

Inclusion body myositis

**Genetic, clinical, and
epidemiological aspects**

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Cover illustration: Cytochrome c oxidase-deficient muscle fibers appear blue in double staining for cytochrome c oxidase and succinate dehydrogenase.

Inclusion body myositis – Genetic, clinical, and epidemiological aspects

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To my family

ABSTRACT

The inflammatory myopathy inclusion body myositis (IBM) is characterized by progressive muscle weakness and dysphagia in individuals over 45 years of age. Muscle biopsy shows inflammatory infiltrates, vacuoles with protein accumulation and cytochrome c oxidase (COX)-deficient muscle fibers. Multiple rearrangements are seen in mitochondrial DNA (mtDNA) in muscle. Inclusion body myositis is rare and larger population-based studies few. The aim of this thesis was to describe aspects of IBM in a population-based cohort in Region Västra Götaland (VGR), Sweden, from 1985 to 2017. Methods included analysis of muscle biopsies, DNA analysis, and review of medical records.

The relative amount of mtDNA deletions in muscle was associated with the amount of COX-deficient muscle fibers ($p < 0.001$) in 26 patients (Paper I). Mean mutation load in muscle mtDNA was 10% (range 1-35%) in 21 patients with IBM and 1% (range 0.2-3%) in controls (Paper II). We saw no increase in variants in nuclear genes associated with mitochondrial myopathies and multiple mtDNA deletions (Papers I, II, IV).

Including 128 patients fulfilling strict diagnostic criteria, the prevalence of IBM December 31, 2017, was 32 per million inhabitants in VGR (19 per million women and 45 per million men). Mean incidence was 2.5 per million inhabitants and year. Cumulative survival was moderately decreased. The most common first symptom was quadriceps weakness. Dysphagia occurred in 77% and autoantibodies to cytosolic 5'-nucleotidase 1A were present in 40% of patients (Paper III). Six additional patients were <46 years of age at symptom onset. They had reduced survival, progressive muscle weakness, high frequencies of swallowing difficulties and ventilation assistance and high mtDNA mutation load compared with controls (Paper IV).

In conclusion, IBM is a severe inflammatory myopathy that reduces cumulative survival. Patients are affected by progressive muscle weakness, dysphagia, and COX-deficiency caused by high levels of mtDNA rearrangements.

Keywords: inclusion body myositis, mitochondrial DNA, epidemiology, cytochrome c oxidase, inflammatory myopathy

SAMMANFATTNING PÅ SVENSKA

Inklusionskroppsmysosit (inclusion body myositis, IBM) är en ovanlig inflammatorisk muskelsjukdom (myosit). Typiska symtom är tilltagande muskelsvaghet i handgrepp och knästräckare samt sväljsvårigheter. Symtombilden i kombination med mikroskopisk analys av muskelbiopsi (provtagning av muskelvävnad) används för att ställa diagnos. Man kan bland annat se inflammatoriska celler och proteinansamlingar i muskelvävnaden.

Cellens energi produceras av cellens ”kraftverk”, mitokondrier. Mitokondrier har sitt eget DNA (mtDNA), som samverkar med cellkärnans DNA (nDNA) för normal mitokondriefunktion. Vid IBM kan man i muskelbiopsier se tecken på mitokondriepåverkan både i mikroskop och vid analys av mtDNA.

IBM är en ovanlig sjukdom och har därför varit svår att studera strukturerat. Denna avhandling baserar sig på en av de största populationsbaserade kohorterna med patienter med IBM. Metoderna inkluderar analys av muskelbiopsier, DNA och antikroppar samt studier av journaldata och epidemiologi.

I delarbete I studerade vi 26 patienter med IBM och kunde visa ett samband mellan mängden mutationer i mtDNA och mängden muskelfibrer med påverkad mitokondriefunktion. Detta undersökte vi vidare i delarbete II, där mtDNA kartlades i detalj hos 21 patienter. Även i detta arbete fann vi att patienter med IBM hade betydligt fler mutationer i mtDNA än matchade kontroller. Vi fann inget stöd i delarbete I eller II för att förändringar i nDNA skulle bidra till uppkomsten av mtDNA-förändringarna i muskel vid IBM.

I delarbete III studerades alla 128 patienter som uppfyllt strikta diagnoskriterier för IBM i Västra Götalandsregionen 1985–2017. Förekomsten av IBM var 32 patienter per miljon invånare 31/12 2017, och i genomsnitt insjuknade 2,5 individer per miljon invånare och år. Överlevnaden var tydligt förkortad för patienter med IBM. Könsskillnader kunde ses, bland annat var sväljsvårigheter vanligare hos kvinnor. De nuvarande diagnoskriterierna för IBM förutsätter symtomdebut efter 45 års ålder. I delarbete IV undersökte vi sex patienter med tidigare symtomdebut. De hade förkortad överlevnad, högre förekomst av andningshjälpmedel och sväljsvårigheter var vanligt. Ökad mängd mutationer i mtDNA jämfört med kontroller förekom redan i ung ålder.

Sammanfattningsvis är IBM en allvarlig inflammatorisk muskelsjukdom som leder till progressiv svaghet och minskad överlevnad. Den ökade mängden skador i mtDNA som är karakteristisk för IBM orsakar defekt funktion i mitokondrierna som en del i sjukdomen.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

- I. **Lindgren U**, Roos S, Hedberg-Oldfors C, Moslemi A-R, Lindberg C, Oldfors A.
Mitochondrial pathology in inclusion body myositis.
Neuromuscular Disorders, 2015;25(4):281-288.
- II. Hedberg-Oldfors C, **Lindgren U**, Basu S, Visuttijai K, Lindberg C, Falkenberg M, Larsson Lekholm E, Oldfors A.
Mitochondrial DNA variants in inclusion body myositis characterized by deep sequencing.
Brain Pathology, 2021;31(3):e12931.
- III. **Lindgren U**, Pullerits R, Lindberg C, Oldfors A.
Epidemiology, survival, and clinical characteristics of inclusion body myositis.
Annals of Neurology, 2022;92(2):201-212.
- IV. **Lindgren U**, Hedberg-Oldfors C, Pullerits R, Lindberg C, Oldfors A.
Inclusion body myositis with early onset – a population-based study.
Manuscript.

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CONTENT

- ABBREVIATIONSIV
- DEFINITIONS IN SHORT.....VII
- 1 INTRODUCTION 1
- 2 AIMS 5
- 3 OUTLINE 7
- 4 MITOCHONDRIAL ALTERATIONS..... 9
 - 4.1 Deficiencies in oxidative phosphorylation 13
 - 4.1.1 Conclusions..... 14
 - 4.2 Variants in mitochondrial DNA 15
 - 4.2.1 Multiple deletions and duplications..... 15
 - 4.2.2 Small somatic mitochondrial DNA variants..... 17
 - 4.2.3 Mitochondrial DNA copy number..... 17
 - 4.2.4 Conclusions..... 18
 - 4.3 Nuclear DNA genes associated with mitochondrial DNA rearrangements..... 19
 - 4.3.1 *POLG*..... 20
 - 4.3.2 Conclusions..... 20
- 5 CLINICAL FEATURES 21
 - 5.1 Signs and symptoms 23
 - 5.1.1 First reported symptom..... 23
 - 5.1.2 Muscle weakness 24
 - 5.1.3 Dysphagia 25
 - 5.1.4 Respiratory dysfunction..... 27
 - 5.1.5 Conclusions..... 27
 - 5.2 Comorbidities 29
 - 5.2.1 Autoimmunity..... 29
 - 5.2.2 Malignancy 30
 - 5.2.3 Conclusions..... 31
 - 5.3 Laboratory features..... 32

5.3.1	Anti-CN1A.....	32
5.3.2	Myositis-specific and myositis-associated autoantibodies	34
5.3.3	Conclusions.....	34
5.4	Treatment.....	35
5.4.1	Conclusions.....	36
6	DIAGNOSTIC CRITERIA AND EPIDEMIOLOGY	37
6.1	Diagnostic criteria	37
6.1.1	Conclusions.....	39
6.2	Age at onset and diagnosis	40
6.2.1	Diagnostic delay	41
6.2.2	Conclusions.....	41
6.3	Survival	42
6.3.1	Cause of death.....	43
6.3.2	Conclusions.....	43
6.4	Prevalence and incidence	44
6.4.1	Conclusions.....	45
7	METHODOLOGICAL CONSIDERATIONS.....	47
7.1	Study design	47
7.1.1	Population-based studies	47
7.1.2	Retrospective studies	48
7.1.3	Diagnostic criteria.....	48
7.2	Methods	50
7.2.1	DNA analyses	50
7.2.2	Review of medical records	50
7.2.3	Statistical analysis and calculations.....	51
7.3	Ethical considerations.....	52
8	CONCLUSIONS.....	53
8.1	Future directions.....	54
	ACKNOWLEDGEMENTS	55
	REFERENCES	57

ABBREVIATIONS

Anti-cN1A	Anti-cytosolic 5'-nucleotidase 1A
ATP	Adenosine triphosphate
CK	Creatine kinase
COX	Cytochrome c oxidase (complex IV)
ENMC	European Neuromuscular Centre
EO-IBM	Early-onset inclusion body myositis
HLA	Human leukocyte antigen
IBM	Inclusion body myositis
IBM-FRS	Inclusion body myositis functional rating scale
MHC-I	Major histocompatibility complex I
MitoSAlt	Mitochondrial Structural Alterations (bioinformatic tool)
MRC	Medical Research Council
mtDNA	Mitochondrial DNA
<i>MT-ND1</i>	Mitochondrial DNA gene coding for NADH-ubiquinone oxidoreductase chain 1
<i>MT-ND4</i>	Mitochondrial DNA gene coding for NADH-ubiquinone oxidoreductase chain 4
NADH	Nicotinamide adenine dinucleotide
nDNA	Nuclear DNA
OH	Origin of mitochondrial DNA heavy strand replication
OL	Origin of mitochondrial DNA light strand replication

PCR	Polymerase chain reaction
<i>POLG</i>	Mitochondrial DNA polymerase gamma
qPCR	Quantitative polymerase chain reaction
SDH	Succinate dehydrogenase (complex II)
TnT	Troponin T
ULN	Upper limit of normal
VGR	Region Västra Götaland, Sweden

DEFINITIONS IN SHORT

Clinicopathological IBM ¹	Clinical and laboratory features: duration >12 months, age at onset >45 years, knee extension weakness \geq hip flexion weakness and/or finger flexion weakness > shoulder abduction weakness, CK no greater than 15 x upper limit of normal. Pathological features: all the following: endomysial inflammatory infiltrate, rimmed vacuoles, protein accumulation or 15–18 nm filaments.
Clinically defined IBM ¹	Clinical and laboratory features: duration >12 months, age at onset >45 years, knee extension weakness \geq hip flexion weakness and finger flexion weakness > shoulder abduction weakness, CK no greater than 15 x upper limit of normal. Pathological features: one or more, but not all, of: endomysial inflammatory infiltrate, up-regulation of MHC class I, rimmed vacuoles, protein accumulation or 15–18 nm filaments.
Early-onset IBM	Clinicopathological IBM except the age criteria.
Probable IBM ¹	Clinical and laboratory features: Duration >12 months, age at onset >45 years, knee extension weakness \geq hip flexion weakness or finger flexion weakness > shoulder abduction weakness, CK no greater than 15 x upper limit of normal. Pathological features: one or more, but not all, of: endomysial inflammatory infiltrate, up-regulation of MHC class I, rimmed vacuoles, protein accumulation or 15–18 nm filaments.

1 INTRODUCTION

The human body contains three types of muscle tissue, skeletal, cardiac, and smooth, all with different functions and features. Most abundant is skeletal muscle tissue. Skeletal muscle tissue consists of muscle cells - muscle fibers - which in adults measure 40-80 μm in diameter and are up to 10 centimeters long.² Each muscle fiber contains multiple contractile units called sarcomeres, which form myofibrils that are surrounded by the sarcoplasmic reticulum. The muscle fiber also contains multiple peripherally located nuclei and multiple mitochondria spread throughout the cell (Figure 1).

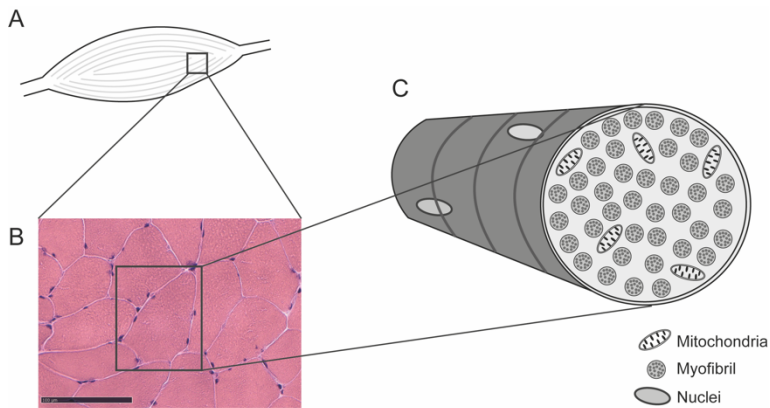


Figure 1. Skeletal muscle. A. A schematic muscle. B. Normal skeletal muscle tissue with multiple cross-sectioned muscle fibers, stained with hematoxylin and eosin. Scale bar 100 μm . C. Schematic cross-section of a muscle fiber.

