECG) showed sinusal rhythm with supraventricular extra systoles, without repolarization abnormalities, and no signs of left-sided heart failure but low voltages in II, aVR, aVF and precordial derivations, suggesting pericardial effusion. Chest radiography showed signs of pleuro-pericardial effusion. A PET-CT scan revealed a pseudo nodular hyper metabolic lesion in the anterior cervical region, suggesting a thyroidal nodule. A fine needle aspiration puncture was performed, compatible with papillary thyroid carcinoma (Bethesda V). Initially, the patient's condition was considered an IIM associated with my pericarditis. The pericardial effusion evolved to cardiac tamponade that required pericardiocentesis. The diaphragm weakness led to restrictive respiratory insufficiency requiring non-invasive mechanic ventilation at intensive care unit. She was treated with methylprednisolone 1mg/kg/day for 4 days and intravenous immunoglobulin for 5 days, with good response. A month after admission, the patient was discharged with residual muscle weakness. She continued tacrolimus 2mg/12h and prednisone 60mg/day as maintenance treatment. A month later, a hemi thyroidectomy was performed. Its biopsy confirmed a papillary carcinoma stage pT1bN0 with G2 histological grade. The indirect immunofluorescence on HEp-2 cells showed a speckled pattern (AC-4,5) at 1/1280 titter. Anti-Ro60, anti-La, anti-Sm and anti-U1RNP were negative. She was screened for MSA/MAA with EUROLINE-profile-16Ag (IgG) (Lübeck, Germany). She was also tested for anti-HMGCR by ELISA (Inova, Bedford, USA) and for anti-MDA5, TIF-1γ and nucleotidase-1A (cN1A) using a homemade western-blot as previously described8. Only anti-Ku autoantibodies were positive, confirmed by protein immunoprecipitation western blot, recognizing 70 and 80 KDa bands (Supplementary Data S1). Muscle biopsy was from the right biceps was performed. It showed variable fibre size,

increased internal nuclei, and abundant necrotic and split fibres with phagocytosis. Moreover, there were endomysia inflammatory infiltrates that were immunophenotyped (anti-CD68, anti-CD4, anti-CD8, anti-CD20, anti-CD138, anti-C5b9, anti-HLA-ABC, and HLA-DR). Infiltrates were composed of B cells with occasional plasma cells, macrophages, and isolated T CD4 and CD8 cells. There were membrane attack complex (MAC) deposits in capillary walls and at the sarcolemma of muscle fibres. We did not observe class I or II HLA expression. These pathological features were compatible with IMNM (Supplementary Figure S1). Immunohistochemistry against Ku70 (Santa Cruz Biotechnology, Dallas, USA) was performed in the muscle biopsy. The patient's sample was processed in parallel with a healthy individual and another patient with IMNM but with anti-HMGCR as controls [8]. Both controls showed homogeneous staining of some muscle fibres. However, our patient showed different staining pattern, with redistribution of Ku towards the sarcolemma (Figure 1). Immunohistochemistry against Ku70 was also performed on the patient's thyroid tumour, a normal thyroid sample, and three other samples from same-stage papillary carcinomas as controls. The healthy control showed homogeneous staining of the nuclei of follicular cells and little or no staining of the cytoplasm, similar to what was seen in the patient's healthy thyroid tissue (not shown). The control papillary carcinomas showed two different nuclear patterns: one with weak homogeneous staining and one with perinuclear staining. Additionally, we observed some immunoreaction at the cytoplasm in all the cells. However, our patient showed a strong homogeneous Ku70 staining in the nuclei of nearly all cells, and little or no staining within the cytoplasm (Figure 2).

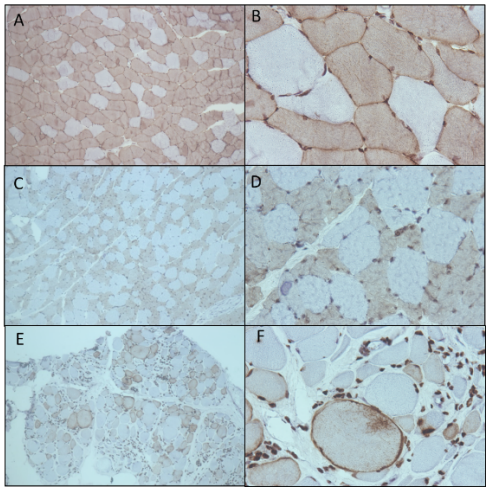

Figure 1: (A) Immunohistochemistry of the muscle with anti-Ku70 antibodies. Both the healthy control at x10 (B) and x40 (C) and a patient with immune mediated necrotizing myopathy (IMNM) and anti-HMGCR autoantibodies at x10 (D) and x40 (E) show Ku positive fibres with homogeneous staining. Our patient with IMNM and anti-Ku antibodies shows a distinctive pattern with greater intensity at its outer rim membrane x10 (F) and x40.

