Review

Diagnostic and prognostic value of anti-cN1A antibodies in inclusion body myositis

S. Salam[1], M.M. Dimachkie[2], M.G. Hanna[1], P.M. Machado[1]

[1]Department of Neuromuscular Diseases, UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, UK; [2]Department of Neurology, University of Kansas Medical Center, Kansas City, KS, USA.

Sharfaraz Salam, MD, MRCP
Mazen M. Dimachkie, MD
Michael G. Hanna, MD, FRCP
Pedro M. Machado, MD, FRCP, PhD

Please address correspondence to:
Pedro M. Machado,
Department of Neuromuscular Diseases,
1st Floor, Russell Square House,
10-12 Russell Square,
London WC1B 5EH, United Kingdom.
E-mail: p.machado@ucl.ac.uk

Received on January 13, 2022; accepted in revised form on January 21, 2022.

Clin Exp Rheumatol 2022; 40: 384-393.

Key words: inclusion body myositis, autoimmunity, antibodies, anti-cN1A, diagnosis, prognosis, outcome

Funding: S. Salam is supported by a UCL Queen Square Institute of Neurology & Cleveland Clinic London MPhil/PhD Neuroscience Fellowship.
P.M. Machado and M.G. Hanna are supported by the National Institute for Health Research (NIHR) University College London Hospitals (UCLH) Biomedical Research Centre (BRC).
M.G. Hanna is supported by a Medical Research Council (UK) strategic award to establish an International Centre for Genomic Medicine in Neuromuscular Diseases (ICGNMD) (MR/S005021/1).
Competing interests: page 392.

ABSTRACT

Inclusion body myositis (IBM) is an acquired idiopathic inflammatory myopathy more commonly seen in individuals aged above 50. Unlike other idiopathic inflammatory myopathies, there is no response to immunosuppression/immunomodulation. The lack of response to such therapies led the focus away from considering IBM as a purely immune-mediated condition. However, the discovery of antibodies against cytosolic 5´-nucleotidase 1A (cN1A) in patients with IBM has reinvigorated interest in autoimmunity as a key role in its pathogenesis. Over the last decade different methods have been developed to detect anti-cN1A antibodies. There has been an interest in whether these assays can be utilised in the diagnosis of IBM. Furthermore, there has been focus on whether anti-cN1A antibodies can be used to prognosticate and predict the clinical phenotype in IBM. Anti-cN1A antibodies appear to have a high specificity and moderate sensitivity for IBM. There have been some exploratory clinicopathological associations described in seropositive IBM patients, but sample sizes in most studies have been small so far. Antibody testing is yet to be standardised; which somewhat limits our ability to draw robust conclusions from current investigations. In this article we review the literature on anti-cN1A antibodies and discuss whether they have a role in clinical practice.

Introduction

Inclusion body myositis (IBM) was first described in detail within a case series reported in 1978 (1). IBM is an acquired myopathy and commonly grouped into a spectrum of myopathies known as idiopathic inflammatory myopathies. It is often reported as the most common acquired muscle disease in individuals aged above 50 (2, 3). The classic pattern of weakness described is characterised by involvement of the long finger flexors, quadriceps and foot dorsiflexors (4-6). Patients can often develop dysphagia which can have a significant impact on prognosis.

The pathogenesis behind the condition is uncertain and is likely to be multifaceted. Given the inflammatory features on muscle biopsy such as marked $CD8^+$ T cell infiltration and increased major histocompatibility complex (MHC) class II staining, an autoimmune aetiology has been hypothesised (2). However, this hypothesis has been under scrutiny given the lack of response to immunosuppressive and immunomodulatory therapies. Therefore, given its progressive nature, it has been suggested that perhaps IBM is driven by degenerative processes. Postulated mechanisms contributing to IBM pathogenesis include abnormal mitochondrial function, myonuclear degeneration and abnormal protein homeostasis resulting in the accumulation of aberrant proteins within the muscle, such as TAR DNA-binding protein 43 (TDP-43), a highly conserved nuclear RNA/DNA-binding protein involved in the regulation of RNA processing, and sequestosome-1 ([SQSTM1], also known as ubiquitin-binding protein p62), a cargo protein involved in the degradation of misfolded proteins via selective autophagy (7). Abnormal nucleic acid metabolism has been suggested to play a role in IBM pathogenesis. The expression of microRNAs, a class of non-coding RNAs that play important roles in regulating gene expression, has been shown to be reduced in immune-mediated myopathies including IBM (8). This may represent a common mechanistic link to other myositis given many of these involve autoantibodies directed against

important components of nucleic acid metabolism.

In 2013, two groups confirmed the presence of an antibody against a 43 to 44 kilodalton protein, cytosolic 5´-nucleotidase 1A (cN1A; synonyms: cN-1A, Mup44, NT5C1A, NT5c1A, NT5C1a) in patients with IBM (9, 10). cN1A is an enzyme which is involved in the nucleic acid metabolism (11). The discovery of the antibody has re-invigorated an interest in the immune-mediated nature of IBM. Furthermore, isolating cN1A as an autoantigen highlighted again the notion of disordered muscle metabolism playing an important role in IBM pathophysiology. Subsequently there has been great interest into whether anti-cN1A antibodies may have a role in diagnosis and help stratify disease course.

In this review we provide an overview of anti-cN1A antibodies and discuss whether these antibodies have a role in clinical practice in relation to IBM.

Cytosolic 5´-nucleotidase 1A (cN1A) function

cN1A is an enzyme highly expressed in skeletal muscle and belongs to a class of enzymes known as 5'-nucleotidases. cN1A has a role in physiological processes such as cell replication and metabolic regulation including regulation of deoxynucleotides after nucleic acid breakdown (11). cN1A is involved in the hydrolysis of adenosine monophosphate into adenosine and inorganic phosphate. Previously, lead salt-based staining for 5´-nucleotidases was used to aid in the diagnosis of inflammatory myopathies (12). Silencing cN1A expression has been shown to increase activation of the enzyme anti-phosphorylated adenosine monophosphate-activated protein kinase (AMPK) (13). Activated AMPK seems to play a role in upregulating catabolic pathways in skeletal muscle tissue (14, 15).

Anti-cN1A antibodies and their role in pathogenesis

In 2011 Salajegheh *et al*. were able to identify circulating antibodies in the plasma of IBM which bind to a 43 kilodalton muscle autoantigen (16). They were able to demonstrate autoantibody binding to this protein in 52% of patients with IBM. This antibody was not present in the sera of 15 healthy volunteers and 25 autoimmune myositis (16). cN1A was identified to be this autoantigen in 2013 by two groups using mass spectrometry (9, 10). Interestingly, immunohistochemistry illustrated the co-localisation of anti-cN1A antibodies to perinuclear regions, rimmed vacuoles and areas of myonuclear degeneration in muscle specimens from IBM patients (9).

Three isotypes of the antibody have been identified; IgG, IgA and IgM isotypes of anti-cN1A antibodies (17). With regards to antibody binding, three peptide epitopes on the cN1A have been described in the literature thus far (9, 10, 18). Synthetic peptides derived from these immunodominant epitopes were used to develop initial enzyme-linked immunosorbent assays (ELISA) for detecting anti-cN1A antibodies (10, 18).