Analysis of muscle tissue is commonly performed by muscle biopsy, typically from the vastus lateralis, biceps, deltoid or tibialis anterior muscle. The biopsy is fresh-frozen and morphology is analyzed using histological and histochemical methods, including enzyme histochemistry and immunohistochemistry (Figure 1B).² The muscle tissue can also be used for DNA analysis, including analysis of mitochondrial DNA (mtDNA).

Muscle weakness is a common symptom that may be the result of numerous different processes. There are multiple diseases causing muscle weakness including malfunction of other organ systems which indirectly affects muscle strength and diseases affecting the central and peripheral nervous systems as

well as the neuromuscular junction. Muscle weakness can also be caused by diseases directly affecting the muscle fiber itself – myopathies.³

Myopathies can be divided into hereditary and acquired and include a wide range of diseases, of which almost all are rare or very rare.² Myopathies can present during the entire life and patients can display a large variety of symptoms depending on the cause of the disease. Among the acquired myopathies, the inflammatory myopathies are most common and include among several others inclusion body myositis (IBM), dermatomyositis and immune mediated necrotizing myopathy. Inclusion body myositis is rare but is considered the most common acquired myopathy presenting in individuals over 40 years of age.⁴

The term “inclusion body myositis” was first described in 1971 by Yunis and Samaha,⁵ and the first case series was published in 1978 by Carpenter et al.⁶ Reported prevalence for IBM varies between 0.68 patients per million inhabitants in Turkey to 50.5 per million inhabitants in South Australia.^{7,8} The prevalence is at least twice as high in men than in women.⁹ Most IBM cases are sporadic. During the last few years, emerging data have shown that IBM has a negative impact on life expectancy.¹⁰⁻¹²

The diagnosis of IBM is based on the combination of characteristic clinical symptoms and histopathological muscle changes. Typically, a patient with IBM develops progressive muscle weakness and atrophy in finger flexors and quadriceps muscle. Tibialis anterior and neck flexor muscles are also frequently affected. The weakness is often asymmetric. Smooth and cardiac muscle seems unaffected.¹³⁻¹⁵ Many patients with IBM will either present with or develop swallowing difficulties during the disease course.¹⁶ Pain is uncommon.¹⁷

Muscle biopsies from patients with IBM typically show multiple mononuclear inflammatory infiltrates invading non-necrotic muscle fibers, rimmed vacuoles, protein aggregations, cytochrome c oxidase (COX) deficient muscle fibers and ragged red fibers (Figure 2).² In severely affected muscles, fat and connective tissue can replace the muscle tissue, which can also be seen using magnetic resonance imaging.

The inflammatory cells in IBM muscle have been characterized as predominantly clonally expanded CD8 positive cytotoxic T cells (Figure 2A).¹⁸⁻²³ Patients with IBM have been shown to have an increased level of CD8 positive T cells and lower amounts of regulatory T cells in peripheral blood than healthy controls.²⁴ It is not known if the CD8 positive T cells themselves

are pathogenic or if they appear as a reaction to a process that was initiated by other changes in the muscle tissue. Another sign of inflammation is the up-regulation of major histocompatibility complex I (MHC-I) in both the sarcolemma and cytoplasm in IBM muscle (Figure 2B).

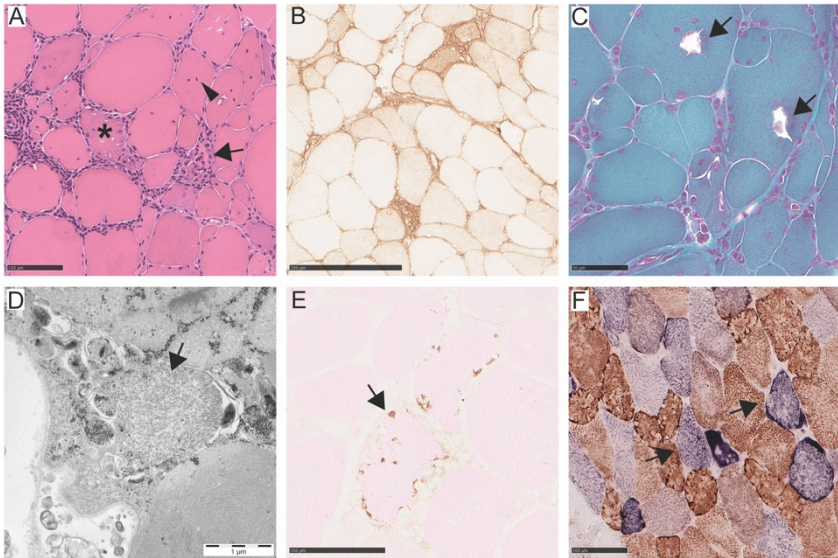


Figure 2. Muscle biopsy in IBM. A. Inflammatory cell infiltrate invading a non-necrotic muscle fiber (arrow). One fiber contains multiple rimmed vacuoles (). Also note the internal nuclei (arrowhead) and variation in fiber size. Hematoxylin and eosin. B. Upregulation of MHC-I (major histocompatibility complex I). C. Gomori trichrome staining shows rimmed vacuoles (arrows). D. Electron micrograph showing filamentous inclusions (arrow). E. Multiple inclusions positive for p62/sequestosome-1 (arrow). F. Combined staining for cytochrome c oxidase (COX) and succinate dehydrogenase (SDH) visualizes COX-deficient fibers as blue (arrows). A, E, F. Scale bar 100 μ m. B. Scale bar 250 μ m. C. Scale bar 50 μ m. D. Scale bar 1 μ m.*

Rimmed vacuoles are a characteristic feature of IBM and not found in other inflammatory myopathies (Figure 2C).² Associated with rimmed vacuoles are aggregated proteins which form the inclusions giving IBM its name. The inclusions are located in the cytoplasm and sometimes in the nuclei and are formed of 15-18 nm tubulofilaments. The proteins have been extensively studied and include among many others β -amyloid, phosphorylated tau and p62/sequestosome 1.^{25,26} The tubulofilaments were earlier often visualized with electron microscopy (Figure 2D), but now p62 immunohistochemistry has emerged as a common method for visualization of inclusions in IBM (Figure 2E).^{27,28} Although p62 positive protein aggregates are part of the diagnostic

criteria of IBM,¹ it should be noted that several other muscle diseases with rimmed vacuoles may show similar p62 positive inclusions.²

Inclusion body myositis has by several leading investigators been considered to be a probable degenerative disease, but more observations are now pointing towards an association with autoimmunity as an important factor.^{29,30} The presence of auto-invasive T cells in muscle tissue,¹⁹ the association to the human leukocyte antigen (HLA) haplotypes HLA-DRB1*03:01, DRB1*01:01 and DRB1*13:01,^{31,32} and the presence of autoantibodies against cytosolic 5'-nucleotidase 1A (cN1A) in 33-80% of patients with IBM all support an autoimmune origin.³³⁻³⁶ This is also supported by an increased prevalence of autoimmune comorbidities in patients with IBM.¹¹ Contradicting this hypothesis, immunomodulating treatment have not proven efficient in larger cohorts.^{29,30,37}

The hypothesis of a degenerative origin is supported by the presence of rimmed vacuoles and accumulation of misfolded proteins in muscle tissue.³⁸⁻⁴⁰ The role of mitochondria and COX-deficient muscle fibers in IBM pathogenesis is not clear but a relation with both inflammation and degeneration has been discussed.⁴¹

The rarity of IBM has resulted in difficulties studying larger patient cohorts. The focus of this thesis is to further characterize and describe genetic, clinical, and epidemiological aspects of inclusion body myositis in a well-defined cohort. The first part covers aspects of mitochondrial and mtDNA dysfunction in IBM. The second part covers signs and symptoms from a more clinical perspective, and the last part diagnostic criteria and epidemiology.

2 AIMS

The aim of this thesis was to characterize and describe different aspects of inclusion body myositis in a well-defined material.

The specific aims for each paper were:

- I. To examine the association between inclusion body myositis and variants in nuclear DNA affecting mitochondrial DNA, as well as the association between cytochrome c oxidase deficient muscle fibers and mitochondrial DNA deletions.
- II. To characterize mitochondrial DNA variants in inclusion body myositis muscle.
- III. To analyze survival and describe epidemiology, signs, symptoms, and laboratory data in inclusion body myositis.
- IV. To describe survival, epidemiology, signs, symptoms, biopsy data and mitochondrial DNA variants in early-onset inclusion body myositis.

3 OUTLINE

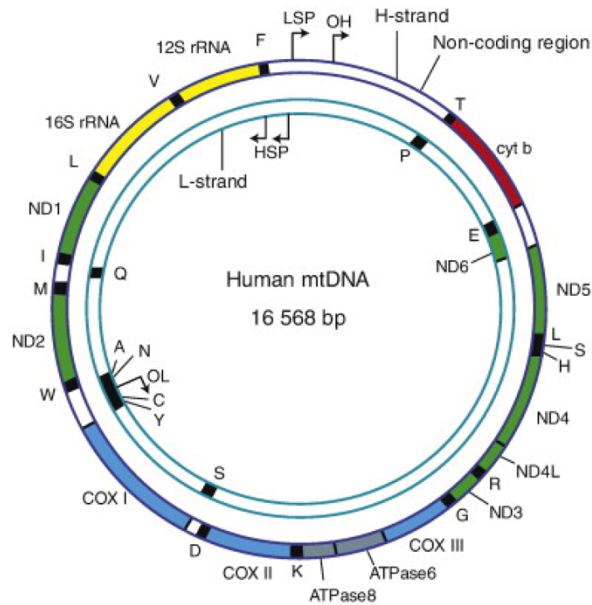
This thesis is outlined with the following subheadings:

- **Mitochondrial alterations** present Papers I, II and the genetic parts of Paper IV, and review cytochrome c oxidase-deficient muscle fibers and changes in mitochondrial and nuclear DNA in IBM.
- **Clinical features** present Papers III and IV and reviews clinical signs and symptoms in IBM including laboratory data and comorbidities.
- **Diagnostic criteria and epidemiology** continue the presentation of Papers III and IV and reviews diagnostic criteria, epidemiology, and survival in IBM.
- **Methodological considerations** discuss strengths and limitations in the study design, methods, and the use of diagnostic criteria.
- **Conclusions and future directions** summarize the conclusions from this thesis and suggest directions for further research.

4 MITOCHONDRIAL ALTERATIONS

This chapter presents Papers I and II and the genetic parts of Paper IV. After a brief introduction on mitochondrial DNA and the oxidative phosphorylation, it aims to review deficiencies in the oxidative phosphorylation as well as variants in mitochondrial and nuclear DNA in IBM muscle.

Mitochondrial function is essential in the production of adenosine triphosphate (ATP), supplying the cells with energy through the citric acid cycle and the oxidative phosphorylation. Tissues with high energy demand, such as muscles, brain, liver, and kidneys are at higher risk of developing symptoms due to mitochondrial dysfunction.⁴²



*Figure 3. Mitochondrial DNA. Major arc is located clockwise from OH (origin of heavy strand replication) to OL (origin of light strand replication) and contains the gene coding for NADH-ubiquinone oxidoreductase chain 4 (MT-ND4). Minor arc is located clockwise from OL to OH and contains the gene coding for NADH-ubiquinone oxidoreductase chain 1 (MT-ND1). Reprinted from Wanrooij S and Falkenberg M, The human mitochondrial replication fork in health and disease, *Biochim Biophys Acta*. 2010;1797(8):1378-88, with permission from Elsevier.*

Each mitochondrion contains multiple copies of the circular mitochondrial DNA (mtDNA) (Figure 3). The mtDNA is small compared with the nuclear DNA (nDNA) and consists of 16.6 kilo bases (kb).⁴³ There are 37 genes

encoded by mtDNA, of which 13 are encoding proteins. All 13 mtDNA encoded proteins are part of the process of oxidative phosphorylation. In the same cell, the mtDNA molecules can carry different genetic variants. This is known as heteroplasmy.⁴²

The mitochondrial oxidative phosphorylation is located at the inner mitochondrial membrane (Figure 4). It consists of complex I-IV, forming the electron transport chain, and ATP synthase (complex V). Complex I, complex III, complex IV (cytochrome c oxidase (COX)) and ATP synthase are all partially encoded by mtDNA and may be affected in diseases with mtDNA deletions.^{2,44-46} Complex II, succinate dehydrogenase (SDH), is completely encoded by nDNA.

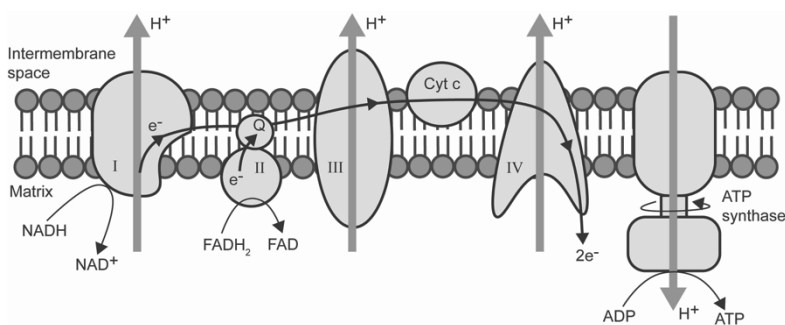


Figure 4. The oxidative phosphorylation with the electron transport chain at the inner mitochondrial membrane. I-IV, complex I-IV; NAD, nicotinamide adenine dinucleotide; H, hydrogen; e⁻, electron; FAD, flavin adenine dinucleotide; Q, coenzyme Q10 (ubiquinone); Cyt c, cytochrome c; ATP, adenosine triphosphate; ADP, adenosine diphosphate.