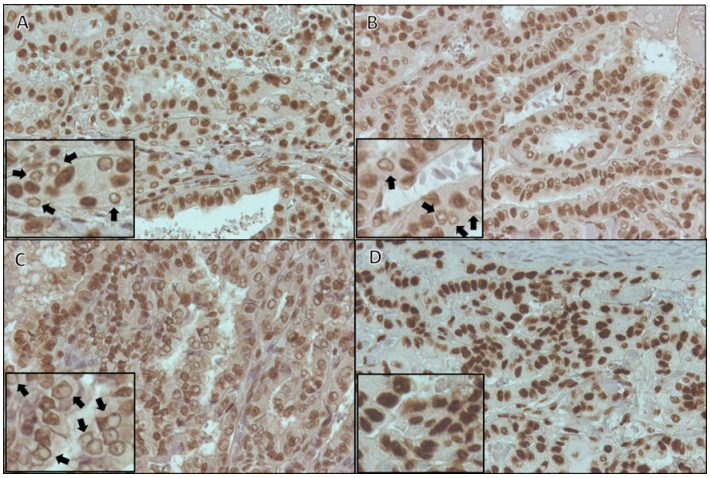

Figure 2: Immunohistochemistry of the thyroid with anti-Ku70 antibodies. The three papillary carcinomas stained as controls from patients without Ku autoantibodies (x40 A, B, C) showed some immunostaining of the cytoplasm and two different patterns of the nuclei: one with weak homogeneous staining and one with perinuclear staining (arrow). The papillary carcinoma of our patient (x40, D) shows strong homogeneous nuclear staining with little or no cytoplasmic staining.

Discussion

Our patient presented severe weakness, weight loss, dysphagia, and high CK levels associated with my pericarditis. The differential diagnosis of these clinical manifestations includes a wide range of muscle disorders. However, the severe and subacute presentation and CK levels suggested IIM. Particularly, the absence of skin rash but extra-muscular involvement indicated IMNM and discarded IBM although overlap myositis, DM or polymyositis still had to be considered [1,9]. Therefore, she was screened for autoantibodies and a muscle biopsy was performed. Laboratory tests revealed anti-Ku autoantibodies and the muscle biopsy suggested IMNM. She was simultaneously diagnosed with papillary thyroid carcinoma. Interestingly, Ku70-immunostaining of the muscle and thyroid showed different patterns compared to controls. A high proportion of false positive anti-Ku autoantibodies detected by Euro line has been reported, thus, we confirmed it by protein immunoprecipitation western blot [10]. In our patient, the finding of anti-Ku antibodies pointed towards overlap IIM with RA. However, her muscle biopsy was compatible with IMNM. Previous association of IIM with isolated anti-Ku antibodies, elevated CK and ILD has been described in two cohorts [11,12].

More recently, Yang et al reported a small IIM cohort in which, although anti-Ku antibodies were infrequent (1.7%), a significant proportion (86%) fulfilled criteria for IMNM [13].

Studies with larger cohorts are still needed. Regarding the Ku70-immunostaining, the patient's skeletal muscle and the controls showed Ku positive fibres. However, while the controls showed a homogeneous pattern, our patient had some fibres with a higher intensity at the sarcolemma. The expression of Ku70 at the membrane has been described, as well as its participation in cell adhesion processes [14]. These observations suggest that Ku70 overexpression at the sub sarcolemma could be targeted by the immune system. Supporting this idea, Mammen et al have reported increased expression of Mi-2 in myofibers within fascicles affected by per fascicular atrophy in DM [15]. Furthermore, other studies have found that anti-SRP and anti-HMGCR antibodies adhere to the muscle membrane and have a pathogenic role in patients with IMNM [2,16]. More experiments will be necessary to decipher the pathogenic role of anti-Ku antibodies in IMNM. Our patient's muscle biopsy showed classical IMNM findings, nevertheless, it also showed a considerable infiltrate composed mainly of CD20 cells with some plasmatic cells, and isolated CD4 and CD8 T cells. Allenbach et al described that a significant proportion of patients with IMNM and anti-SRP or anti-HMGCR showed CD3 infiltrates comparable to those of patients with DM and anti-Jo [12]. Thus, some T and B cell infiltrates as well as IgG deposits could be expected in IMNM [2].