At the time of this manuscript there has been limited investigation into the precise role of anti-cN1A antibodies in IBM pathogenesis. In an experimental murine investigation, Tawara *et al*. compared the effect of passive immunisation of mice using IgG extracted from IBM patients positive for anti-cN1A, IgG extracted from IBM patients negative for anti-cN1A, control IgG obtained from healthy subjects without the autoantibodies, and phosphate buffered saline (PBS) as negative control (19). They observed the formation of p62/SQSTM1-positive sarcoplasmic aggregates in myofibres of mice injected with anti-cN1A positive IgG, which was significantly greater in comparison to mice injected with IgG from anti-cN1A negative IBM patients, control IgG or PBS. Mice injected with anti-cN1A positive IgG also showed higher infiltration of CD68+ macrophages; however, this difference was not statistically significant. Furthermore, myocytes treated *in vitro* with anti-cN1A positive IgG showed significantly greater expression of p62 and significantly reduced cN1A expression, compared to cells treated with anti-cN1A negative IgG, control IgG, PBS, or naïve cells. Finally, AMPK levels tended be higher and cN1A expression was reduced in muscle extracted from IBM patients, but these findings were not statistically significant.

Another supportive finding for an autoimmune process driving IBM is that the strongest genetic risk lies within the MHC region, in particular the human leukocyte antigen (HLA)-DRB1*03:01 allele (20). The largest genetic association study in IBM to date has also identified other two candidate HLA alleles: DRB1*01:01 and DRB1*13:01 (20). No significant association with anti-cN1A seropositivity has been found to be independent of the HLA-DRB1*03:01 allele (20, 21). This observation may reflect the high frequency of this allele in the IBM population. When seropositive IBM patients were compared against their seronegative counterparts, no significant differences in HLA associations were observed (20). Recently, highly differentiated effector $CD8^+$ cytotoxic T-cells, relatively resistant to apoptosis and expressing the killer cell lectin-like receptor G1 (KLRG1), have been described in association with IBM, and proposed as a potential treatment target in IBM (and T-large granular lymphocytic leukaemia [T-LGLL]) (22).

Anti-cN1A antibody detection

Since the initial identification of anti-cN1A antibodies various techniques have been developed for their detection. At the time of this publication, we were able to identify two assays in the literature that have been developed for commercial use. The Washington University Neuromuscular Laboratory have provided commercial testing using a method involving western blotting followed by confirmatory ELISA with recombinant cN1A polypeptide (23). Another whole recombinant polypeptide ELISA was developed by Kramp *et al*. at Euroimmun labs, Lubeck, Germany, and has also been used commercially by the Rheumatology Diagnostics Laboratory (RDL) in the USA (24, 25).

In earlier studies detection of anti-cN1A antibodies was performed using techniques such as immunoblotting and immunoprecipitation using lysates derived from extracted human skeletal muscle tissue (10, 16). Alternatively, other groups have used immunoblotting

with Human Embryonic Kidney (HEK) 293 cell lysates (26). Such assays have had sensitivities varying from 33% to 70.2% (Table I) (9, 10, 16, 26).

ELISA remains the most common method of detecting anti-cN1A antibodies in studies so far. As mentioned earlier, three major epitopes have been identified on the cN1A antigen. These identified peptide sequences were used to manufacture three linear synthetic peptides (peptides 1, 2 and 3) for a peptide ELISA (18, 27). Positivity was determined if sera demonstrating reactivity above the cut-off value for at least one of the three peptides. Sensitivity from these peptide assays have varied between 32.8 to 37% (18, 20, 27). Different combinations of peptide binding were observed between individuals and within different disease groups (18). Some sera testing positive for anti-cN1A using immunoprecipitation were not showing any reactivity against the three peptides (18). Therefore, some individuals may have circulating antibodies that do not bind to these specific epitopes and are at risk of producing false negative results (18, 28). Evidence suggests that, in addition to linear epitopes, there may be antibody binding to 'conformational' epitopes in a fully synthesised cN1A protein. Therefore it has been proposed that ELISAs using an entire cN1A polypeptide rather than epitope peptides in isolation, may improve the ability to detect circulating anti-c1NA antibodies. It may also be more advantageous in terms of ease; using one ELISA rather then running a three separate peptide ELISAs.

Kramp *et al*. developed an ELISA using whole recombinant cN1A polypeptide at Eurroimmun, Lubeck, Germany, and this assay has also been used at the RDL(24). The sensitivity of this assay has varied from 35.5% to 66.7% (Table I) (24, 29). The Washington University Neuromuscular Laboratory have achieved sensitivities varying between 63.9% to 72% using a technique involving western blotting followed by a confirmatory whole recombinant cN1A polypeptide ELISA (23, 30).

Herbert and Pruijn described early pilot work comparing the peptide ELISA with a whole recombinant cN1A ELISA in 55 IBM patients (28). This study showed a moderate correlation in seropositivity between the two assays (r^2=0.54). They found that 27.3% (15/55) of patients demonstrated seropositivity with both assays, 23.6% (13/55) for the peptide ELISA alone, 9.1% (5/55) testing positive for the recombinant cN1A ELISA alone and 40% (22/55) testing negative for both assays. Essentially 54% (15/28) of those testing using the peptide ELISA showed reactivity to the polypeptide ELISA. A possible explanation for these observations is that linear peptides may be less accessible to antibodies due to the folding of the full-length protein. The authors suggest a potential utility in using anti-cN1A assays combining peptide and whole protein ELISAs. However the authors note a bias towards selecting patients testing positive for anti-cN1A using the peptide ELISA in their cohort. A more optimal study design would be to test both techniques on an unselected group of samples. Kramp *et al*. found a higher and significant correlation in seropositivty (r=0.79) between recombinant cN1A and peptide ELISAs in 51 IBM patients (24). Whole recombinant cN1A ELISAs do appear to have the potential to achieve higher sensitivities than peptide ELISAs (Table I). However, more detailed work directly comparing these ELISAs techniques using larger sample sizes is needed.

The majority of ELISAs developed detect the IgG isotype of anti-cN1A antibodies. However, IgA and IgM isotypes have been shown to exist (17). Greenberg was able to develop separate ELISAs to detect these three isotypes separately with similar sensitivities (IgM=53%, IgA=49%, IgG=51%) and specificities (IgM=96%, IgA=95%, IgG=94%). An ELISA to detect all three isotypes was developed using a recombinant c1NA polypeptide antigen, improving the sensitivity to 76% (17). However when this technique was used in another cohort the sensitivity obtained was lower, with 34.8% of IBM patients testing positive (21). Authors of this study suggested the lower seropositivity in their cohort may be in part attributed to use of immunosuppression in 70% of IBM patients in their cohort (21).