Mitochondrial DNA replication, transcription and translation are under dual genomic control and are partly regulated and performed by proteins encoded by nDNA. Variants in these nuclear genes, as well as genes regulating the mtDNA deoxyribonucleotide (dNTP) pools, can cause accumulation of somatic mtDNA variants or reduced mtDNA copy numbers (mtDNA depletion). Genes known to be associated with multiple large-scale mtDNA deletions and duplications include *SPG7*, *DNA2*, *RNASEH1*, *SLC25A4*, *OPA1*, *TWNK*, *POLG*, *POLG2*, *TYMP*, *RRM2B*, *TK2* and *MPV17*.² Accumulation of variants in mtDNA can occur during life and cause heteroplasmy, which implies a variable amount of variant mtDNA together with normal (wild-type) mtDNA.^{47,48} When the amount of variant mtDNA reaches a threshold, it can affect the mitochondrial function.

In muscle tissue each fiber contains multiple mitochondria (Figure 1). Some muscle fibers with defective mitochondrial function contain an increased number of mitochondria and can be visualized as “ragged red fibers” using Gomori trichrome staining. Ragged red fibers, which are found in many mitochondrial disorders and considered a hallmark of mitochondrial myopathy, can also be found in IBM muscle.^{2,42}

In the muscle fiber, one of the effects of accumulated pathogenic mtDNA variants is that the cell loses normal function of cytochrome c oxidase and becomes COX-deficient. Double staining for SDH and COX can be used to visualize COX deficiency (Figure 2F). The number of COX-deficient fibers increase with age and occasional COX-deficient fibers can occur in otherwise healthy older individuals.^{47,49} High numbers of COX-deficient muscle fibers can be caused by variants in both nDNA and mtDNA and are a common sign of mitochondrial myopathies.²

Multiple mtDNA deletions associated with COX deficiency were first described in 1993 in IBM muscle.⁵⁰ While IBM is not regarded as a mitochondrial myopathy, COX-deficient fibers are a common histopathological finding in IBM muscle and highly suggestive of IBM in muscle biopsies with inflammatory myopathy without rimmed vacuoles.^{51,52} The origin as well as the role of COX-deficient muscle fibers and mtDNA rearrangements in IBM pathogenesis and symptoms are unknown.⁴¹

In Papers I, II and IV, we aimed to further describe rearrangements in mtDNA in IBM, and the possible relation to variants in nuclear DNA. In Paper I, we also examined the hypothesis that the proportion of COX-deficient muscle fibers was associated with the relative amount of mtDNA deletions.

In Paper I, two groups of patients with IBM were identified from a registry of approximately 150 individuals with IBM. The two groups either had few or unusually many COX-deficient muscle fibers (Table 1). Diagnostic criteria used were typical clinical presentation, and muscle biopsy with inflammation, rimmed vacuoles, and p62-positive protein aggregates. Quadriceps femoris vastus lateralis was the muscle most commonly biopsied in both groups.

The patients in Papers II and IV were identified as described in chapter 5 Clinical features (Figure 7). All patients with IBM in Papers II and IV fulfilled the European Neuromuscular Centre (ENMC) 2011 research diagnostic criteria for clinicopathological IBM, and patients with early-onset IBM fulfilled all criteria for clinicopathological IBM except the age criteria (Table

1).¹ In Paper IV, a total of 19 muscle biopsies from patients with early-onset IBM were examined, with up to six biopsies from each individual.

Controls for Papers I, II and IV consisted of age-matched individuals who had undergone muscle biopsy, for whom the diagnostic work-up did not show any evidence of muscle- or mitochondrial disease (Table 1).

Table 1. Patients and muscle biopsy controls in Papers I, II and IV.

	Group	n (M:W)	Mean age at biopsy (years)	Age range at biopsy (years)
Paper I	IBM high proportion	15 (11:4)	66	50-80
	IBM low proportion	11 (9:2)	67	48-79
	Controls	6 (2:4)	72	44-89
Paper II	IBM	21 (13:8)	65	50-74
	Controls	10 (5:5)	68	56-80
Paper IV	EO-IBM	6 (3:3) [†]	47	38-60
	IBM (disease controls)	16 (11:5)	65	51-75
	Controls EO-IBM	9 (4:5)	50	41-60
	Controls IBM	8 (4:4)	70	61-80

[†]19 biopsies. n, number of individuals; M, men; W, women; EO-IBM, early-onset IBM.

The clinical and epidemiological parts of Paper IV are presented in chapters 5 Clinical features and 6 Diagnostic criteria and epidemiology. The ENMC 2011 diagnostic criteria are described in Definitions in short.

4.1 DEFICIENCIES IN OXIDATIVE PHOSPHORYLATION

Complex I, III and IV in the oxidative phosphorylation in mitochondria are all partially encoded by mtDNA and can therefore be affected by mtDNA alterations (Figure 4). In IBM muscle, complex IV, COX, is the most studied of the five complexes in the oxidative phosphorylation. Mitochondrial dysfunction indicated by COX-deficient muscle fibers is one of the most common histopathological findings in IBM muscle,⁵¹ although seldom included in diagnostic criteria.⁵³ Some patients with IBM have very large numbers of COX-deficient fibers, similar to what is seen in mitochondrial myopathies caused by mtDNA mutations.^{50,54} As in many other mitochondrial myopathies, COX-deficiency occurs in 75-1000 μm long segments along the length of the muscle fiber in IBM.⁵⁵

Earlier studies have shown large differences in the number of COX-deficient muscle fibers between individual patients with IBM.^{54,56} In Paper I, we identified a group of 15 patients with a high proportion of COX-deficient fibers and a group of 11 patients with a low proportion of COX-deficient fibers (Table 1). The group with a high proportion had a mean value of 12.7% (range 5.81-23.5%) COX-deficient fibers and the group with a low proportion 1.4% (range 0.48-2.65%) COX-deficient fibers. The hypothesis was that in patients with very high numbers of COX-deficient fibers, nuclear gene variants might contribute to the accumulation of mtDNA rearrangements (see 4.3 Nuclear DNA genes associated with mitochondrial DNA rearrangements).

A COX-deficient muscle fiber appears blue in double staining for COX/SDH. Some fibers appear blue-brown with COX/SDH-staining and can be interpreted as COX “intermediate” fibers, with decreased COX activity.⁵⁴ These were not included as COX-deficient fibers in Paper I. If COX-deficient and COX “intermediate” fibers were both included, 1.5% to 79.1% of all muscle fibers in IBM showed signs of COX-deficiency in 16 patients with IBM studied by Rygiel et al.⁵⁴ COX-deficient fibers were more often atrophic, and they were also associated with a higher number of immune cells in the tissue.⁵⁴

All patients included in this thesis displayed an increased number of COX-deficient fibers compared with normal for their age. In Papers IV, there were no signs of increasing COX-deficiency in muscle biopsies over time in the four patients with multiple muscle biopsies. This is similar to earlier studies with no clear correlation between the number of COX-deficient fibers and severity or disease progression.⁵⁴

Deficiency of complex I has long been known to occur in IBM muscle.⁵⁷ Rygiel et al demonstrated complex I-deficiency to occur in 1.7-89% of all muscle fibers in IBM muscle and to be more common than COX-deficient fibers. Complex I- and COX-deficiency did not correlate with age at disease onset or strength decline.⁵⁴ While complex I-deficiency can occur in a muscle fiber with normal COX/SDH appearance, the simultaneous occurrence of complex I-deficiency in COX-deficient muscle fibers and COX “intermediate” fibers led to the hypothesis that complex I-deficiency precede COX-deficiency. In Paper II, a reduction of complex I was shown in muscle homogenate in seven patients with IBM using western blot. Complex III and IV were variably reduced, while complex II and V did not show any reduction in patients with IBM compared with controls. This supports the hypothesis that complex I is more vulnerable than complex IV in IBM muscle.

4.1.1 CONCLUSIONS

Inclusion body myositis muscle displays a disturbance of the function of complex I and IV (COX). Some individuals with IBM have high levels of COX-deficient muscle fibers with levels in the same range as primary mitochondrial disorders. Deficiency of complex I and IV can be seen at both muscle fiber-level and in IBM muscle homogenate, suggesting an overall impact on oxidative phosphorylation in IBM muscle.

4.2 VARIANTS IN MITOCHONDRIAL DNA

4.2.1 MULTIPLE DELETIONS AND DUPLICATIONS

Multiple mtDNA deletions have been shown in IBM muscle by different methods including polymerase chain reaction (PCR), single-fiber PCR, quantitative PCR (qPCR), long extension PCR and in situ hybridization.^{50,54,56,57} In IBM, mtDNA variants are usually clonally expanded in each COX-deficient muscle segment.^{56,58} The deletions most often involve the mtDNA major arc,⁵⁵ and the distribution of mtDNA deletions seems similar to other diseases with multiple mtDNA deletions.⁵⁹

Mitochondrial DNA deletion load (level of heteroplasmy) is higher in COX-deficient than COX-normal IBM muscle fibers when analyzed with single fiber qPCR.⁵⁴ In Paper I, the association between the proportion of COX-deficient muscle fibers and deletions in mtDNA in muscle tissue homogenate was studied with qPCR. Exclusion of high levels of deletions in mtDNA minor arc was performed by long extension PCR before further analysis. For qPCR, *MT-ND1*, which is located in the minor arc, was used to estimate the total amount of mtDNA. *MT-ND4*, located in the major arc and usually affected by multiple deletions, was used to estimate the amount of mtDNA with deletions (Figure 3).

Patients with a high proportion of COX-deficient fibers had a significantly higher amount of mtDNA deletions i.e., higher levels of heteroplasmy compared with patients with a low proportion of COX-deficient fibers ($p < 0.001$) and age-matched controls ($p < 0.01$) as seen in Figure 5.

Duplications in mtDNA have initially been suspected and then shown to occur in IBM muscle,^{59,60} but methodological limitations have made it difficult to analyze the ratio between deletions and duplications and to map rearrangements in detail. The development of whole genome sequencing (WGS) has provided new opportunities of further characterization of mtDNA in muscle tissue homogenate, including analysis of deletions, duplications, and breakpoints. Using WGS data, rearrangements in mtDNA can be mapped by bioinformatic tools including the Mitochondrial Structural Alterations (MitoSAIt) pipeline.⁶¹ This pipeline has several advantages, including the possibility to detect duplications and visualize rearrangements in mtDNA (Figure 6). Rearrangements are classified as duplications if overlapping replication origins (Figure 3).^{61,62}

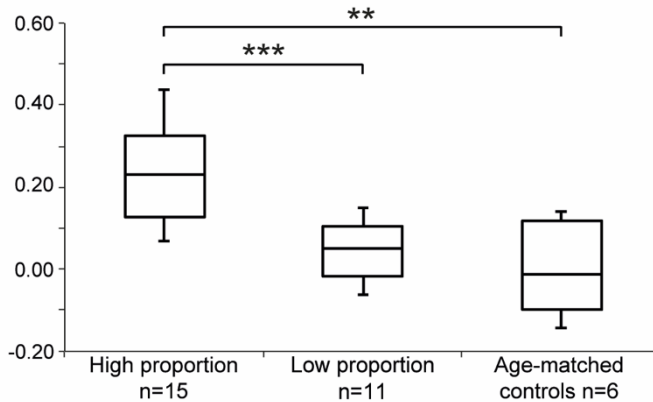


Figure 5. Relative amount of mtDNA deletions in patients with high and low proportion of COX-deficient muscle fibers and age-matched controls. The mean value of age-matched controls was set to 0. The median value for patients with a high proportion of COX-deficient muscle fibers was 0.23, for patients with a low proportion of COX-deficient muscle fibers 0.053 and for age-matched controls - 0.012. n, number of individuals; **, $p < 0.01$; ***, $p < 0.001$. Reprinted from Lindgren U, et al. Mitochondrial pathology in inclusion body myositis. *Neuromuscul Disord.* 2015;25(4):281-288 with permission according to Elsevier Author Rights.

Deletions and duplications in mtDNA were characterized with WGS in 21 patients with clinicopathological IBM and 10 age-matched controls in Paper II. Disease duration in the patients was 1-11 years. The mean depth of mtDNA coverage was 46,000x. Patients with IBM had a mean heteroplasmy level of large-scale mtDNA rearrangements of 10% (range 1-35%), and controls 1% (range 0.2-3%) (Figure 6). The number of different rearrangements in each individual patient correlated to the heteroplasmy level.

