Chemokine CXCL10 and Cytokine GDF15 Associate with Autoimmune Myositis and Serum Levels Support Diagnosis and Subtyping

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Article

Chemokine CXCL10 and Cytokine GDF15 Associate with Autoimmune Myositis and Serum Levels Support Diagnosis and Subtyping

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Abstract: Implementation of novel blood-based biomarkers is desired to reduce diagnostic delay and burden for myositis patients. In this retrospective study, the potential of C-X-C motif chemokine ligand 10 (CXCL10) and growth differentiation factor 15 (GDF15) was explored in an established patient cohort diagnosed with immune-mediated necrotizing myopathy (IMNM; n=21), sporadic inclusion body myositis (IBM; n=18), polymyositis (PM; n=3), dermatomyositis (DM; n=2), and anti-synthetase syndrome (ASS; n=1), comparing with healthy controls (n=10) and patients with a hereditary neuromuscular disorder (n=14). CXCL10 and GDF15 were quantified in sera with enzyme-linked immunosorbent assays and immunolocalized in skeletal muscle tissue. In myositis patients, serum CXCL10 levels were significantly increased 9.6--fold compared to healthy and 4.2-fold compared to disease controls. Mean levels in IBM (929±658 pg/ml) were significantly higher than in IMNM (425±324 pg/ml). With the threshold set to 180pg/ml of CXCL10, myositis patients could be differentiated from healthy and disease controls with a sensitivity of 0.80 and a specificity of 0.71. Incorporating a threshold of 300 pg/ml for GDF15 reduced false negatives to two IMNM patients only. Subsets of muscle-infiltrating immune cells expressed CXCL10, and serum levels correlated with muscle inflammation grade. We propose adding circulating CXCL10 and GDF15 to the blood-based diagnostic toolkit for myositis as a valuable patient-friendly approach.

Keywords: biomarker; C-X-C motif chemokine ligand 10; growth differentiation factor 15; idiopathic inflammatory myopathy; immune-mediated necrotizing myopathy; myositis; sporadic inclusion body myositis

1. Introduction

The idiopathic inflammatory myopathies (IIM) represent a heterogeneous group of distinct autoimmune conditions jointly termed myositis. Subclassification of patients is a necessary effort to develop appropriate disease management and for disease prognosis. Methodologies have been developed for accurate classification, yet they continue to evolve and debate persists over definitions and validation of diagnostic criteria. Since the subgroups of polymyositis (PM) and dermatomyositis (DM) were first described based upon clinical and myopathological criteria [1,2], deepened understanding of IIM pathophysiology and heterogeneity led to further inclusion of in-depth diagnostic imaging and laboratory testing. Autoantibody profiles and muscle magnetic resonance imaging (MRI) have been incorporated successfully in the diagnostic arsenal [3]. The distinct subgroup of sporadic inclusion body myositis (IBM) was recognized, characterized by specific clinical features and presence of endomysial auto-aggressive inflammation and muscle fiber vacuoles and amyloid deposits [4], and frequent presence of anti-cytosolic 5'–nucleotidase 1A (CN1A) autoantibodies [5]. The subgroup of immune-mediated necrotizing myopathy (IMNM) has also been recognized and is characterized by muscle necrosis predominating over inflammation in the diagnostic biopsies [6], and association with anti-signal recognition particle (SRP) or anti-3-hydroxy-3-methylglutaryl-coA reductase (HMGCR) autoantibodies in part of the patients [7]. Autoantibodies

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directed against aminoacyl tRNA synthetases reveal myositis as part of the antisynthetase syndrome (ASS), a subgroup of patients who frequently suffer from interstitial lung disease (ILD) [8]. Myositis may also occur in overlap with other connective tissue diseases.

Conclusive diagnosis of the IIM may require specialized and elaborate clinical, genetic, histological and biochemical evaluation, and for many patients means taking a diagnostic muscle biopsy as a necessary yet invasive and time-consuming effort for which standardized diagnostic procedures have been proposed [9]. Further implementation of blood-based disease biomarkers therefore represents a convenient alternative approach with the potential to further reduce the need for diagnostic muscle biopsies in the myositis patient population. This is a very plausible approach, as a blood sample is routinely taken from patients for measurement of skeletal muscle markers (including the inevitable creatine kinase) and autoantibody typing, the latter already an established part of the diagnostic process. This study focusses on two stress-related proteins and their biomarker potential for identifying and subtyping the IIM. C-X-C chemokine ligand 10 (CXCL10), also known as interferon γ-induced protein 10 (IP-10) is a chemokine with a pathogenic role in autoimmune diseases that features among the main myokines involved in the pathogenesis and progression of myositis [10]. Damaged muscle expresses higher levels of CXCL10, yet the chemokine is dispensable for effective muscle regeneration [11]. A strong association of CXCL10 with the IIM has been known for two decades, with documented expression in skeletal muscle [12–14] and elevation of circulating levels in the blood [15–18]. Growth differentiation factor 15 (GDF15) is a transforming growth factor β superfamily cytokine implicated in age-related disorders, inflammation and cognitive decline [19]. Elevated GDF15 was only recently described in IIM [20,21], with GDF15 levels associated to an increased risk of myocardial injury [22].