Tawara and Yamshita developed a novel assay using cell-based immunofluorescence cytochemistry (19, 31). In this method, the presence of anti-cN1A autoantibodies is detected based on colocalisation of green fluorescent protein (GFP) labelled cN1A and the signal detected from Alexa Fluor 594-labelled human IgG. The sensitivity and specificity for this technique was 35.8% and 91.8% respectively (19). Amlani *et al*. developed a method of antibody detection using an Addressable laser bead immunoassay (ALBIA) using a recombinant cN1A protein (32). They were able to achieve a sensitivity of 48.8% in IBM patients. Eura *et al*. employed a histopathological approach, in which patients were deemed to be positive if the muscle biopsy showed evidence of anti-cN1A antibody staining in perinuclear areas or vacuoles (33). Seropositivity has been shown to fluctuate in some assays with no apparent relevance to the clinical status so far (9, 30).

The heterogeneity of assays used in studies assessing anti-cN1A antibodies impacts on how we interpret the difference in results reported in observational studies looking at the association between seropositivity and IBM features. There is a need for the testing of anti-cN1A to become standardised so more reliable interpretations can be made about its clinical utility.

Another aspect that adds variability and needs to be addressed moving forward, is identifying suitable cut offs. Setting higher cut offs has clearly improved specificity in some assays but increases risk of false negative results (and decreases sensitivity) (9, 10, 28). In the future it may be helpful to investigate the use of anti-cN1A antibody titre levels when testing for seropositivity rather than just abiding by arbitrary cut-offs (28).

A recent meta-analysis has investigated the role of anti-cN1A antibody as a diagnostic marker using a Bayesian methodology (39). Out of 17 studies that were reviewed, seven were pooled together for this meta-analysis (10, 16, 18, 19, 26, 32, 34). Case reports, duplicate reports and reviews were removed from the analysis. Based on these data the authors suggest that anti-cN1A antibodies

are not a useful diagnostic biomarker, with a positive predictive value of 0.75 for those aged above 50 and 0.25 in the general population. When interpreting these results, it should be noted that different assay techniques were used in the studies included in this meta-analysis. Furthermore, analysis was not adjusted for factors such as age, gender, ethnicity, disease severity and comorbidities.

Anti-cN1A antibodies and histopathological correlation

Attempts have been made to determine associations between anti-cN1A antibody positivity and histopathological changes.

Lloyd *et al*. were able to demonstrate that IBM patients testing positive for anti-cN1A antibodies had significantly lower levels of rimmed vacuoles on histology compared to those patients testing negative for the antibody (26).

Lilleker *et al*. were able to show that muscle from those IBM patients testing positive for anti-cN1A antibodies demonstrate significantly more cytochrome c (COX) negative fibres (27). Despite adjustment for age at disease onset, gender, co-morbidities and age at biopsy, there was a significant excess of COX deficient fibres in antibody positive patients. Ikenaga *et al*. made a similar observation of significantly more COX deficient fibres in seropositive IBM patients (30).

Tawara *et al*. report a significantly smaller mean area of type 2 myofibres in seropositive IBM patients (19). Pinto *et al*. describe seropositive patients having significantly higher number of regenerating fibres (29).

Paul *et al*. found seropositive patients were more likely to have inflammatory changes (36). However, this was either in the presence of one or more other classic IBM pathological findings, or not statistically significant in the absence of such features. No other studies have shown a relationship between seropositivity and the degree of muscle inflammation (19, 25-27, 30).

Although not statistically significant, Tawara *et al*. observed increased perinuclear colocalisation of autophagy related proteins aggregates containing p62 and the anti-cN1A antibody (19). However, the sample sizes in this study were small with three antibody positive IBM patients and six seronegative patients, therefore limiting the ability to extrapolate these findings. Eura *et al*. noted that in 80% of their IBM cohort there was anti-cN1A positivity within vacuoles; in all such patients p62/SQSTM1 was co-expressed in these vacuoles (33). They also observed 89% of patients had anti-cN1A staining in the perinuclear region. Colocalisation of anti-cN1A at these sites had been noted in earlier studies (9). This colocalisation supports the hypothesis that altered nucleic acid metabolism at these sites may be involved in IBM pathogenesis.

No statistically significant relationship between seropositivity and other histological features such as MHC expression, congophillic or tubofilamentous inclusions and focal infiltration have been noted thus far (25-27, 30, 36).

Anti-cN1A antibody and clinical phenotype or other disease features

There have been attempts to determine whether anti-cN1A antibodies can help predict a specific clinical phenotype and if seropositive patients belong to a clinically distinct subset of IBM.

Investigating correlations between anti-cN1A antibody positivity and other investigations have shown variable findings. We have already discussed the potential associations between seropositivity and pathological hallmarks noted on biopsy. Creatinine Kinase (CK) levels are classically only mildly elevated (or normal) in IBM but can be a useful tool in formulating the diagnosis. There appears to be no relationship noted thus far in seropositive IBM patients and CK levels (25, 27, 30, 32, 36, 37). Interestingly, in one study, CK levels were found to be significantly lower in seropositive patients diagnosed with inflammatory myopathies other than IBM (30). Seropostivity has recently been shown to be associated with shorter motor unit potentials (29). No significant relationship has been observed between seropositive IBM patients and other myopathic changes on electromyography (EMG), such as myotonic discharges and denervation (19, 36).

Muscle MRI is used as a clinical and research tool in IBM; fatty infiltration can be observed in T1-weighted sequences, reflecting chronic changes, and water deposition or muscle oedema as part of muscle inflammation can be detected using T2-weighted sequences with fat suppression, such as the short tau inversion recovery (STIR) sequence (40, 41). Detecting such changes in characteristic muscle groups can be useful in clinical practice. Our group were able to demonstrate higher degree of fat infiltration and greater STIR hyperintensity in seropositive IBM patients (42).

Certain antibodies tested in autoimmune screening have been shown to be significantly higher in IBM patients testing positive for anti-cN1A antibodies; this includes anti-La/SSB, anti-Ro52, anti-signal recognition particle (SRP) and anti-isoleucyl-tRNA synthetase (anti-OJ) (24, 27). No significant relationship between anti-cN1A positivity and antinuclear antibody staining patterns in IBM has been demonstrated (32). Certain immune-mediated myopathies have been shown to be associated with a high risk of cancer, namely anti-transcriptional intermediary factor-1 gamma (TIF-1γ) and anti-nuclear matrix protein 2 (NXP2) associated myositis (43). Unlike these myositis-specific antibodies, anti-c1NA positivity does not appear to be associated with malignancy (21, 30).

The role of specific viral infection resulting in a predilection to develop IBM has previously been suggested (4). Viruses suggested to have an association with IBM, including hepatitis C virus (HCV), human immunodeficiency virus (HIV) and human T-cell lymphotropic virus type 1 (HTLV-1). Interestingly, Tawara *et al*. noted that in their Japanese cohort those patients testing positive for anti-cN1A antibodies were less likely to have evidence of previous hepatitis C infection (19). Oyama *et al*. found no such relationship between antibody status and previous hepatitis C infection (35).

There does not appear to be an association between gender and seropositivity (19, 23, 27, 30, 36, 37). In one report the risk of seropositivity was shown to be higher when the age of diagnosis was

Table I. Anti-cN1A antibody assays used in studies testing positivity in inclusion body myositis.