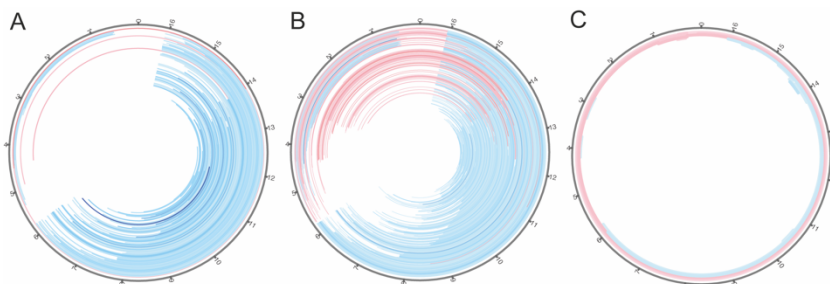


Figure 6. Circular map of mitochondrial DNA rearrangements in IBM. Deletions are visualized in blue and duplications in red. More intense color relates to higher frequency of the rearrangement. A, B. Individuals with clinicopathological IBM. C. An age-matched control.

In Paper IV, the mtDNA rearrangements in six patients with early-onset IBM were analyzed with WGS, with a mean depth of mtDNA coverage of 51,300x. Patients with early-onset IBM showed a similar pattern of large-scale mtDNA rearrangements as patients with IBM. Both patients with early-onset IBM and clinicopathological IBM had significantly higher heteroplasmy levels compared with their respective age-matched control group (see Paper IV, Figure 3 for more detailed results).

Multiple different deletions in mtDNA are present in the individual patient,⁵⁶ and occasionally also in the same muscle fiber.⁶⁰ In Paper II, more than 200 different rearrangements were found in two or more patients with IBM. The most common were in mtDNA regions m.6330-13993 (present in all 21 patients), m.534-4429, and m.8636-16087. Deletions were more common than duplications. In patients with clinicopathological IBM as well as early-onset IBM, most rearrangements were located in mtDNA major arc, but rearrangements were also present in minor arc.

4.2.2 SMALL SOMATIC MITOCHONDRIAL DNA VARIANTS

Whole genome sequencing also enables analysis of small somatic mtDNA variants including single-nucleotide variants and small insertions and deletions (indels). Point mutations in mtDNA including single base substitutions and small indels were found in 10 of 21 patients with clinicopathological IBM and in two of ten controls in Paper II. Heteroplasmy levels of each variant varied from 1.01-5.53% in the patients with IBM and from 1.06-1.31% in the controls (a cut-off level for heteroplasmy was set at 1% to exclude sequencing errors). The low level of heteroplasmy in patients compared with levels in individuals with inherited point-mutations in mtDNA suggest that the described small variants are probably of little biochemical or clinical significance in IBM.

4.2.3 MITOCHONDRIAL DNA COPY NUMBER

A slight decrease of mtDNA copy number has been shown in IBM,^{63,64} and was also seen in Papers II and IV calculated as the mitochondrial genome coverage $\times 2$ / nuclear genome coverage.⁶⁵ However, the analysis of mtDNA copy number in muscle tissue homogenate from patients with IBM might interfere with the nDNA present in the inflammatory cells and should be interpreted with care. Statistical analysis for comparison of mtDNA copy number between groups was therefore not performed in Papers II and IV.

4.2.4 CONCLUSIONS

The use of WGS and bioinformatics has facilitated mapping of mtDNA deletions and duplications. Patients with IBM harbor multiple deletions and duplications in mtDNA. Cytochrome c oxidase-deficiency is associated with multiple mtDNA deletions in IBM muscle. Since not all muscle fibers in IBM muscle tissue are COX-deficient, the heteroplasmy seen in analysis of tissue homogenate suggests that the heteroplasmy levels in the affected fibers are even higher. Small single nucleotide variants or indels in mtDNA are probably not a significant part of IBM pathology.

4.3 NUCLEAR DNA GENES ASSOCIATED WITH MITOCHONDRIAL DNA REARRANGEMENTS

Pathogenic variants in nuclear genes can cause multiple mtDNA deletions through disturbance of replication and maintenance of mtDNA. The nuclear genes *SPG7*, *DNA2*, *RNASEH1*, *SLC25A4*, *OPA1*, *TWNK*, *POLG*, *POLG2*, *TYMP*, *RRM2B*, *TK2* and *MPV17* are all known to be associated with multiple mtDNA deletions through this mechanism.² Many of them can cause patterns of multiple mtDNA deletions and COX-deficient muscle fibers similar to IBM.

Eight nDNA genes associated with multiple mtDNA deletions were analyzed with whole exome sequencing in Paper I, and the found variants were analyzed with Sanger sequencing in all 26 patients (Table 2). In Papers II and IV all twelve now known genes associated with multiple mtDNA deletions were analyzed with WGS. The only significantly increased variant in IBM compared with controls from the Exome Variant Server was the synonymous variant *RRM2B* c.207c>T p.(V69=) in Paper I.⁶⁶ The previously not reported synonymous variant *TWNK* c.1572C>t p.(H524=) was found in one patient. In Paper II, mostly common variants and/or single nucleotide variants were found in the twelve nDNA genes using WGS. The 21 patients with clinicopathological IBM had an average of 6.3 variants and age-matched controls 6.4 variants. In both Papers II and IV, none of the patients harbored any likely pathogenic variants after filtering.

Variant	% of alleles	
	Patients	Controls
<i>TWNK</i> p.(H524=) [†]	1.9	-
<i>DNA2</i> p.(T657=)	7.7	5.2
<i>DNA2</i> p.(C718F)	1.9	0.42
<i>MGME1</i> p.(Q90=)	7.7	2.7
<i>POLG2</i> p.(G416A)	1.9	1.7
<i>RRM2B</i> p.(V69=)	14***	2.9
<i>TYMP</i> p.(G428=)	5.8	-
<i>POLG</i> p.(A194V)	1.9	-
<i>POLG</i> p.(L752=)	1.9	1.1
<i>POLG</i> p.(E1143G)	1.9	3.9
<i>POLG</i> p.(Q1236H)	11.5	8.1

[†] previously not reported; ***, p<0.001.

Table 2. Frequency of nDNA variants in TWNK, DNA2, MGME1, POLG2, RRM2B, TYMP and POLG in 26 patients with IBM compared with the Exome Variant Server control population (Paper I).⁶⁶

4.3.1 *POLG*

Mitochondrial DNA is replicated by mitochondrial DNA polymerase gamma, of which the catalytic part is encoded by *POLG*. Pathogenic variants in *POLG* can generate multiple mtDNA deletions and/or duplications as well as mtDNA depletion through defective replication.^{62,67,68} One of the more common manifestations of pathogenic variants in *POLG* in adults is progressive external ophthalmoplegia (PEO). As a nuclear gene, *POLG* is present in all nucleated cells and variants can cause multi organ dysfunction. Manifestations in adults are often due to accumulation of mtDNA rearrangements in postmitotic tissues such as muscle and brain.^{2,69}

The progressive nature of IBM, combined with the typical onset at an older age and the protein accumulation in muscle tissue has rendered speculations that there may be similarities between IBM and neurodegenerative disorders. In both Parkinson's disease and aged controls, high levels of mtDNA deletions have been seen in neurons in substantia nigra.⁷⁰ *POLG* contains a polyglutamine tract (poly-Q), consisting of a trinucleotide repeat (CAG). The poly-Q tract normally has 10 or 11 repeats,⁷¹ but variants in length have been reported as associated with Parkinson's disease in North America, Finland, Norway, and Sweden.⁷²⁻⁷⁵

POLG including the length of the poly-Q tract was analyzed with Sanger sequencing in the 26 patients with IBM in Paper I. No differences in the length of the poly-Q tract (non 10/11 repeats) were seen between patients with more or less COX-deficient fibers, nor between patients with higher or lower relative amount of mtDNA deletions or between patients with IBM and the Swedish control population described in Anvret et al.⁷⁵ Heterozygous *POLG* variants were found in eight of the patients and included a not previously reported missense variant (c.581C>T p.(A194V)) predicted as probably not pathogenic (Table 2).

4.3.2 CONCLUSIONS

There is no evidence that variants in nDNA are part of the pathogenesis of mtDNA rearrangements in IBM.

5 CLINICAL FEATURES

This chapter presents Papers III and IV and aims to review signs and symptoms, comorbidities, laboratory signs and frequencies of immunomodulating treatment in inclusion body myositis.

Due to the rarity of the disease, systematic studies on natural history in inclusion body myositis are often including only a small number of patients. The hope to halt the progression of the disease has resulted in many patients with IBM using immunomodulating treatment, with the result that studies do not describe the “true” natural history. However, the lack of effect of treatment,^{29,30,37} implies that also studies including patients with treatment can be regarded as close to the true natural history.

Different diagnostic criteria and different methods for identifying and including patients can affect the results of the studies. When studying the frequency of signs and symptoms, the cohort’s construction, e.g., patients from a national referral center, can cause bias in the results. See chapters 6 Diagnostic criteria and epidemiology, and 7 Methodological considerations for further discussion on these matters.

In Papers III and IV, a population-based cohort from Region Västra Götaland (VGR), Sweden, diagnosed with IBM during 1985-2017 was identified (Figure 7). The European Neuromuscular Centre Research (ENMC) diagnostic criteria for IBM from 2011 was used,¹ and the date of the first biopsy needed to fulfill histopathological criteria was considered the date of diagnosis. Paper III focuses on the 128 patients fulfilling all criteria for clinicopathological IBM, and Paper IV on the six patients fulfilling all criteria for clinicopathological IBM except the age criteria who also had a first muscle biopsy showing inflammation before 50 years old. The mean follow-up time from diagnosis was 8 years in Paper III and 12 years in Paper IV. This cohort also formed the basis for the 21 patients with clinicopathological IBM included in Paper II.

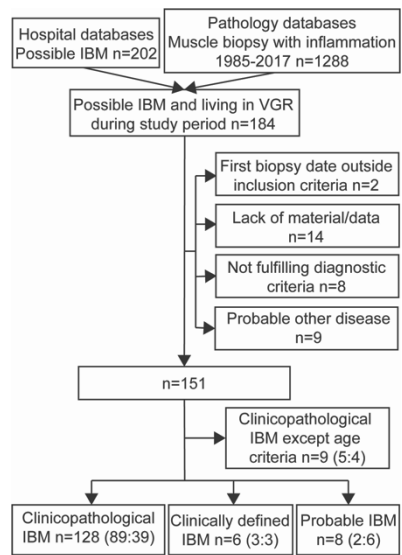


Figure 7. Identification of a population-based cohort of 151 patients with inclusion body myositis in Region Västra Götaland, Sweden. n, number of individuals (men:women). Adapted from Lindgren U et al. Epidemiology, survival, and clinical characteristics of inclusion body myositis. *Ann Neurol.* 2022;92(2):201-212 under the terms of the Creative Commons Attribution-NonCommercial License (CC BY-NC).

5.1 SIGNS AND SYMPTOMS

The pattern of asymmetrical progressive muscle weakness and atrophy primarily affecting quadriceps and finger flexors is typical for IBM. This combined with swallowing difficulties should bring the IBM diagnosis to mind.

5.1.1 FIRST REPORTED SYMPTOM

It is important to identify IBM as early as possible to reduce unnecessary investigations, medication, and misdiagnosis. A hypothesis on the inefficiency of IBM treatment is that it is started too late in the disease course, and therefore it would be interesting to study treatment in patients identified in an early stage of the disease.⁷⁶ As a progressive disease, the presenting symptom usually has a successive onset. The disease may well be asymptomatic for years, delaying diagnosis. In Papers II-IV, the information on symptom onset is based on what the patients recalled as their first symptom as stated in their medical records.

Quadriceps weakness, finger flexor weakness and dysphagia are the most common presenting symptoms in most cohorts (Figure 8), while the individual frequencies vary between cohorts. Quadriceps weakness was the most common presenting symptom in all cohorts.

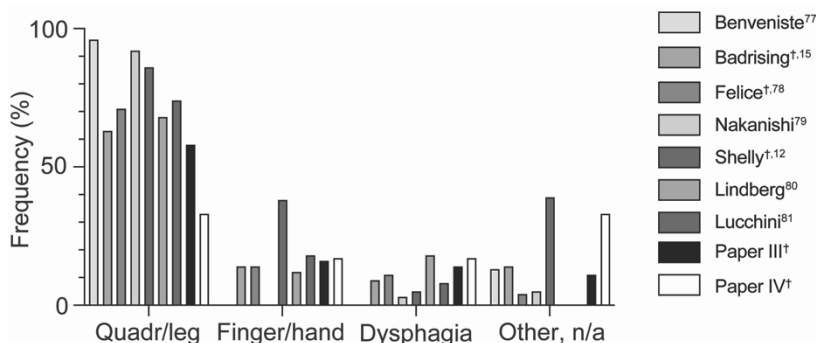


Figure 8. First reported symptom in retrospective studies on clinical characteristics of IBM published 2000 or later and including >20 patients. [†]population based. *Quadr*, quadriceps femoris muscle; *Dysphagia*, including dysphagia or other complaints of swallowing difficulties; *Other*, including unspecified muscle weakness and proximal arm weakness; *n/a*, not available.

In Paper III, we were able to study men and women separately. Quadriceps weakness was more common in men, while finger flexor weakness and dysphagia were more common in women.

Atypical IBM onset has been described with a range of more uncommon features including foot drop, axial weakness, proximal upper limb weakness, facial weakness, and isolated elevated levels of creatine kinase (CK).^{15,82,83} Most of these patients later develop typical symptoms of IBM.⁸² Another atypical presentation is IBM with early onset as described in Paper IV, where all patients had muscle weakness and/or dysphagia before 46 years of age.