The mechanism at fault might be the activation of the classical pathway of the complement, beginning with antibody adhesion [2,16]. However, the amount of CD20 cells found in our patient was unexpectedly high. Such B lymphocyte infiltrates the presence of plasma cells and the MAC deposit is consistent with an immune response towards the muscle in a patient with evidence of inadequate control of self-tolerance, as shown by her RA. Simultaneously with the IIM, this patient was diagnosed with thyroid papillary carcinoma. It is well established that seronegative IMNM has a higher rate of malignancy [3]. However, MAA may be present in seronegative IMNM. Thus, anti-Ku antibodies may be related with IMNM. Further studies are necessary to elucidate an association between anti-Ku IMNM and malignancy. There are several hypotheses on the underlying mechanism of IIM as a PNS. First, some patients have shown overexpression of common antigens both in their tumour and their muscle. The immune response against the cancer could trigger an autoimmune response against the shared antigen in the muscle [7,15,17]. Second, the tumour DNA somatic mutations hypothesis postulates that mutations from tumours elicit antibodies against the neoantigens, which cross-react with wild-type antigens. Thereafter, the tumour might escape by losing the mutation, redirecting the orphan immune response against the wild-type antigen in healthy tissues [7]. We found that Ku70 expression in the patient's papillary carcinoma differed from that in other papillary carcinomas of the same histological type and staging but without anti-Ku antibodies. Our patient's tumour showed strong homogeneous staining of the nuclei and little or no staining of the cytoplasm, while the control tumours showed weaker central nuclear expression and stronger cytoplasmic staining. Ku plays critical roles in processes such as non-homologous end joining repair, V(D)J recombination, apoptosis, telomere maintenance and DNA replication [5]. Its function and localization inside the cell vary with the cell cycle: during interphase, it appears dispersed throughout the nucleus, bound to chromatin, but during metaphase, it dissociates from chromatin and localizes to the periphery of chromosomes [5]. This delocalization has been related to Ku70 phosphorylation, which allows dissociation from DNA [18].

Moreover, Ku70 translocate between nucleus and cytoplasm through acetylation [19]. In the cytoplasm, it senses DNA, acting as a pattern-recognition receptor to trigger IFN responses [20]. Our controls seemed to have little Ku70 bound to chromatin in their tumoral cells, so, Ku70 might have been migrating to the cytoplasm. However, in our patient's tumoral cells, Ku70 was only in the nuclei. This phenomenon could have several explanations. Ku70 phosphorylation or acetylation could have been impaired, preventing either its dissociation from the chromatin or migration to the cytoplasm. Sui et al suggest that inhibiting cytoplasmic translocation of Ku70 could be a treatment for autoimmune diseases, as it would limit the sensing of cytosolic DNA, therefore downregulating IFN responses [20]. However, we hypothesize that the abnormal nuclear/cytoplasmic localization within the tumoral environment could have triggered our patient's autoimmune response to Ku. Alternatively, a mutation in Ku might have caused both the abnormal cell localization and the immune response. In our case, both the nuclear overexpression of Ku or a Ku mutation could have broken the tolerance towards Ku in the context of a tumoral environment. Further studies are needed to elucidate the mechanisms involved in the association of anti-Ku, IMNM and the tumour. In conclusion, we report a patient with autoimmune background and neoplasm with abnormal expression of Ku that might have acted as the trigger to develop IMNM with autoantibodies against Ku. No specific funding was received from any bodies in the public, commercial or not-for-profit sectors to carry out the work described in this article. The authors declare that they have no relevant or material financial interests that relate to the research described in this paper. In this case, ethics committee approval was not required since the studies performed were part of clinical practice and the patient gave written informed consent for the procedures and the use of the clinical data and extra biological material. The authors confirm that the data supporting the findings of this study are available within the article or its supplementary materials.

Key Messages

- Anti-Ku antibodies may be related with IMNM.
- There might be an association between anti-Ku positive IMNM and malignancy.

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