Year	Authors	Assay technique *(± commercial laboratory)*	Number of IBM patients tested	Total number of non-IBM individuals tested	Number of non-IBM individuals tested in each subgroup	Sensitivity (%)	Specificity (%)
2011	Salajegheh *et al*. (16)	Immunoprecipitation using muscle lysates	25	40	DM (n=10), PM (n=10), MG (n=5), HC (n=15)	52	100
2013	Larman *et al*. (9)	Immunoblotting using muscle lysates	47	153	HC (n=35), PM (n=26), Necrotising Myositis (n=14), DM (n=36), MG (n=13), Muscular dystrophy (n=10), Myotonic dystrophy (n=4), LGMD (n=4), Myofibrillar myopathy (n=1), Distal myopathy with rimmed vacuoles (n=1), Other muscle diseases (n=19)	70.2 (34.0 at a higher cut off)	92.4 (98.3 at a higher cut-off)
2013	Pluk *et al*. (10)	Immunoprecipitation using muscle lysates	94	172	HC (n= 32), DM (n=24), PM (n=22), NMD (n=94)	59.6 (33.0 at a higher cut off)	91.3 (97.1 at a higher cut-off)
2014	Greenberg (17)	Recombinant cN1A ELISA for three Ig isotypes	50	155	HC (n= 34), DM (n=36), PM (n=27) Muscular dystrophy (n=9), MG (n=13), Necrotising myositis (n=13), Other myopathies (n=23)	76	91
2016	Lloyd *et al*. (26)	Immunoblotting of lysates from transfected HEK293 cells	117	383	HC (n=42), PM (n=42), DM (n=159), SLE (n= 96), SS (n=44)	60.7	86.7
2016	Herbert *et al*. (18)	Three peptide ELISA	238	524	PM/DM (n=185), PM/SSc overlap (n=12), NMD (n=93), SS (n=22), SLE (n=44), SSc (n=44), RA (n=4), Multiple sclerosis (n=40), Type 1 Diabetes (n=40)	37.0	93.7
2016	Limaye *et al*. (21)	Recombinant cN1A ELISA for three Ig isotypes	69	0	0	34.8	NA
2016	Kramp *et al*. (24)	Recombinant cN1A ELISA *(Euroimmun)*	Group A = 31 (*RDL*)	Group A =255	*Group A* HC (n=52), DM (n =4), PM (n=7), Unspecified myositis (n=94) Muscle atrophy (n=1), Myonecrosis (n=4), SLE (n=33), SS (n=20), SSc (n=20), RA (n=9)	Group A = 35.5	Group A = 96.1
			Group B = 51 (Lubeck, Germany)	Group B =202	*Group B* HC (n=202)	Group B = 39.2	Group B = 96.5
2016	Goyal *et al*. (23)	Western Blotting followed by recombinant cN1A ELISA *(WUNL)*	25	0	0	72	NA
2016	Eura *et al*. (33)	Perinuclear or rimmed vacuole anti-cN1A staining in muscle	35	20	PM (n=10), DM (n=10)	88.6	80.0
2017	Muro *et al*. (34)	Recombinant cN1A ELISA	10	356	HC (n=42), DM (n=144, 62 with classic, 48 with clinically amyopathic, 22 with cancer-associated, 12 with juvenile), SLE (n=50), SSc (n=50), SS (n=50), PM (n=10), Mixed connective tissue disease (n=10)	80	91.9
2017	Lilleker *et al*. (27)	Three peptide ELISA	311	0	0	32.8	NA
2017	Tawara *et al*. (19) (Yashita and Tawara 2019) (31)	Cell based immunofluorescence assay	67	158	HC (n=10). PM (n=36), DM (n=31), Immune-mediated necrotising myopathy associated with anti–signal recognition particle autoantibody (n=8), Non-inflammatory muscle diseases (41), fasciitis (51), Autoimmune diseases including SLE and SS (n=15), and Neurogenic muscular atrophy (16)	35.8	91.8
2017	Rothwell *et al*. (20)	Three peptide ELISA	104	0	0	34.6	NA
2018	Felice *et al*. (25)	Recombinant cN1A ELISA *(RDL)*	40	0	0	50 (42.5 when weakly positive patients excluded)	NA

Year	Authors	Assay technique *(± commercial laboratory)*	Number of IBM patients tested	Total number of non-IBM individuals tested	Number of non-IBM individuals tested in each subgroup	Sensitivity (%)	Specificity (%)
2019	Amlani *et al*. (32)	ABIA using recombinant cN1A	43	615	HC (n=78), IIM (DM/PM n=142), SLE (n=199), SSc (n=50), SS (n=19), Juvenile DM (n=40) Osteoarthritis (n=47), RA (n=27)	48.8	91.7
2020	Oyama *et al*. (35)	Recombinant cN1A ELISA	83	0	0	33.7	NA
2021	Ikenaga *et al*. (30)	Western Blotting followed by Recombinant cN1A ELISA *(WUNL)*	249	344	DM (n = 53), Anti-synthetase syndrome (n = 27), Necrotising myositis (n = 76), Non-specific myositis (n = 84). VCP-related multisystem proteinopathy (n = 26), LGMD (n = 19), Myotonic dystrophy (n = 9), Mitochondrial myopathy (n = 5), Facioscapulohumeral muscular dystrophy (n = 6), Becker muscular dystrophy (n = 5), McArdle's disease (n = 3), GNE myopathy (n = 3), myofibrillar myopathy (n = 3), Pompe disease (n = 2), dystroglycanopathy (n = 1), Poland syndrome (n = 1), and centronuclear myopathy (n = 1), Idiopathic rhabdomyolysis (n = 20).	63.9	84.6
2021	Paul *et al*. (36)	Recombinant cN1A ELISA *(RDL)*	92	0	0	51.1	NA
2021	Lucchini *et al*. (37)	Recombinant cN1A ELISA *(Euroimmun)*	62	62	DM (n=20), PM (n=10), Necrotising myositis (n=28), Overlap Myositis (n=4)	37.1	96.8
2021	Levy *et al*. (38)	Recombinant cN1A ELISA *(Euroimmun)* (79 myositis patients were tested. 26 out of these 79 myositis patients also had SS. Of the SS patients, 6 had IBM, 3 had DM and 17 had PM.)	9	70	DM (21), PM (49)	55.6	88.6
2021	Pinto *et al*. (29)	Recombinant cN1A ELISA *(RDL)*	30	0	0	66.7 (Of all IBM patients tested; 35% were weakly positive, 25% were moderately positive and 40% were strongly positive	NA

cN1A: 5'-cytosolic nucleotidase 1A; ELISA: enzyme-linked immunosorbent assay; LGMD: limb-girdle muscular dystrophy; NA: not applicable (when specificity not quoted in the article or could not be inferred the value has been marked as NA); RDL: Rheumatology Diagnostics Laboratory; HC: healthy controls; IIM: idiopathic inflammatory myopathies; DM: dermatomyositis; PM: polymyositis; MG: myasthenia gravis; NMD: neuromuscular diseases; RA: rheumatoid arthritis; SLE: systemic lupus erythematous; SS: Sjögren's syndrome; SSc: systemic sclerosis; WUNL: Washington University Neuromuscular Laboratory.

above 60 (25). However, most studies have not identified such an association with age. An association between dysphagia and anti-cN1A seropositivity has been noted in two studies. In one cross-sectional study using the IBM functional rating scale (IBMFRS) scale item one to assess swallowing function, seropositive patients had lower item 1 scores and were significantly more likely to have more severe swallowing problems (defined as an item score below or equal to 2) (37). Goyal *et al*. also found that seropositive patients more frequently had dysphagia (23), with dysphagia being defined based on patient reported symptoms during assessment. Both studies did not use validated dysphagia-specific questionnaires or any radiographic studies to determine the degree of dysphagia or any subclinical evidence of dysphagia in absence of reported symptoms. This potential relationship between anti-cN1A seropositivity and dysphagia has not been confirmed in other studies (19, 25, 27, 30, 32, 36). When dysphagia has been assessed with videofluroscopy in addition to speech and language therapist review, there was no evidence that anti-cN1A antibodies were associated with the presence of dysphagia (36).