An interesting question is if different subgroups of IBM can be identified by the first symptom. Lindberg et al described a tendency of faster decline in muscle strength in patients with bulbar onset, in a cohort of 66 patients partially overlapping with Papers III and IV,⁸⁰ while Cox et al described no difference in weakness progression or life expectancy depending on the presenting symptom in 15 patients.¹³

In Paper III, patients with dysphagia were the oldest at reported symptom onset, 68 years, and had the shortest mean survival of 12 years from symptom onset after omitting an outlier value (40 years). Patients reporting finger flexor weakness as the presenting symptom were the youngest at symptom onset, 62 years, and had the longest mean survival of 15 years from diagnosis. In the six patients with early-onset IBM presented in Paper IV, there was no apparent different distribution of presenting symptoms compared to patients with clinicopathological IBM.

5.1.2 MUSCLE WEAKNESS

The progression of inclusion body myositis over time can be measured in different ways, including using functional scales such as the IBM functional rating scale (IBM-FRS),⁸⁴ the Medical Research Council (MRC) Scale,⁸⁵ and manual and quantitative testing of muscle strength. Several studies have shown progressive muscular weakness over time, where the decline in quadriceps strength seems to be most evident.^{86,87} The difference between individuals is large. Decline in knee extension strength has been reported by Lindberg et al as 1.22 Newton or 1.1% per month. It is unknown if the decrease in strength is linear or follows another pattern.^{13,14,80,87,88} In normal aging, strength loss is approximately 3-4% per year,⁸⁹ and lower limb weakness is associated with falls.⁹⁰ We studied the progression rate in IBM with early onset in Paper IV and there was no evident difference to other patients with IBM.

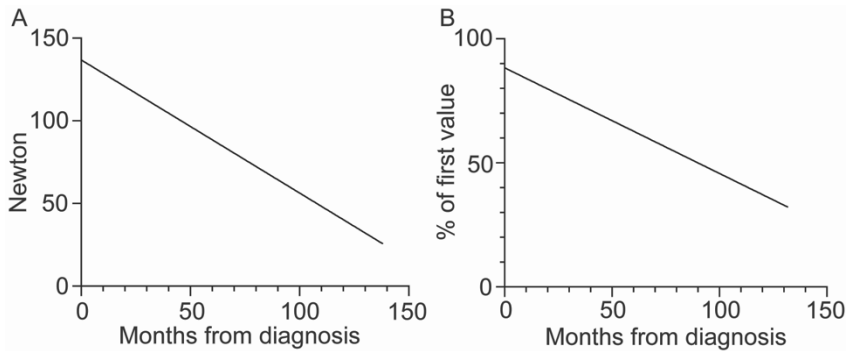


Figure 9. Progress of knee extension weakness over time analyzed with linear regression in six patients with early-onset IBM. A. Absolute strength in Newton. B. Strength as % of first available measurement. For more detailed values, see Paper IV.

While the relationship between knee extension strength and walking ability is not linear,⁸⁷ the use of a wheelchair is an indication of a significant muscle weakness. Median and mean times from symptom onset to start of wheelchair use has been reported as 11-15 years in IBM. The frequency of wheelchair usage varies greatly between studies, from 14% to 100%, and this variation is probably, at least partially, dependent on the time from symptom onset in the studied patients.^{12,13,15,77,88} Falls have been reported in 93% of 57 patients in a study by Needham et al., and 46% of patients had suffered from fractures or extensive soft-tissue injury.⁸³

Among the 128 patients with clinicopathological IBM in Paper III, 61% were using a wheelchair with a mean time of 11 years from symptom onset to first reported use. In the subgroup of deceased patients, 77% had documented use of a wheelchair. In Paper IV, five of six patients with early-onset IBM were using a wheelchair, with a mean time to wheelchair use of 14 years from symptom onset.

5.1.3 DYSPHAGIA

Dysphagia can both occur at symptom onset as well as develop during the disease course of IBM. It is a severe symptom that can cause malnutrition, weight loss, aspiration pneumonia and ultimately death.^{11,82,91} Prevalence has been reported as varying between 40% and 80% in patients with IBM,^{13,15,16,77,82,92-94} similar to Paper III in which 77% of all patients had dysphagia. Swallowing difficulties were significantly more common as symptom of onset in IBM than in other inflammatory myopathies in a study of 62 patients with dysphagia associated with inflammatory myopathy.⁹²

Dysphagia with radiologic confirmation of cricopharyngeal bar has been associated with higher age in a group of 45 patients with IBM (Figure 10).⁹⁵

While not uncommon in the older population, dysphagia is rare amongst healthy young individuals. Despite this, five of six patients in Paper IV had dysphagia.

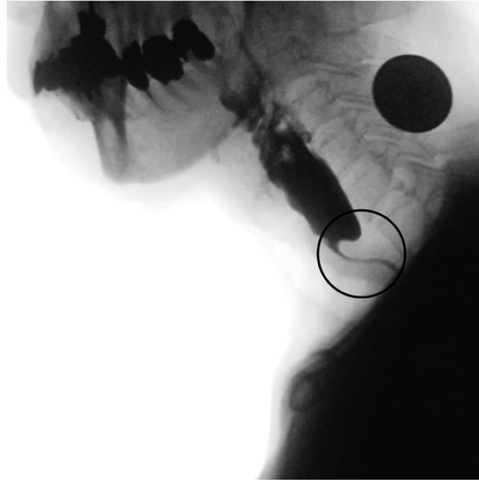


Figure 10. Cricopharyngeal bar visualized with video fluoroscopic examination of swallowing. From Taira K, et al. Clinical characteristics of dysphagic inclusion body myositis. Neuromuscul Disord. 2023;33(2):133-138. Reprinted under the terms of the Creative Commons Attribution-NonCommercial-No Derivatives License (CC BY-NC-ND).

The prevalence of dysphagia in IBM can be compared with a questionnaire-based survey in Sweden where 35% of 556 individuals from the general population aged 50-79 years reported any type of swallowing complaint,⁹⁶ and as study from Northern England where 11.4% in a cohort of 634 otherwise healthy persons with a mean age of 81 years reported symptoms consistent with significant dysphagia.⁹⁷

The cause of dysphagia in IBM is not fully clear, but muscle fibrosis and inflammation in cricopharyngeal muscle,^{98,99} pharyngeal muscle weakness and impaired opening of the upper esophageal sphincter have been described.^{95,100} Common treatments of dysphagia in IBM include adjustments of food texture, cricopharyngeal myotomy and esophageal dilatation, but may also include gastrostomy.⁹¹ In Paper III, 31 of the 128 patients had at least one invasive treatment of dysphagia with a mean time from IBM symptom onset of 9.5 years. Dysphagia seemed more common in women than men, and of women

with dysphagia, 24% had a gastrostomy. The higher frequency of dysphagia in women was also seen in an American non-population-based cohort described by Michelle et al.¹⁰¹

The high frequency of swallowing difficulties as the first symptom in IBM emphasizes the importance of awareness of IBM in primary health care as well as in ear, nose, and throat clinics, enabling early referral for further evaluation and diagnostics when needed.

5.1.4 RESPIRATORY DYSFUNCTION

Like dysphagia, respiratory dysfunction is a severe symptom with potentially harmful effects. Both hypoventilation and decreased lung vital capacity have been reported in IBM in non-population-based cohorts.^{78,102} Disorders in the respiratory system are an over-represented cause of death in patients with IBM.¹³

Shelly et al described noninvasive ventilation use in three of 21 patients (14%), with a median time of 64 months from symptom onset to dyspnea.¹² In Paper III, the use of ventilation assistance devices was documented in 10 patients (8%) of whom three had never or very rarely used it. Mean time from symptom onset of IBM to start of ventilation assistance was 11 years. Six patients initiated BiLevel treatment, and four of them were deceased within a year from the initiation. In Paper IV, three of six patients with early-onset IBM (50%) had some kind of ventilation assistance. Within four years after starting ventilation assistance, all three patients were deceased.

5.1.5 CONCLUSIONS

The most frequent initial symptom of IBM is muscle weakness in the quadriceps muscles or “leg weakness”. Whether the various onset symptoms are associated with different progression and prognosis remains unclear, and more extensive population-based studies are needed. Knowledge on atypical presentations including early onset is important in the clinical setting to optimize care for the individual patient.

Muscle weakness in IBM is progressive over time. Wheelchair use is common and usually begins 11-15 years after symptom onset.

Dysphagia is common in patients with IBM, and more frequent among women than men. Fibrosis and inflammation in the cricopharyngeal muscle and the high frequency of dysphagia in early-onset IBM underlines that the dysphagia is caused by the disease itself and not just a symptom of increasing age.

Respiratory insufficiency is a severe symptom of IBM affecting up to 50% of patients. The results from Papers III and IV suggest that respiratory dysfunction develops late during the disease course.

5.2 COMORBIDITIES

It is difficult to study comorbidities in IBM. Several reports studying associations of diseases including hepatitis C, HIV and chronic T cell large granular lymphocytic leukemia in IBM are available.¹⁰³⁻¹⁰⁵ However, due to the rarity of the disease, the number of population-based studies are small.

The association between different autoimmune diseases due to shared risk factors is well known. Some autoimmune diseases are also associated with a higher malignancy risk. If the malignancy risk is increased, it calls for further examinations of the patient. It is therefore important for clinical practice to explore the possible association between malignancy and IBM.

5.2.1 AUTOIMMUNITY

The definition of autoimmune diseases and the investigated population are central parts when studying the prevalence of autoimmunity. The use of different definitions cause difficulties in comparing frequencies of autoimmune diseases between studies. It is even more difficult when the studies are also using different definitions of IBM.

Regarding how common autoimmune diseases are in the general population Eaton et al reported a lifetime prevalence of 5.3% in Denmark using a definition including 31 autoimmune diseases.¹⁰⁶ Hayter et al estimated a prevalence of 4.5% using a definition including 81 autoimmune diseases.¹⁰⁷

In 1998, Koffman et al studied 99 patients with IBM and found that 13 patients had one or more of the defined 11 autoimmune diseases.¹⁰⁸ Cortese et al described a frequency of autoimmune disease of 16% in a cohort of 51 patients with IBM.⁸⁸ More recent population-based studies including autoimmunity are reviewed in Table 3 (see Paper IV for more details on early-onset IBM). All studies used different definitions of autoimmune disease except Papers III and IV that both used the definition described in Eaton et al.¹⁰⁶

Of the patients described in Paper III, four individuals had more than one autoimmune disease. According to the definition of autoimmune disease, six patients with Raynaud syndrome, three patients with hypogammaglobulinemia and three patients with sicca symptoms were not included. In Paper IV, three patients with diseases commonly regarded as autoimmune or possibly autoimmune were not included as having an autoimmune disease.

The most prevalent autoimmune diseases in patients with IBM include Sjogren's syndrome, rheumatoid arthritis, and hypothyroidism.^{11,12,109} In

patients with myositis of any type, the presence of Sjogren's syndrome has been shown to be associated with IBM.¹¹⁰

Table 3. Prevalence of autoimmune diseases in IBM in population-based studies published 2000 or later using the ENMC 2011 diagnostic criteria.

First author, country	Number of patients (M:W)	Mean age (years)		Autoimmune disease (%)		Diseases included (n)
		Onset	Diagnosis	IBM	Control population	
Dobloug, Norway ¹⁰⁹	100 (60:40)	61 [†]	67	24	-	3
Shelly, USA ¹²	21 (11:10)	67 [‡]	68 [‡]	48	-	6
Naddaf, USA ¹¹	50 (29:21)	-	-	16	5	4
Paper III	128 (89:39)	64	70	21	5.3 [§]	30

[†]Calculated from data in the paper; [‡]Median; [§]Lifetime prevalence in the Danish population from Eaton et al. n, number; M, men; W, women.

5.2.2 MALIGNANCY

Some inflammatory myopathies are associated with malignancy, where the relative risk is highest in dermatomyositis.^{10,111,112} An Australian study described an increased risk of malignancy in patients with IBM.¹¹³ Contradicting this, Dobloug et al used a population-based approach to study 100 patients with IBM and 150 matched controls in south-east Norway. A total of 25 patients with IBM had cancer, with a mean time from symptom onset to cancer of 3.7 years. The cancer risk and the frequency distribution of different cancer types did not differ from age- and sex- matched controls.¹⁰

Further studies on malignancy in IBM include Naddaf et al, who studied 50 patients with IBM. Of them, 10% had a hematologic malignancy, and 40% had a malignancy of any type. There were no significant differences to patients with other inflammatory myopathies or matched controls.¹¹ Shelly et al described 21 patients of whom 10 had a malignancy, with a mean time between IBM diagnosis and cancer diagnosis of 1.5 years. The cancer incidence did not differ significantly from matched controls.¹² Cortese et al described malignancy in 10% of 51 patients with IBM with a mean age of 68 years at assessment.⁸⁸

This is in line with the findings in Papers III and IV. In Paper III, a total of 31% of patients with IBM had a malignancy using the NORDCAN definition which includes basal-cell carcinoma.^{114,115} Among the 97 patients followed until the age of 75 years or until death before the age of 75 years, 22% had a

malignancy. In the Western Region, Sweden, the malignancy risks before 75 years old during 2012-2016 were 31% for men and 28% for women.^{114,115} See Paper IV for more detailed data on early-onset IBM.