In this study, we explore the potential of CXCL10 and GDF15 evaluation in patient sera for diagnosing and subdividing the IIM.

2. Materials and Methods

2.1. Subjects and materials

This retrospective study included sera and muscle biopsies from an established cohort of 45 adult IIM patients with confirmed clinical and myopathological diagnosis of IMNM (n=21), IBM (n=18), PM in overlap with other autoimmune diseases (n=3), DM (n=2) and ASS (n=1) (Table 1).

Table 1. Patient clinical data

Diagnosis	ID	Gender	Age	BMI	CK	Autoantibodies	Medication	Associated disease/ comorbidities
IMNM	01	F	67	33	1417	HMGCR+	GC IVIG	myocard infarct, Hashimoto's thyroiditis, diabetes
	02	F	77	25	2614	HMGCR+		
	03	F	60	24	6923	HMGCR+		cancer
	04	M	61	27	851	HMGCR+ PL7+	STAT	cerebellar ataxia, diabetes, obstructive sleep apnea
	05	F	76	30	7000	HMGCR+	STAT GC	Sjogren's, diabetes
	06	F	74	22	10899	HMGCR+	STAT	diabetes
	07	M	73	31	7855	HMGCR+	STAT	
	08	F	70	25	9356	HMGCR+	STAT	pneumocystis pneumonia, ischemic heart disease, diabetes
	09	F	72	20	5572	HMGCR+	STAT	
	10	M	68	26	1889	HMGCR+	STAT	coronary heart disease
	11	M	73	>25	4876	HMGCR+	STAT	coronary heart disease, diabetes
	12	F	60	22	5749	HMGCR+	GC	
	13	F	56	23	6144	SRP+	GC IVIG TNF	
	14	F	65	20	6168	SRP+	STAT	
	15	M	67	23	609	SAE1+	STAT GC	diabetes

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	16	F	68	31	100	Ro52+	GC	Sudeck dystrophy, diabetes, diverticulitis
	17	F	53	22	233	PM/Scl75+		
	18	F	74	21	3000	ND	GC	hypothyroidism, heart failure
	19	M	46	26	10264	ND	GC	RA
	20	M	57	29	400	ND	GC	RA, atherosclerosis
	21	F	53	31	150	ND	GC	
IBM	01	M	73	21	170	cN1A+	GC	
	02	F	68	19	262	cN1A+		
	03	M	72	22	128	cN1A+	GC	
	04	M	76	25	186	cN1A+		
	05	M	62	25	513	cN1A+, EJ+		
	06	F	61	20	717	cN1A-		
	07	F	75	21	290	cN1A-		RA
	08	F	82	24	160	cN1A-		
	09	F	70	25	658	cN1A-		cancer
	10	M	76	>25	399	cN1A-	GC β-BL	pericarditis
	11	M	72	25	68	cN1A-	STAT GC	hypercholestrolemia
	12	M	66	24	579	cN1A-	IVIG	psoriasis, diabetes, atherosclerosis
	13	F	64	31	134	cN1A-	STAT	Hashimoto's thyroiditis
	14	M	70	22	118	ND	GC	RA, COPD
	15	M	73	<25	303	ND		
	16	M	66	<25	626	ND		diabetes, hypercholesterolemia
	17	M	61	26	356	ND		
	18	M	84	>25	180	ND		myocard infarct, Hashimoto's thyroiditis
PM	01	F	42	23	542	PL7+	GC	Sjogren's
	02	M	56	24	462	cN1A-		spondyloarthritis, coronary heart disease
	03	M	70	27	308	SSA+ Ro52+		RA, cancer, diabetes
DM	01	F	57	<25	2139	Mi2+	GC	
	02	M	44	ND	1616	ND		
ASS	01	F	61	23	3046	Ro52+ Jo1+		ILD

Abbreviations: anti-synthetase syndrome (ASS), β-blockers (β-BL), body mass index (BMI), chronic obstructive pulmonary disease (COPD), creatine kinase (CK), cytosolic 5'-nucleotidase 1A (cN-1A), dermatomyositis (DM), female (F), glucocorticoids (GC), hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR), immune-mediated necrotizing myopathy (IMNM), sporadic inclusion body myositis (IBM), interstitial lung disease (ILD), intraveneus immunoglobuline (IVIG), male (M), not determined (ND), polymyositis (PM), rheumatoid arthritis (RA), small ubiquitin-like modifier 1 activating enzyme (SAE), signal recognition particle (SRP), Sjogren's-syndrome-related antigen A (SSA), statins (STAT), TNF inhibitors (TNF). Age is given in years. Only medication taken prior to sampling is listed.