Only one study so far has noted facial weakness to be significantly more likely in seropositive patients, even after adjusting for other variables such as age, gender and comorbidities (27). One group found seropositive patients to score higher on the modified oral bulbar facial respiratory scale, however this relationship was not statistically significant (p=0.06) (23). No other studies have described an association between anti-cN1A seropositivity and facial weakness. Clearly it would be

of great benefit to have a biomarker to help predict the pattern or severity of weakness in IBM. Patients testing positive for the antibody have been shown to be less likely to present with proximal upper limb weakness in comparison to seronegative IBM patients in two studies (27, 30). However, again other reports have not found a significant relationship. The potential of anti-cN1A to predict more severe muscle weakness has been noted using the sum score of the Medical Research Council (MRC) scale for muscle strength (23). Amlani *et al*. found that seropositive patients were more likely to have more severe weakness (assessed using dynamometry testing of the finger flexors and quadriceps) (32). Further multivariate analysis did not show a statistically significant association. A relationship between anti-cN1A antibodies and limb weakness has not been demonstrated in other studies (36).

Difficulties with mobility affects most patients with IBM at some point in their disease course. In one study, seropositive patients were found to have an increased risk of requiring a walker or a wheelchair (23). The median time to stand up from a standard chair was 15 seconds and was also significantly longer in the seropositive group. Dejthevaporn *et al*. found seropositive patients to score significantly lower on the IBMFRS item for their ability to climb stairs compared to their seronegative counterparts (42). Apart from these studies, other groups have been unable to detect a significant relationship between being anti-cN1A antibody positive and the level of disability. After adjusting for age at disease onset, gender and comorbidities, the risk for mobility aid requirement was just outside the significance threshold (p=0.056) in a study by Lilleker *et al*. (27).

Respiratory compromise is often seen as an end stage feature in IBM. Goyal *et al*. found that seropositivity is associated with a greater probability of respiratory compromise, with these patients achieving lower forced vital capacities (23). Furthermore, seropositivity has been shown to be associated with greater risk of death from respiratory complications (27). These associations with respiratory function are yet to be replicated.

The correlation between phenotype and antibody status is being prospectively investigated across 12 US sites (NCT05046821). The Sporadic Inclusion Body Myositis Natural History Study (INSPIRE-IBM) is a prospective natural history study on 150 patients fulfilling the ENMC 2011 criteria for diagnosis of IBM. Participants will be followed up every 6 months over 2 years and at baseline and will be testing for cN1A antibody status. This study will assess the rates of disease progression and severity as measured by rates of decline in IBMFRS score and TUG, and will quantify decline in respiratory function. Additional studies on muscle and blood derived lymphocytes are also planned.

Anti-cN1A antibody and predicting survival in IBM

There has been interest in whether anti-cN1A antibodies could be used as prognostic marker. Lilleker *et al*. were able to demonstrate that testing positive for anti-cN1A antibody increases the risk of death (27). The median survival time after diagnosis in seropositive group was 17.6 years compared to 24.2 years in the seronegative group which was significantly less. The risk of death was 65% higher in seropositive patients. Even after adjusting for age at diagnosis, gender and comorbidities, the risk of death was significantly higher in seropositive patients. However other studies looking at the impact of anti-cN1A antibodies on survival has not found any statistically significant associations (30). Caution is required when interpreting the results of these studies (27, 30), as they have retrospective study designs and inherent limitations with possibility of bias and spurious results.

Anti-cN1A antibody in other autoimmune diseases and muscle conditions

Anti-cN1A antibody positivity was shown to be significantly lower in immune-mediated myopathies in comparison to IBM (Table I) (10, 17, 18, 24, 26). Anti-cN1A antibody positivity could be particularly useful in distinguishing IBM from polymyositis or dermatomyositis, as IBM is commonly misdiagnosed as these conditions (10, 17, 18, 24, 26). Testing positive for the antibody may be helpful in the clinic if muscle biopsy is contraindicated or inconclusive though the current ENMC 2011 criteria do not include antibody status. In one report seropositive inflammatory myositis patients (other than IBM) were shown to have lower CK levels and higher levels of antinuclear antibodies. Seropositivity in juvenile myositis (present in 26.8%) was found to be associated with greater clinical severity with more frequent hospitalisations and more respiratory disease (44). In this study, 27.0% of juvenile dermatomyositis, 11.1% of juvenile polymyositis and 12.0% of healthy children tested positive for anti-cN1A. A recent study has described seropositivity in anti-synthetase syndrome and other interstitial lung diseases including hypersensitivity pneumonitis and idiopathic pulmonary fibrosis (45). Interestingly, Rietveld *et al*. and Amlani were unable to detect anti-cN1A antibodies in their cohort of juvenile dermatomyositis (32, 46). Seropositivity has been investigated in necrotising myositis; Tawara *et al*. noted positivity in 25% and Ikenaga *et al*. noted positivity in 11.8% of necrotising myositis patients in their studies, whereas Larman *et al*. did not detect any anti-cN1A antibodies in the 14 patients tested (9, 17, 19, 30, 37).

Patients with other autoimmune conditions have been shown to test positive for anti-cN1A antibodies. This observation lends support to the argument that IBM does have an autoimmune aetiology. However, seropositivity in such conditions adds another layer of complexity when considering anti-cN1A as a diagnostic marker calling into question its specificity to IBM. In particular patients with Sjögren's syndrome (SS) and systemic lupus erythematous (SLE) have been shown to test positive for anti-cN1A antibodies fairly frequently (18, 26, 32, 34, 38, 47). In the largest study investigating anti-cN1A positivity in SLE and SS, the frequency of seropositive patients ranged from 6 to

Table II. Studies with available anti-cN1A antibody positivity rates in autoimmune disease and muscle conditions other than IBM.