5.2.3 CONCLUSIONS

Autoimmune diseases seem more frequent in patients with IBM than the general population. This supports the hypothesis that IBM is an autoimmune disease. Due to different definitions, it is difficult to compare studies. Larger population-based studies on epidemiology would be interesting to further analyze the relationship between IBM and autoimmune diseases. There is no apparent association between IBM and malignancy.

5.3 LABORATORY FEATURES

The effect of IBM on regularly used laboratory tests is important to be aware of in the clinical setting. Creatine kinase (CK) is well known to be normal or slightly increased in IBM, as demonstrated also in Paper III with the mean first value 2.1 x the upper limit of normal (ULN). High values are excluded in many diagnostic criteria, for example the ENMC 2011 criteria with a limit of CK no greater than 15 x ULN.¹

Troponin T (TnT) has earlier been described as increased in the absence of acute myocardial infarction in IBM as well as other muscular disorders including dermatomyositis and Duchenne muscular dystrophy. In IBM, the increased TnT level remains over time, shown by repeated sampling up to 17 months.¹¹⁶ The mean first documented level of TnT in Paper III was 4.3 x ULN when values for admission for probable myocardial infarction were excluded. The cause of the increased TnT levels is unknown and the cardiac muscle seems unaffected by IBM. TnT might leak from damaged, regenerating skeletal muscle, and can also be increased in patients with impaired renal function.^{117,118} Rarely elevated without cardiac involvement, troponin I is regarded as more reliable than TnT when evaluating acute myocardial infarction in patients with IBM.¹¹⁷

Creatinine is a breakdown product of creatine phosphate in muscles and while commonly used to evaluate kidney function, it also correlates to muscle mass.¹¹⁹ In Paper III, the mean first creatinine level was 72 $\mu\text{mol/L}$ (reference interval 60-110 $\mu\text{mol/L}$) and the mean lowest level during the disease course was 50 $\mu\text{mol/L}$. The low creatinine values and decrease in creatinine value over time for an individual patient with IBM may reflect the muscle atrophy.

5.3.1 ANTI-cN1A

The 5'-nucleotidases are involved in the regulation of nucleotides and nucleosides and disturbance can affect the balance in NTP and dNTP pools.¹²⁰ Circulating autoantibodies against cytosolic 5'-nucleotidase 1A (cN1A) in individuals with IBM were first described in the early 2010s.^{33,34,121} The clinical effect of autoantibodies against cN1A including the possible role in the pathogenesis of IBM is still unknown.¹²² Two studies have reported a higher frequency of COX-deficient fibers in patients with positive anti-cN1A.^{123,124} The presence of anti-cN1A has a high specificity for IBM, but antibodies against different cN1A epitopes have been described in Sjogren's syndrome, systemic lupus erythematosus as well as in other inflammatory myopathies.^{35,81,123,125-127} In patients with myositis, Sjogren's syndrome is associated with anti-cN1A regardless of if the patient has IBM.¹¹⁰

In Paper III, a panel of myositis-specific and myositis-associated auto-antibodies was analyzed in 50 patients with IBM and 28 matched blood donor controls. Anti-cN1A was positive in 40% of the patients with IBM and 3.6% of the controls. This is in line with previous studies that indicate a varying anti-cN1A frequency of 33-80% among different IBM cohorts.^{33-36,123,124,128} The positive frequency was higher in the tested men than women. See Paper IV for data on patients with early-onset IBM.

Several studies address the question of subgrouping patients with IBM depending on anti-cN1A status. Among the patients in Paper III, dysphagia was a more common first symptom in the 20 patients with positive anti-cN1A than the 30 patients without anti-cN1A. Patients with positive anti-cN1A did not seem to differ in symptom duration or frequency of immunomodulating treatment at the time for blood sampling compared to patients without expression of anti-cN1A. In a previous cross-sectional study including 25 patients, more frequent dysphagia, reduced forced vital capacity and more pronounced functional motor deficits was observed in 18 patients with positive anti-cN1A compared to seven patients without anti-cN1A.¹²⁸ An Italian study including 62 consecutive patients with IBM reported swallowing difficulties as more common among the 23 patients with anti-cN1A compared to the 39 patients without anti-cN1A.⁸¹ On the other hand, Ikenaga et al showed no significant difference in frequency of dysphagia between 47 patients with and 193 patients without anti-cN1A.¹²³

A higher risk of death among patients with positive anti-cN1A was seen in an European multicenter study including 311 patients of whom 33% were positive for anti-cN1A.¹²⁴ Ikenaga et al showed no such risk in an American study of 249 patients with IBM.¹²³ Due to the study design where only living patients were analyzed for anti-cN1A, it was not possible to evaluate the mortality risk for patients with anti-cN1A in Papers III and IV.

Three other studies showed little or no impact of anti-cN1A-status. Paul et al found no correlation between anti-cN1A status, clinical phenotype and CK in 92 patients,¹²⁹ and Felice et al found no correlation between anti-cN1A and clinical, laboratory or histopathological findings except with a higher age than 60 years at symptom onset.¹³⁰ Michelle et al described higher median CK levels in patients with anti-cN1A but no influence on clinical characteristics, pattern of weakness, muscle biopsy or electrodiagnostic findings.¹⁰¹

5.3.2 MYOSITIS-SPECIFIC AND MYOSITIS-ASSOCIATED AUTOANTIBODIES

Myositis-specific autoantibodies and myositis-associated autoantibodies can be associated with different clinical subgroups and histopathological profiles in dermatomyositis,^{131,132} or to specific disease entities such as myopathy with autoantibodies against 3-hydroxy-3-methylglutaryl coenzyme A (HMGCR).¹³³ No such association has been described in IBM.

In a Swedish study using a panel with 16 myositis-specific and myositis-associated autoantibodies, Sjogren's syndrome-related antigen (SSA/Ro-52) was found in four of ten patients with IBM.¹³⁴ The same panel was used for evaluation in Papers III and IV. In Paper III, anti-SSA/Ro52 was positive in 8 (16%) of the 50 studied patients and in none of the 28 controls. In Paper IV one of two patients were positive for anti-SSA/Ro52 and none of the six controls were. In Shelly et al, two of 14 patients were positive for anti-SSA.¹²

Anti-SSA/Ro52 is associated with many autoimmune diseases, among them dermatomyositis, Sjogren's syndrome and systemic lupus erythematosus.¹³⁵ While the role of anti-SSA/Ro52 in the pathogenesis of autoimmune disease is unknown, Ro52 has a role in innate immunity.¹³⁵ The increased frequency of anti-SSA/Ro52 in IBM might be part of the disease itself or related to the increased frequency of autoimmune diseases in patients with IBM.

5.3.3 CONCLUSIONS

CK and TnT is commonly increased in IBM. Creatinine is unreliable as a measurement of renal function in IBM. Increasing or stable creatinine values, also within or below the normal range, should call for further evaluation of renal function. Anti-cN1A is positive in 33-80% of patients with IBM. The role of anti-cN1A in pathogenesis or subgrouping patients with IBM is unknown.

5.4 TREATMENT

Treatment in IBM is a wide subject and this thesis does not aim to cover all aspects. Important aims are to minimize suffering and provide as good quality of life as possible for patients with IBM. The hope for the future is to find a treatment that can cure or at least halt the progression of IBM. Studies on treatment have the same challenges and limitations as other studies on IBM, with few patients, slow progression of the disease and increasing muscle weakness and mortality over time also in matched control groups.

Several immunomodulating therapies have been studied with the goal of altering the progression of the disease. Therapies tried over the years include among others methotrexate, mycophenolate mofetil, azathioprine, sirolimus, immunoglobulin and corticosteroids. No pharmacological treatment has proven efficient in larger cohorts, but many of the studies have been short or underpowered.^{30,37,76,136}

The frequency of immunomodulating therapies differs in studied cohorts and are often separated into treatment with corticosteroids and other immunomodulating agents. In Paper IV, all six patients had tried both corticosteroids and other immunomodulating agents. In Paper III, 50% had tried corticosteroid treatment and 82% other immunomodulating agents. In other cohorts, 44-89% of patients had tried corticosteroids and 33-40% of patients other immunomodulating agents.^{11,12,14,77,88,109} Median treatment times for corticosteroids and/or other immunomodulating therapies have been reported as 41 and 67 months respectively.^{12,77}

The value of immunomodulating treatment in the individual patient with IBM is unclear. Both corticosteroids and other immunomodulating agents have potentially severe side effects. On the other hand, there are reports on individuals or smaller groups responding to treatment.^{14,66,80,137,138} Earlier treatment studies reporting beneficial treatment outcomes might include patients not fulfilling more recent diagnostic criteria or with simultaneous autoimmune diseases potentially confounding treatment outcome.^{139,140} Dose, treatment time and other comorbidities may also affect outcome. Regarding corticosteroids, both positive and negative effects of treatment have been reported.^{11,77}

Non-pharmacological treatments include physiotherapy, treatment of dysphagia, medical dietitian advice, respiratory devices, occupational therapy and more. Physiotherapy aims to prevent complications such as contractures, but also to lessen the decline in muscular weakness. Individually adapted

exercise does not seem to result in harmful effects on muscular function.¹⁴¹⁻¹⁴⁴ However, long-term data are sparse. Dysphagia and ventilation assistance devices are further discussed under 5.1.3 Dysphagia, and 5.1.4 Respiratory dysfunction.

5.4.1 CONCLUSIONS

While studies involving treated patients cannot be regarded as true natural history studies, the effect of both pharmacological and non-pharmacological treatment on the disease course can be considered low and the studies can therefore be regarded as close to the natural history. Further studies on treatment including larger groups and long-term outcomes are warranted.

6 DIAGNOSTIC CRITERIA AND EPIDEMIOLOGY

This chapter will continue the presentation of Papers III and IV and starts with a short overview of different diagnostic criteria and their performance in IBM cohorts. It then aims to review age at onset, diagnostic delay, survival, age at death, incidence, and prevalence in IBM.

6.1 DIAGNOSTIC CRITERIA

The diagnosis inclusion body myositis is based on diagnostic criteria. Depending on the set of criteria used, the required symptoms, signs and morphology required for diagnosis will vary, and because of that the number of patients included and their clinical appearance will also differ. During the years, different sets of diagnostic criteria have been used, attempting to describe the unique characteristics of IBM, and differentiating it from other myopathies. Initially the criteria focused on histopathology, but more recent criteria include clinical signs, symptoms and sometimes also laboratory data.

An overview of the different items included in some of the more commonly used diagnostic criteria is presented in Table 4. There is an overlap between many of the diagnostic criteria, but in some cases also “gaps” where patients included in one criteria set do not fulfill requirements for diagnosis according to other sets of criteria.¹⁴⁵ The combination of finger flexor weakness, rimmed vacuoles and inflammatory cells invading non-necrotic muscle fibers have been described as highly performing.¹⁴⁵ In assessment of muscle biopsies with inflammatory myopathy, p62, rimmed vacuoles, upregulation of MHC-I and mitochondrial changes have been described as important.^{51,52} The need for muscle biopsy and the utilization of anti-cN1A as part of the diagnostic criteria has been debated.^{146,147} Approximately half of the patients with IBM lack anti-cN1A (see 5.3.1 Anti-cN1A) and its absence can therefore not exclude IBM.

Table 4. Diagnostic criteria for inclusion body myositis used in papers referred to in this thesis.

	ENMC 2011 ¹			Lloyd ¹⁴⁵		ENMC 1997 ¹⁵⁰			Needham and Mastaglia ⁴³			MRC (Hilton-Jones) 2010 ⁴⁹			Griggs ¹⁴⁸		
	Cp	Cli	Pr			Def	Pr	Pos	Def	Pr	Pos	Pat	Cli	Pos	Def	Pos	
Temporal, demographic	x	x	x			x	x	x	x	x	x	x	x	x		x	
Age at onset	x	x	x			x	x	x	x	x	x	x	x	x		x	
Sporadic disease						x	x										
Slowly progressive course						x	x	x	x	x	x						
No response immunosuppr treatment						con	com	x									
Other autoimmune diseases						com	com	com									
Muscular weakness present						x	x	x									
Pattern of weakness	x	x	x		x	a	a		x	x	a/o		x	x		x	
Dysphagia is common									x	x	a/o						
CK	x	x	x			x	x	x		x	a/o						x
EMG										x	a/o						
Inflammatory cells	x	a/o	a/o		x	x	x	x	x	a/o	a/o	x	or	or	x	x	
Rimmed vacuoles	x	a/o	a/o		or [†]	x	b		x	a/o	a/o	x	or	or	x		
Protein accumulation	x	a/o	a/o			b			or	a/o	a/o	x	abs	abs	x		
MHC-I		a/o	a/o						x	a/o	a/o		or	or			
Necrosis and regeneration										a/o	a/o						
COX-negative fibers						com	com		x	a/o	a/o						
Ragged red fibers																	

[†] Rimmed vacuoles or invasion of nonnecrotic muscle fibers. Cp, clinico-pathologically defined; Cli, clinically defined; Pr, probable; Def, definite; Pos, possible; immunosuppr, immunosuppressive; CK, creatine kinase; EMG, electromyography; a/o, and/or; con, confirms; com, compatible; abs, absent. ENMC 1997: must have a or b.

The ENMC 2011 IBM research diagnostic criteria are commonly used in recent studies, including Papers II-IV.¹ The distribution of patients fulfilling the strict diagnostic criteria for clinico-pathologically defined IBM varies between published cohorts, with Paper III having a large proportion (Table 5). One possible explanation is the decreased impact of sampling effects when renewed examination including new sections are performed. There was no difference in the frequency of anti-cN1A between the diagnostic subgroups in an Italian cohort.⁸¹

Table 5. Distribution of patients fulfilling the different subgroups of the ENMC 2011 IBM research diagnostic criteria (source: manuscripts listed in PubMed as referring to Rose et al.¹ April 13, 2023).

First author	Patients (n)	Clinico-path (%)	Clinically def. (%)	Probable (%)	Excluded age at onset (%)
de Camargo ¹⁵¹	18	-	-	-	3
Dobloug ¹⁰⁹	95	17	-	-	-
Goyal ¹²⁸	25	0	76	24	-
Lucchini ⁸¹	62	66	23	11	-
Michelle ¹⁰¹	335	1	53	78	-
Naddaf ¹¹	50	62	32	6	-
Shelly ¹²	21	48	43	5	5
Paper III	151	85	4	5	6

n, number of patients; Clinocopath, clinicopathological; def, defined.

6.1.1 CONCLUSIONS

The result of epidemiological studies can be influenced by the diagnostic criteria and methods used. Given the rarity of IBM, identification of even a small number of additional patients can significantly change the epidemiological calculations. Hence, direct comparisons of prevalence, incidence, and survival between different cohorts with patients with IBM may not be possible, but trends and estimates can be observed.

6.2 AGE AT ONSET AND DIAGNOSIS

Typical symptom onset in IBM is after 45 years of age, although earlier onset occurs.^{15,78,101,152} There is no apparent difference between men and women in age at symptom onset.^{4,9,101,109} In Paper III, mean age at symptom onset was 63 years in women and 65 years in men. In Figure 11, age at symptom onset, diagnosis and death are presented in the 151 patients fulfilling the ENMC 2011 criteria for IBM except the age criteria and included in Papers III and IV. None of the 151 patients had symptom onset before 30 years of age.

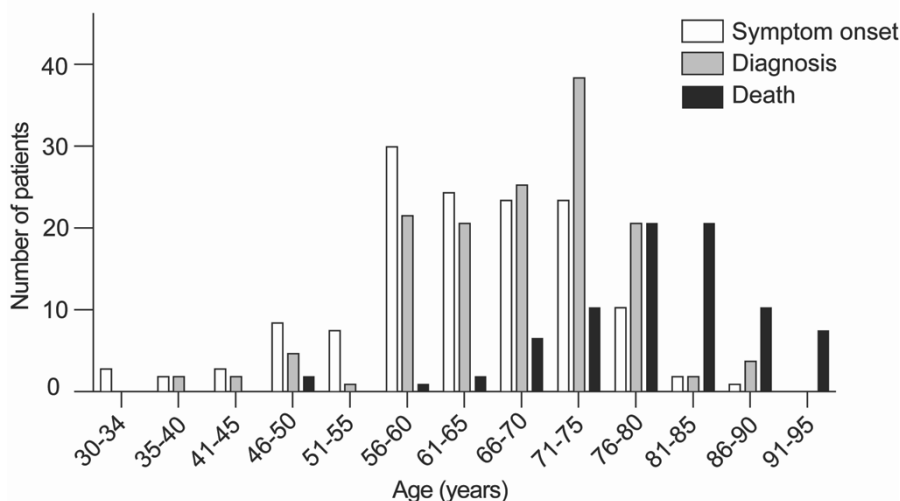


Figure 11. Age at symptom onset, diagnosis, and death in 151 patients with clinicopathological, clinically defined or probable IBM, according to the ENMC 2011 criteria except the age criteria. Data for symptom onset missing in four patients.¹

Mean age at diagnosis varies between 67 and 70 years in population-based cohorts including Paper III (Figure 11).^{8,9,12,78,109} All patients fulfilling the ENMC 2011 criteria are required to be at least 45 years of age at symptom onset and 46 years of age at diagnosis.¹ The mean age at diagnosis was only 45 years of age in the six patients with early-onset IBM in Paper IV.

The definition of diagnosis date differs between studies and can also affect the result. Frequently, the date of the muscle biopsy is considered the date of diagnosis, including in Papers III and IV.^{12,77} The date the treating physician made the IBM diagnosis is also used in some studies.¹⁰¹

6.2.1 DIAGNOSTIC DELAY

The mean and median times from symptom onset to diagnosis varies between 2-8 years in population-based cohorts,^{9,12,78,109} with a systematic review reporting a mean diagnostic delay of 5.2 years.¹⁵³ A slightly longer diagnostic delay has been reported in women than men in both Paper III, Badrising et al and in Michelle et al (the latter cohort based on patients in a tertiary care referral center).^{9,101} Early onset is also associated with a longer diagnostic delay.¹⁰¹ The median diagnostic delay was longer in patients with early-onset IBM than in patients with IBM in Papers III and IV. Other uncommon onset symptoms are also reported having longer diagnostic delays.⁸²

Many factors can affect the diagnostic delay. Delay has been reported both in time from symptom onset to seeking medical attention, and in receiving the correct diagnosis from medical practitioners.⁸³ Elderly patients may expect some muscular weakness,¹⁵⁴ and not seek medical care when noticing early symptoms. A muscular overcapacity in younger individuals might lead to extensive muscle loss before the individual notice any symptoms. Doctor's delay can be caused by low susceptibility of IBM because of the rarity of the disease and atypical presentation. The progressive nature of the disease can contribute to both patient's and doctor's delay. The diagnostic criteria used is also important as several criteria requires symptoms for more than six or twelve months, predefining the minimum time between symptom onset and diagnosis.^{1,4,53,146,148,155}

Several studies describe misdiagnosis including polymyositis, motor neuron disease, arthritis and "old age" in 31-100% of patients before the correct diagnosis of IBM.^{15,77,78,83,101} Polymyositis with inflammation and mitochondrial pathology (and no rimmed vacuoles) progresses to IBM in most patients and has been suggested as a part of the IBM "spectrum" or an early stage of IBM.¹⁵⁶⁻¹⁵⁸ In Paper III, 8% of patients initially had a diagnosis of polymyositis or motor neuron disease. The low numbers of patients with initial incorrect diagnosis in Paper III may be influenced by both the centralized clinical care and muscle biopsy diagnostics of neuromuscular diseases in western Sweden.

6.2.2 CONCLUSIONS

Symptom onset in IBM rarely occurs before 45 years of age. The diagnostic delay is slightly longer in women than men and a more atypical clinical presentation including early onset is associated with a longer diagnostic delay. Difficulties in initial diagnostics with high rates of misdiagnosis in some cohorts may account for part of the diagnostic delay.

6.3 SURVIVAL

Previously, the survival rate of patients with IBM was regarded as unaffected.^{9,13,77,80} However, more recent studies have been able to show a decreased survival.¹⁰⁻¹² In Paper III, cumulative survival was decreased both from symptom onset and from diagnosis date. The large material made it possible to study men and women separately. In women, survival was significantly affected already in the third year from diagnosis and in men from year 13 after diagnosis. Although survival is affected, many patients live for several years with IBM as seen in Figure 11.

In Figure 12, cumulative survival in all 151 patients with IBM including early-onset IBM, and the corresponding age- and sex- matched population in Region Västra Götaland, Sweden is visualized. The decrease in cumulative survival becomes more evident during the long follow-up time.

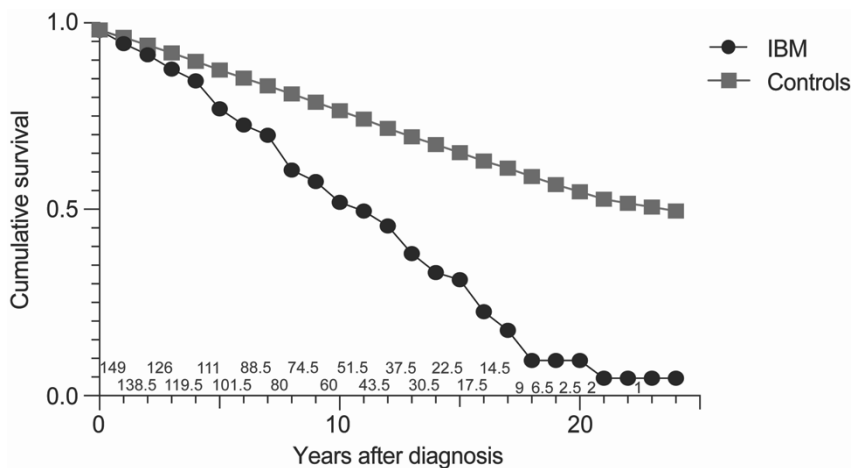


Figure 12. Life table estimate of observed cumulative survival in 151 patients with IBM and matched population controls from Statistics Sweden.^{159,160} Mean number of patients at risk shown above the x-axis.

The mean age at the time of death among patients with clinicopathological IBM in Paper III was 80 years. For population controls who had reached 65 years of age, the expected age at the time of death was 82 years for men and 85 years for women. Mean and median age at death has been reported as 79-84 years in other cohorts including 21 to 66 patients with IBM (Lindberg et al partially overlapping the cohort in Papers III and IV).^{11-13,80} Patients with early-onset IBM have a longer survival from diagnosis as shown in Paper IV, but due to young age at diagnosis, the mean age at death is still lower

compared with the 73 deceased patients with clinicopathological IBM in Paper III.

6.3.1 CAUSE OF DEATH

One study including 64 patients of whom 46 were deceased reported patients with IBM having a significantly increased risk for diseases in the respiratory system, especially pneumonia, and cachexia as cause of death.¹³ Additionally, a slightly elevated risk of malignancy as a cause of death was also observed. This was not confirmed in other studies, even though respiratory failure and pneumonia were the most common causes of death among the deceased patients.^{11,12} Dysphagia, female sex and age at diagnosis have all been described as risk factors for death.^{10-12,77} The largest available population-based study on risk factors for death in IBM included 100 patients of whom 31 were deceased. Age at diagnosis was the only independent risk factor for mortality.¹⁰ Oropharyngeal muscle dysfunction was ranked the most common factor contributing to death in a questionnaire study including physicians from seven countries.¹⁶¹

6.3.2 CONCLUSIONS

Inclusion body myositis is associated with a slightly to moderately reduced cumulative survival. More extensive population-based studies are needed to further describe potential differences in the causes of death compared with the general population.

6.4 PREVALENCE AND INCIDENCE

The reported prevalence of IBM varies between 0.68 patients per million inhabitants in Turkey to 50.5 patients per million inhabitants in South Australia.^{7,8} When including only individuals over 50 years of age, reported prevalence is up to 182 per million inhabitants.¹² Higher prevalence numbers are reported in Europe, USA and Australia (Table 6).¹⁶² Genetic factors including HLA-type probably affect the susceptibility of IBM.^{31,32,163,164} Study design and diagnostic criteria can also affect the prevalence values. Some of the more commonly used diagnostic criteria are outlined in Table 4.

Table 6. Prevalence of IBM per million inhabitants in population-based studies published 2000 or later including ≥ 20 patients.

First author	n (M:W)	Country and area	Diagnostic criteria	Prevalence		
				All ages	≥ 50 years	Year
Badrising ⁹	76 (50:26)	Netherlands	ENMC 1997 ¹⁵⁰	4.9	16	1999
Felice ⁷⁸	35 (23:12)	Connecticut	Griggs 1995 ¹⁴⁸	10.7	28.9 [†]	1992-2002
Needham ⁴	31 (19:12)	Western Australia	Needham and Mastaglia ⁵³	14.9	51.3	2007
Tan ⁸	126 (60:66)	South Australia	Local criteria [‡]	50.5	139	2009
Dobloug ¹⁰⁹	100 (60:40)	Southeast Norway	ENMC 2011 ¹ and 1997 ¹⁵⁰	35	-	2012
Lefter ¹⁶⁵	149	Republic of Ireland	Hilton-Jones ¹⁴⁹	32.5 [§]	117	2013
Shelly ¹²	21 (11:10)	Minnesota, USA	ENMC 2011 ¹	-	182	2010
Paper III	128 (89:39) 142 (94:48) [¶]	VGR, Sweden	ENMC 2011 ¹	31.9 33.7 [¶]	85.2 94.7 [¶]	2017

[†] ≥ 45 years; [‡]Local criteria: Rimmed vacuoles, inflammatory infiltrate of CD45+ lymphocytes and CD68+ macrophages in interstitial space, MHC-1 sarcolemmal positivity (strong), electron microscopy findings: tubulofilamentous inclusions, TDP 43, Tau, amyloid immunopathological abnormalities (not seen in all); [§]Calculated from values in the manuscript; ^{||}ENMC 2011 clinicopathological IBM; [¶]ENMC 2011 total prevalence. M, men; W, women.

Adapted from Lindgren U et al. Epidemiology, survival, and clinical characteristics of inclusion body myositis. *Ann Neurol.* 2022;92(2):201-212 under the Creative Commons Attribution-NonCommercial License (CC BY-NC).