Year	Authors	Assay technique *(± commercial laboratory)*	Healthy controls positivity (%)	PM positivity (%)	DM positivity (%)	NM positivity (%)	SLE positivity (%)	SS positivity (%)	SSc positivity (%)	Non-autoimmune NMD positivity (%)
2011	Salajegheh *et al*. (16)	Immunoprecipitation using muscle lysates	0	0	0	NA	NA	NA	NA	NA
2013	Larman *et al*. (9)	Immunoblotting using muscle lysates	NA	7.69 3 (3.8 at higher cut off)	16.7 (2.7 at higher cut off)	0	NA	NA	NA	5.3 (0 at higher cut off)
2013	Pluk *et al*. (10)	Immunoprecipitation using muscle lysates	0	13.6 (4.2 at higher cut off)	20.8 (4.5 at higher cut off)	NA	NA	NA	NA	7.4 (3.2 at higher cut off)
2016	Lloyd *et al*. (26)	Immunoblotting of lysates from transfected HEK293 cells	4.8	4.8	15.1	NA	13.5	22.7	NA	NA
2016	Herbert *et al*. (18)	Three peptide ELISA	NA	NA[a]	NA[a]	NA	20.5	36.4	2.3	4.3
2016	Kramp *et al*. (24)	Recombinant cN1A ELISA *(Euroimmun)*	1.9	0	0	NA	6.1	0	10	0
2016	Eura *et al*. (33)	Perinuclear or rimmed vacuole anti-cN1A staining in muscle	NA	30	1 0	NA	NA	NA	NA	NA
2017	Muro *et al*. (34)	Recombinant cN1A ELISA	2.4	10	11.1 (16.7 in JDM)	NA	6	2	4	NA
2017	Tawara *et al*. (19)	Cell based immunofluorescence assay	0	13.9	12.9	25	0	0	NA	3.5
2018	Rietveld *et al*. (47)	Recombinant cN1A ELISA *(Euroimmun)*	NA	NA	NA	NA	10.3	11.9	NA	NA
2018	Yeker *et al*. (44)	Immunoblotting of lysates from transfected HEK293 cells	12 (children)	11.1 (JDM only)	27.0 (JDM only)	NA	NA	NA	NA	NA
2019	Amlani *et al*. (32)	ABIA using recombinant cN1A	5.1	NA[b]	NA[b] (0 in JDM only)	NA	13.6	0	6	15.4
2021	Ikenaga *et al*. (30)	Western Blotting followed by recombinant cN1A ELISA *(WUNL)*	NA	NA	20.8	11.8	NA	NA	NA	5.8

[a]PM and DM positivity rate was reported collectively as 4.3% in this study (18). [b]Adult PM and DM positivity rate was reported collectively as 7% in this study (32).
cN1A: 5'-cytosolic nucleotidase 1A; ELISA: enzyme-linked immunosorbent assay; NA: not applicable (when positivity for the condition was not tested, not quoted in the article or could not be inferred the value has been marked as NA); RDL: Rheumatology Diagnostics Laboratory; HC: healthy controls; IIM: idiopathic inflammatory myopathies; DM: dermatomyositis; JDM: dermatomyositis; PM: polymyositis; NMD: neuromuscular diseases; RA: rheumatoid arthritis; SLE: systemic lupus erythematous; SS: Sjögren's syndrome; SSc: systemic sclerosis; WUNL: Washington University Neuromuscular Laboratory.

21% (mean 10.3%) and 7 to 19% (mean 11.9%), respectively, with the range reflecting the different provenance of the serum (cohorts from 4 different countries) (47). In both diseases, seropositive patients were shown to have a greater burden of comorbidities. The highest rate of seropositivity for SS and SLE reported so far is 36.4% and 20.5% respectively (18). SS has been shown to share common pathogenic mechanisms with IBM (22). In a recent study, IBM was diagnosed more freqeuntly in myositis patients with SS, than in myositis patients without SS (38). Although anti-cN1A seropositivity was more frequent in SS patients with concurrent myositis, this association between SS and anti-cN1A was shown to be independent from the diagnosis of IBM. This suggests that seropositvity has limited specificity in the diagnosis of IBM in SS patients. Therefore, it may be important to be more cautious with or even avoid anti-cN1A testing is SS patients suspected to have myositis. As expected, anti-cN1A antibody positivity in IBM patients is significantly higher in comparison to neuromuscular conditions other than myositis including muscular dystrophies, metabolic myopathies, and other neurodegenerative conditions (9, 17-19, 30, 32). Most patients in these subgroups test negative for anti-cN1A antibodies. A recent report described two patients with motor neurone disease that tested positive for anti-cN1A antibody while undergoing investigation for differential diagnoses (48). Seropositivity in such conditions is likely to represent an epiphenomenon and should advocate caution before anti-cN1A testing; only reserving it for cases when there is high index of suspicion for IBM. Valosin containing protein (VCP) related multisystem proteinopathy is a syndrome in which patients develop a myopathy with histological similarities to IBM, in addition to Paget's disease and frontotemporal dementia (2). Animal models of VCP-related multisystem proteinopathy have been used in experimental studies to replicate features of IBM seen in humans (49). Interestingly VCP related myopathy patients had a significantly higher rate of anti-cN1A antibody positivity compared with other non-inflammatory muscle diseases (30). Seropositivity in familial forms of IBM have

been variable thus far and due to rarity of such cases it is difficult to infer any sort of relationship at this stage (9, 50, 51).

In summary, anti-cN1A antibodies have been reported in 0%-20.5% of patients with SLE (18, 19), 0%-36.4% of patients with SS (18, 19, 24, 32), 2.3%-10.0% of patients with systemic sclerosis (18, 24), 0%-20.8% of patients with dermatomyositis (up to 27.0% in juvenile dermatomyositis) (16, 19, 44), 0%-30.0% of patients with polymyositis (16, 24, 32, 33), 8.7%-25.9% of patients with anti-synthetase syndrome (30, 45), 0%-25.0% of patients with necrotising autoimmune myopathy (9, 19), 0%-5.1% of healthy controls (up to 12.0% in healthy children) (10, 16, 19, 32, 44), and 0%-15.4% of patients with non-autoimmune neuromuscular diseases (Table II) (24, 32). Therefore it is critical to guide antibody testing and its interpretation in the clinic based on typical IBM phenotypic presentation, all the while factoring in the potential impact of co-morbid illnesses.

Conclusions

Over the past decade there has been much interest in the role of anti-cN1A antibodies in IBM. There has been a lack of investigation into whether the antibody has a pathogenic role or whether it is secreted as an epiphenomenon in the context of immune-mediated sequalae.

These antibodies overall have a high specificity and moderate sensitivity in IBM. A variety of assay techniques have described in the literature to detect anti-cN1A antibodies. This heterogeneity in the literature limits our ability to draw precise conclusions about the utility of this antibody as a diagnostic marker or prognostic tool. Standardisation of antibody testing and international agreement on cut off values will allow more robust, reliable and reproducible evaluations of clinicopathological associations. Some reports do suggest that seropositive patients may have a more severe phenotype and are at risk of developing specific features. However, it should be noted that such findings are variable and have not been universally replicated. Moreover, these reports are largely either coming from cross-sectional or retrospective studies, often describing univariable rather than multivariable analyses. Such studies are therefore limited in their ability to provide a conclusive understanding of anti-cN1A risk; their results are prone to bias (*e.g.* selection and collider bias, and unmeasured confounding) and cannot be interpreted causally but only as observed associations within a given patient population. Moreover, given the relative rarity and the frequent misdiagnosis of IBM it is difficult to generate large amounts of data on anti-cN1A antibodies and we are still reliant on smaller study populations; prospective data collection and multicentre collaboration should therefore be stimulated and is currently ongoing as part of the INSPIRE-IBM study.