In most materials, IBM is more common in men than women (Table 6).^{9,166} This was also the case in Paper III, where the prevalence for clinicopathological IBM according to ENMC 2011 was 49 patients per million women over 50 years of age and 124 patients per million men over 50 years of age as of December 31 2017. The prevalence for all individuals was 19 per

million inhabitants for women and 45 per million inhabitants for men. Among patients with early-onset IBM in Paper IV, half were men and half were women, suggesting a more pronounced difference in prevalence with age. Prevalence increased over time and then stabilized in both Region Västra Götaland, Sweden in Paper III and in Olmsted County, Minnesota, USA.^{12,167} A possible explanation can be improved diagnostics over time, including for example the use of p62 immunohistochemistry.

Large variations in incidence over years have been described in some geographical areas,^{8,12,167} while a more stable incidence is reported from others.⁷⁹ Similarly to prevalence, the diagnostic criteria, methods, and geographic area probably affect the results. Shelly et al reported incidences varying between 3 and 12 patients per million inhabitants and year in Minnesota, USA during 1980-2019,¹² and Tan et al a mean incidence of 8 patients per million inhabitants in South Australia 1980-2009.⁸ In Paper III, the mean incidence 1985-2017 was 2.5 patients (range 0-5.3 patients) per million inhabitants and year. A large variation in incidence between separate years is not surprising due to the rarity of IBM.

6.4.1 CONCLUSIONS

Prevalence and incidence of IBM varies due to methodology and geographical area. An increase over time has been seen but might be explained by increased awareness among clinicians as well as advancements in diagnostic techniques.

7 METHODOLOGICAL CONSIDERATIONS

This chapter will discuss strengths and limitations in the study design and methods. It will also address some of the ethical considerations.

7.1 STUDY DESIGN

Sweden is suitable for population-based medical studies due to the cost-reduced health care provided to all inhabitants combined with reliable population registries. The care of patients with neuromuscular disorders in Region Västra Götaland, Sweden is centralized to the Department of Neurology and Department of Pathology, both Sahlgrenska University Hospital, Gothenburg, Sweden. In combination with the long tradition of neuromuscular and IBM research in VGR, this has given us unique opportunities to study genetic, clinical, and epidemiological aspects in IBM.

Studying IBM, issues include the risks of misdiagnosis and that patients as well as caretakers do not recognize the late-onset weakness as a pathological process. Elderly individuals might also have medications (e.g., anticoagulant medication) or other diseases that can affect the diagnostic examinations or complicate muscle biopsy. In our material, all patients had undergone at least one muscle biopsy (as defined in the diagnostic criteria). Most probably, there are undiagnosed patients in Region Västra Götaland that are not included in our studies.

Both a limitation and a strength of all papers in this thesis is the number of patients. The groups are, although small, among the larger IBM cohorts.

7.1.1 POPULATION-BASED STUDIES

A population-based cohort is a prerequisite for the epidemiological studies in Papers III and IV. When studying symptoms, a population-based cohort is of importance to receive data that reflects the disease as true as possible. Cohorts describing patients seeking highly specialized care can be biased by for example educational level or income. The population-based approach results in a higher grade of generalizability.

The negative effect of a population-based study is the possible reduction of the number of participants. This affects Papers III and IV, but the advantages of using a population-based material can be considered larger than the slightly smaller number of included individuals. Multi-center studies are a possibility

for studying larger cohorts, but centers need to be chosen with care to not lose the population design.

7.1.2 RETROSPECTIVE STUDIES

All studies were retrospective except analyses of myositis-specific and myositis-associated autoantibodies in Papers III and IV that were cross-sectional. A limitation of all retrospective studies is missing data. To minimize although not eliminating the impact, extensive search of medical records and muscle specimens was performed. The use of checklists at patient visits can reduce missing data, but checklists and routines can change over time in studies covering many years.

7.1.3 DIAGNOSTIC CRITERIA

Depending on the aim, different use of diagnostic criteria can be applicable. In Papers III and IV, the aim was to describe inclusion body myositis, and we chose strict diagnostic criteria to minimize inclusion of patients with other diagnoses. The disadvantage is that stricter inclusion criteria result in a smaller cohort. In Paper III, 23 of 151 (15%) patients were excluded due to not fulfilling all diagnostic criteria (six of these patients are described as a subgroup in Paper IV). In Paper II, the aim was to describe mitochondrial changes in IBM and strict diagnostic criteria were used aiming not to include any individuals incorrectly diagnosed with IBM.

There is a risk of circular reasoning based on diagnostic criteria. Among patients studied in this thesis, histopathological, clinical and laboratory features of IBM are partially predefined by the ENMC 2011 criteria.¹ To lessen the impact of circular reasoning, a possibility can be to apply either clinical or pathological criteria and study the resulting cohort in a more exploratory study. However, our aim was to describe patients as defined by the diagnostic criteria to increase the general knowledge on IBM. In the study of epidemiology, it is important to be adherent to predefined diagnostic criteria.

Diagnosis date in medical records can be dependent on the physician. In Papers II-IV, the date of the first biopsy needed to fulfill diagnostic criteria was used as the diagnostic date. All muscle biopsy charts, and muscle biopsies were reevaluated by two persons who both examined all samples. Renewed staining including p62 and COX/SDH was performed when needed. Starting with the earliest muscle biopsy in each patient, the subsequent biopsies were examined until fulfillment of ENMC 2011 clinicopathological criteria for IBM or all available muscle biopsies reexamined. This is probably the diagnostic date most comparable between patients possible to achieve with present methods.

Exclusion of patients with similar clinical and/or pathological features but other diseases than IBM is also of importance, particularly in patients with atypical clinical presentation of IBM including early onset. Pathogenic gene variants in nDNA were not found in the six patients in Paper IV by WGS. When identifying patients with IBM in Papers II-IV, nine patients were excluded due to probably having another disease (Figure 7).

7.2 METHODS

The thesis spans from laboratory to more clinical methods as reviewed in Table 7. A short discussion on some of the methods and on statistical analysis in this material follows below.

Table 7. Overview of the methods used in Papers I-IV

Method	Paper I	Paper II	Paper III	Paper IV
Morphological analysis	x	x	x	x
DNA analysis	x	x		x
Protein analysis		x		
Analysis of autoantibodies			x	x
Review of medical records			x	x

7.2.1 DNA ANALYSES

During the last decade there has been a rapid development of methods for DNA analysis. Sanger sequencing was the gold standard when the work with Paper I started, and whole exome sequencing was introduced. Later, whole genome sequencing (WGS) including mapping of mtDNA became available. This paved the way for Papers II and IV.

In Paper I, long-extension PCR of mtDNA minor and major arc and qPCR of *MT-ND1* and *MT-ND4* were used to examine multiple mtDNA deletions. These methods do not find duplications, and do not map the mtDNA rearrangements in detail. As seen in Figure 6 and Papers II and IV, some patients with IBM carry duplications in minor arc. Duplications including *MT-ND1* can result in an underestimation of the amount of mtDNA deletions. This could affect the result in Paper I, where no difference was seen between patients with IBM with a low proportion of COX-deficient fibers and age-matched controls. If the study had been repeated today, it would have been possible to use WGS instead and compare heteroplasmy levels between the groups and controls.

7.2.2 REVIEW OF MEDICAL RECORDS

When reviewing medical records, there are several risks. Recall error can be caused both by the patient during the visit and by the physician when writing the records. Both the patient and the physician might also be biased e.g., hoping for a treatment effect. The medical records used in these studies were not written aiming to be used for research and data with less importance in the clinical setting might be missing. An advantage of using data from medical

records is that it has been collected independently from the aims and hypotheses in this thesis.

7.2.3 STATISTICAL ANALYSIS AND CALCULATIONS

Statistical analysis can be problematic when studying small groups and many different outcomes. In Papers II-IV, we decided beforehand to aim for descriptive data rather than statistically significant differences and focus on a limited number of statistical analyses to reduce the effect of multiple testing. In all statistical analyses, data were considered non-parametric.

Paper III included statistical analysis of survival. Survival was calculated from date of diagnosis to avoid immortal time bias.¹⁶⁸ When designing the early-onset IBM study described in Paper IV, the small number of included patients and expected moderate impact on survival resulted in the decision not to use statistical analysis.

A strength is the large control groups, such as the entire matched population in VGR in Papers III and IV and large genetic databases in Paper I.

7.3 ETHICAL CONSIDERATIONS

All studies were designed and performed according to the Declaration of Helsinki. The study in Paper I was performed on archival anonymized muscle biopsy samples, which had been obtained after informed consent. After diagnostic workup, some of the material was selected for the study and anonymized and cannot be traced to individual patients. For each sample only age at biopsy, sex, and the muscle that had been biopsied were recorded. For Papers II-IV, an ethical permit was received from the Regional Ethics committee at the University of Gothenburg (633-15 and addendum T215-16). Consent for deceased patients was waived by the Regional Ethics committee at the University of Gothenburg. All living patients were asked for participation.

All living patients were given oral and written information that their decision to participate or not participate in the study, or decision to withdraw, would not affect their healthcare. However, there is still always a risk that the patient feels obligated to participate.

Individuals with rare diseases can value that the disease is studied, and this can both motivate the individual to participate and have a positive effect on the individual's mental health. The study design implied little or no extra effort for the individual patient. Supplementary blood samples were obtained during regular visits if possible, and otherwise in the local hospital, primary health care, or in the patient's home, depending on the individual. The risk with obtaining a blood sample can be considered low.

The risk for personal integrity is low in these studies. Data were described on group level or anonymized. Genetic data were limited to mtDNA and nDNA genes associated with mtDNA replication and maintenance.

Many of the individuals participating in the studies will have little or no gain from the results. The result of reduced survival can cause a negative effect on mental health for patients and their relatives. On the other hand, the results can improve care for future patients, e.g., by bringing attention to the high frequency of dysphagia. The shortened life span can also give increased interest in IBM diagnostics, pathogenesis and hopefully treatment research.

8 CONCLUSIONS

The aim should be to provide the highest quality care as possible for all patients with IBM, including effective treatment. To reach this, it is of great importance to describe and characterize the disease and pathology. IBM is a rare disease, and long retrospective studies can be an approach to study larger groups of patients. In the studies in this thesis, we have been able to describe both genetic, clinical, and epidemiological aspects of IBM in a well-defined population of 151 patients covering 33 years.

Deletions and duplications in mitochondrial DNA from muscle tissue are increased in patients with IBM. The mitochondrial DNA rearrangements are not associated with any known pathogenic nuclear DNA variants. Both complex I and complex IV in the electron transport chain are affected. Enzyme histochemical COX-deficiency is associated with mtDNA deletions. The mtDNA heteroplasmy levels are high and overlap with those found in myopathy due to single mtDNA deletions. This implies that the mtDNA rearrangements probably affect muscle function. We were able to show extensive mitochondrial changes also in muscle tissue in patients with early-onset IBM, supporting mitochondrial changes being a part of the disease pathobiology. While not included in present diagnostic criteria, the exclusion of potentially pathological variants in myopathy-associated genes in patients with atypical onset including early-onset is important.

An important result in this thesis is that cumulative survival is reduced in patients with IBM, both from symptom onset and diagnosis. The decrease in survival is seen earlier in women. Patients with earlier disease onset possibly have a more pronounced impact on survival. Symptom onset before 45 years of age is uncommon but occurs.

We have also been able to describe possible differences in survival, symptom onset and frequency of dysphagia between men and women although more studies are needed. Our studies support earlier research in that dysphagia is common and anti-cN1A is negative in a substantial part of patients with IBM. Increased attention to dysphagia might improve survival for future patients.

Patients with IBM have a high number of mtDNA rearrangements in muscle fibers, live for a long time with a progressive disease and have a decreased cumulative survival. We hope that our data will improve diagnostics and care for individuals with IBM and that it will be of value when designing and evaluating future diagnostic criteria and treatment studies.

8.1 FUTURE DIRECTIONS

While there are many recent advances in IBM research, numerous questions remain, and new questions emerge from recent results.

The pathogenesis of mtDNA rearrangements in IBM muscle is still unknown, as is the possible connection between mtDNA rearrangements and clinical progression. The possibility of inflammatory changes as cause of the mtDNA rearrangements is also yet to be further explored. Detailed mapping of the rearrangements by deep sequencing and bioinformatic tools may pave the way to understand the pathogenesis behind the rearrangements. Comparing the pattern of deletion breakpoints with other myopathies with COX-deficient fibers would also be of interest, including diseases with known genetic background such as PEO and acquired diseases such as myositis with COX-deficient fibers.

A low level of mtDNA copy number has been described in IBM muscle. However, the presence of inflammatory infiltrates can make it difficult to accurately interpret the ratio between mtDNA and nDNA. It would be of interest to study mtDNA levels in single muscle fibers with and without COX-deficiency, thus avoiding nDNA from inflammatory cells.

Population-based retrospective or long prospective observational studies are needed in combination with use of registries or case-control design to address questions on cause of death, frequency of autoimmune diseases and malignancy. Also important is the patients' own perspectives and quality of life, topics which were not studied in this thesis. Preferably studies should be multi-center to increase the number of patients. This can also shorten the needed study follow-up time. Studies on large cohorts can subgroup patients with IBM depending on sex, age at onset, presenting symptom and anti-cN1A-status to find possible patterns in disease progression and prognosis.

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