At this stage it is difficult to accurately conclude whether anti-cN1A antibodies have a concrete role in clinical practice. Whilst these antibodies are being evaluated, rather than a core diagnostic test they may be useful as supportive tool in aiding diagnosis; for example, in patients with classical features and non-diagnostic findings on repeat muscle biopsy or who cannot undergo biopsy. Diagnosis of IBM should not rely exclusively on isolated disease features. Expert diagnosis is based on the combination of clinical findings and results of investigations (*e.g.* muscle biopsy, imaging, laboratory, autoantibody and EMG evaluations).

Competing interests

M.M. Dimachkie serves or recently served as a consultant for Amazentis, ArgenX, Catalyst, Cello, Covance/Labcorp, CSL-Behring, EcoR1, Janssen, Kezar, Momenta, NuFactor, Octapharma, RaPharma/UCB, Roivant Sciences Inc, RMS Medical, Sanofi Genzyme, Shire Takeda, Scholar Rock, Spark Therapeutics, Third Rock and UCB Biopharma and received research grants or contracts or educational grants from Alexion, Alnylam Pharmaceuticals, Amicus, Biomarin, Bristol-Myers Squibb, Catalyst, Corbus, CSL-Behring, FDA/OOPD, GlaxoSmith-Kline, Genentech, Grifols, Kezar, Mitsubishi Tanabe Pharma, MDA, NIH, Novartis, Octapharma, Orphazyme, Ra Pharma/UCB, Sanofi Genzyme, Sarepta Therapeutics, Shire Takeda, Spark Therapeutics, the Myositis Association, UCB Biopharma/RaPharma, Viromed/Healixmith and TMA.

P.M. Machado has received consulting/speaker's fees from Abbvie, BMS, Celgene, Eli Lilly, Galapagos, Janssen, MSD, Novartis, Orphazyme, Pfizer, Roche and UCB, all unrelated to this manuscript.

The other authors have declared no competing interests.

References

1. CARPENTER S, KARPATI G, HELLER I, EISEN A: Inclusion body myositis: a distinct variety of idiopathic inflammatory myopathy. *Neurology* 1978; 28: 8-8.
2. WEIHL CC: Sporadic inclusion body myositis and other rimmed vacuolar myopathies. *Continuum* (Minneap Minn) 2019; 25: 1586-98.
3. NADDAF E, BAROHN RJ, DIMACHKIE MM: Inclusion Body Myositis: Update on Pathogenesis and Treatment. *Neurotherapeutics* 2018; 15: 995-1005.
4. GREENBERG SA: Inclusion body myositis: clinical features and pathogenesis. *Nat Rev Rheumatol* 2019; 15: 257-272.
5. SANGHA G, YAO B, LUNN D *et al*.: Longitudinal observational study investigating outcome measures for clinical trials in inclusion body myositis. *J Neurol Neurosurg Psychiatry* 2021 Apr 13 Online ahead of print.
6. MACHADO P, BRADY S, HANNA MG: Update in inclusion body myositis. *Curr Opin Rheumatol* 2013; 25 :763-771.
7. MACHADO PM, DIMACHKIE MM, BAROHN RJ: Sporadic inclusion body myositis: new insights and potential therapy. *Curr Opin Neurol* 2014; 27: 591-8.
8. GEORGANTAS RW, STREICHER K, GREENBERG SA *et al*.: Inhibition of myogenic microRNAs 1, 133, and 206 by inflammatory cytokines links inflammation and muscle degeneration in adult inflammatory myopathies. A*rthritis Rheumatol* 2014; 66: 1022-33.
9. LARMAN HB, SALAJEGHEH M, NAZARENO R *et al*.: Cytosolic 5'-nucleotidase 1A autoimmunity in sporadic inclusion body myositis. *Ann Neurol* 2013; 73: 408-18.
10. PLUK H, VAN HOEVE BJ, VAN DOOREN SH *et al*.: Autoantibodies to cytosolic 5'-nucleotidase 1A in inclusion body myositis. *Ann Neurol* 2013; 73: 397-407.
11. HUNSUCKER SA, MITCHELL BS, SPYCHALA J: The 5'-nucleotidases as regulators of nucleotide and drug metabolism. *Pharmacol Ther* 2005; 107: 1-30.
12. EL-SHAMMAA NA, FISHBEIN WN, ARMBRUSTMACHER VW: Interstitial 5'-nucleotidase stain for frozen biopsy specimens of skeletal muscle. A useful adjunct in the diagnosis of polymyositis. *Arch Pathol Lab Med* 1984; 108: 251-6.
13. KULKARNI SS, KARLSSON HK, SZEKERES F *et al*.: Suppression of 5'-nucleotidase en-

zymes promotes AMP-activated protein kinase (AMPK) phosphorylation and metabolism in human and mouse skeletal muscle. *J Biol Chem* 2011; 286: 34567-74.
14. HARDIE DG: AMPK: a key regulator of energy balance in the single cell and the whole organism. *Int J Obes* (Lond) 2008; 32 (Suppl. 4): S7-12.
15. KRAWIEC BJ, NYSTROM GJ, FROST RA, JEFFERSON LS, LANG CH: AMP-activated protein kinase agonists increase mRNA content of the muscle-specific ubiquitin ligases MAFbx and MuRF1 in C2C12 cells. *Am J Physiol Endocrinol Metab* 2007; 292: E1555-1567.
16. SALAJEGHEH M, LAM T, GREENBERG SA: Autoantibodies against a 43 KDa muscle protein in inclusion body myositis. *PLoS One* 2011; 6: e20266.
17. GREENBERG SA: Cytoplasmic 5'-nucleotidase autoantibodies in inclusion body myositis: Isotypes and diagnostic utility. *Muscle Nerve* 2014; 50: 488-92.
18. HERBERT MK, STAMMEN-VOGELZANGS J, VERBEEK MM *et al*.: Disease specificity of autoantibodies to cytosolic 5'-nucleotidase 1A in sporadic inclusion body myositis versus known autoimmune diseases. *Ann Rheum Dis* 2016; 75: 696-701.
19. TAWARA N, YAMASHITA S, ZHANG X *et al*.: Pathomechanisms of anti-cytosolic 5'-nucleotidase 1A autoantibodies in sporadic inclusion body myositis. *Ann Neurol* 2017; 81: 512-25.
20. ROTHWELL S, COOPER RG, LUNDBERG IE *et al*.: Immune-array analysis in sporadic inclusion body myositis reveals HLA-DRB1 amino acid heterogeneity across the myositis spectrum. *Arthritis Rheumatol* 2017; 69: 1090-9.
21. LIMAYE VS, LESTER S, BLUMBERGS P, GREENBERG SA: Anti-C N1A antibodies in South Australian patients with inclusion body myositis. *Muscle Nerve* 2016; 53: 654-5.
22. GREENBERG SA, PINKUS JL, AMATO AA, KRISTENSEN T, DORFMAN DM: Association of inclusion body myositis with T cell large granular lymphocytic leukaemia. *Brain* 2016; 139: 1348-60.
23. GOYAL NA, CASH TM, ALAM U *et al*.: Seropositivity for NT5c1A antibody in sporadic inclusion body myositis predicts more severe motor, bulbar and respiratory involvement. *J Neurol Neurosurg Psychiatry* 2016; 87: 373-8.
24. KRAMP SL, KARAYEV D, SHEN G *et al*.: Development and evaluation of a standardized ELISA for the determination of autoantibodies against cN-1A (Mup44, NT5C1A) in sporadic inclusion body myositis. *Auto Immun Highlights* 2016; 7: 16.
25. FELICE KJ, WHITAKER CH, WU Q *et al*.: Sensitivity and clinical utility of the anti-cytosolic 5'-nucleotidase 1A (cN1A) antibody test in sporadic inclusion body myositis: report of 40 patients from a single neuromuscular center. *Neuromuscul Disord* 2018; 28: 660-4.
26. LLOYD TE, CHRISTOPHER-STINE L, PINAL-FERNANDEZ I *et al*.: Cytosolic 5'-nucleotidase 1A as a target of circulating autoantibodies in autoimmune diseases. *Arthritis Care Res* (Hoboken) 2016; 68: 66-71.
27. LILLEKER JB, RIETVELD A, PYE SR *et al*.: Cytosolic 5'-nucleotidase 1A autoantibody profile and clinical characteristics in inclusion body myositis. *Ann Rheum Dis* 2017; 76: 862-8.
28. HERBERT MK, PRUIJN GJ: Novel serology testing for sporadic inclusion body myositis: disease-specificity and diagnostic utility. *Curr Opin Rheumatol* 2015; 27: 595-600.
29. PINTO MV, LAUGHLIN RS, KLEIN CJ, MANDREKAR J, NADDAF E: Inclusion body myositis: correlation of clinical outcomes with histopathology, electromyography and laboratory findings. *Rheumatology* (Oxford) 2021 Oct 7, Online ahead of print.
30. IKENAGA C, FINDLAY AR, GOYAL NA *et al*.: Clinical utility of anti-cytosolic 5'-nucleotidase 1A antibody in idiopathic inflammatory myopathies. *Ann Clin Transl Neurol* 2021; 8: 571-8.
31. YAMASHITA S, TAWARA N: Determination of cN1A autoantibodies by cell-based immunofluorescence cytochemistry. *Methods Mol Biol* 2019; 1901: 89-94.
32. AMLANI A, CHOI MY, TARNOPOLSKY M *et al*.: Anti-NT5c1A autoantibodies as biomarkers in inclusion body myositis. *Front Immunol* 2019; 10: 745.
33. EURA N, SUGIE K, KINUGAWA K *et al*.: Anti-cytosolic 5'-nucleotidase 1A (cN1A) positivity in muscle is helpful in the diagnosis of sporadic inclusion body myositis: a study of 35 Japanese patients. *J Neurol Neurosci* 2016; 7: 5.
34. MURO Y, NAKANISHI H, KATSUNO M, KONO M, AKIYAMA M: Prevalence of anti-NT5C1A antibodies in Japanese patients with autoimmune rheumatic diseases in comparison with other patient cohorts. *Clin Chim Acta* 2017; 472: 1-4.
35. OYAMA M, OHNUKI Y, INOUE M *et al*.: HLA-DRB1 allele and autoantibody profiles in Japanese patients with inclusion body myositis. *PLoS One* 2020; 15: e0237890.
36. PAUL P, LIEWLUCK T, ERNSTE FC, MANDREKAR J, MILONE M: Anti-cN1A antibodies do not correlate with specific clinical, electromyographic, or pathological findings in sporadic inclusion body myositis. *Muscle Nerve* 2021; 63: 490-6.
37. LUCCHINI M, MAGGI L, PEGORARO E *et al*.: Anti-cN1A antibodies are associated with more severe dysphagia in sporadic inclusion body myositis. *Cells* 2021; 10: 1146.
38. LEVY D, NESPOLA B, GIANNINI M *et al*.: Significance of Sjögren's syndrome and anti-cN1A antibody in myositis patients. *Rheumatology* (Oxford) 2021 May 11, Online ahead of print.
39. MAVROUDIS I, KNIGHTS M, PETRIDIS F *et al*.: Diagnostic Accuracy of Anti-CN1A on the Diagnosis of Inclusion Body Myositis. A Hierarchical Bivariate and Bayesian Meta-analysis. *J Clin Neuromuscul Dis* 2021; 23: 31-8.
40. RIDER LG, AGGARWAL R, MACHADO PM *et al*.: Update on outcome assessment in myositis. *Nat Rev Rheumatol* 2018; 14: 303-18.
41. MACHADO PM, AHMED M, BRADY S *et al*.: Ongoing developments in sporadic inclusion body myositis. *Curr Rheumatol Rep* 2014; 16: 477.
42. DEJTHEVAPORN R, SHAH S, WASTLING S *et al*.: SAT0332 antibodies against cytosolic 5'-nucleotidase 1a in sporadic inclusion body myositis: association with clinical and MRI features. *Ann Rheum Dis* 2020; 79: 1112.1.
43. GOYAL NA: Immune-mediated myopathies. *Continuum* (Minneap Minn) 2019; 25: 1564-85.
44. YEKER RM, PINAL-FERNANDEZ I, KISHI T *et al*.: Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis. *Ann Rheum Dis* 2018; 77: 714-9.
45. MOLL SA, PLATENBURG M, PLATTEEL ACM *et al*.: Prevalence of novel myositis autoantibodies in a large cohort of patients with interstitial lung disease. *J Clin Med* 2020; 9: 2944.
46. RIETVELD A, WIENKE J, VISSER E *et al*.: Anti-cytosolic 5'-nucleotidase 1A autoantibodies are absent in juvenile dermatomyositis. *Arthritis Rheumatol* 2021; 73: 1329-33.
47. RIETVELD A, VAN DEN HOOGEN LL, BIZZARO N *et al*.: Autoantibodies to cytosolic 5'-nucleotidase 1A in primary Sjogren's syndrome and systemic lupus erythematosus. *Front Immunol* 2018; 9: 1200.
48. LIEWLUCK T: Anti-cytosolic 5'-nucleotidase 1A (cN1A) autoantibodies in motor neuron diseases. *Neurology* 2017; 89: 2017-8.
49. AHMED M, MACHADO PM, MILLER A *et al*.: Targeting protein homeostasis in sporadic inclusion body myositis. *Sci Transl Med* 2016; 8: 331ra341.
50. KHOSA S, VAZIRIAN S, MISHRA S *et al*.: Familial inclusion body myositis with negative Anti-cytosolic 5'-nucleotidase 1A (cN1A) antibody test. (P3. 4-016). *AAN Enterprises*, 2019.
51. NICOLAU S, NIU Z, LING K, MILONE MP: 21Genetic analysis of first-degree relatives with inclusion body myositis. *Neuromuscular Disorders* 2019; 29: S47.