Control materials were commercially obtained samples from healthy subjects (Zenbio, Durham, NC) and sera from patients with hereditary muscle disease that were diagnosed in our hospital (supplementary Table S1). Sampling adhered to ethical and privacy regulations.

2.2. Quantification of serum CXCL10 and GDF15 levels

Enzyme-linked immunosorbent assays were performed with human GDF15 (DGD150) and CXCL10 (DIP100) Quantikine ELISA kits from R&D Systems (Bio-Techne, Abingdon, UK) according to the manufacturer's specifications. Based upon preliminary experiments, optimal dilutions were determined (1/10 and 1/20 for control, 1/10 and 1/50 for patient sera). Sera were loaded onto 96-well plates in duplicate. Values were calculated as the mean of duplicates and the two dilutions tested, and reported as mean±SD. Shapiro-Wilk test determined that variables were not normally

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distributed, hence the Kruskal Wallis test by ranks for multiple groups of independent values was used, comparing values pairwise between groups. Asymptotic significance values in 2-sided tests were adjusted by Bonferroni correction for multiple tests, with mean differences considered significant from the 0.05 level. Bivariate Pearson's correlation tests were performed to evaluate correlations between variables. Receiver operating characteristics (ROC) analysis was used to compare diagnostic performances, and graphic representation with area under the curve (AUC) measured separability. All analyses were done with SPSS software version 28 (IBM, New York, NY).

2.3. Immunofluorescence, immunohistochemistry and histochemistry

Immunostaining was performed on six µm frozen muscle sections, first treated with blocking solution containing 5% donkey serum, 10% heat-inactivated human serum and 2% bovine serum albumin in phosphate buffered saline. Immunofluorescent immunolocalization of GDF15 was carried out with 4µg/ml of mouse monoclonal IgG_{2a} anti-GDF15 (clone H-2; Santa Cruz Biotechnology, Santa Cruz, CA), combined with 0.7µg/ml rabbit polyclonal anti-CD68 (H-255; Agilent, Santa Clara, CA) or 1µg/ml rabbit polyclonal anti-CD56 (Fisher Scientific, Waltham, MA) or 1.25 µg/ml rabbit polyclonal anti-LC3B (ab48394, Abcam, Cambridge, United Kingdom), and incubated for 2h at room temperature. Secondary antibodies were used labeled with CY3 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and AlexaFluor488 (Invitrogen, Carlsbad, CA) and slides were mounted with Fluoromount (Southern Biotech, Birmingham, AL). Serial sections were immunostained with mouse monoclonal IgG_{2a} anti-CXCL10 (4D5; Biorad Laboratories, Temse, Belgium), 4µg/ml mouse monoclonal IgG_1 anti-CD68 (KP1, Abcam, Trumpington Cambridge, United Kingdom), and 1.3µg/ml mouse monoclonal IgG_1 anti-SQSTM1 (BD Biosciences, San Jose, CA) for 1h (or 2h for anti-CXCL10) at room temperature. Sections were stained with Envision anti-mouse and DAB substrate (Agilent) according to the manufacturer's specifications, and mounted with aquatex (Merck Life Science, Hoeilaart, Belgium). Muscle tissues were imaged and recorded with a light/fluorescence microscope (Zeiss, Goettingen, Germany) and analyzed with CellF software (Olympus, Antwerp, Belgium). In a selection of patient biopsies, muscle histology and inflammation was evaluated in hematoxylin and eosin (H&E)-stained sections using standard histological procedures, and scored absent (0), intermediate (1) to severe (2) by an experienced myopathologist.

3. Results

3.1. Increased CXCL10 and GDF15 levels in IIM sera

In individual patients and controls, levels of CXCL10 and GDF15 were determined in the same serum sample (supplementary Table S2). Statistical analysis was done with Kruskal Wallis one-way analysis of variance with Bonferroni correction for multiple tests (Figure 1A), and ROC analysis compared diagnostic performance (Figure 1B).

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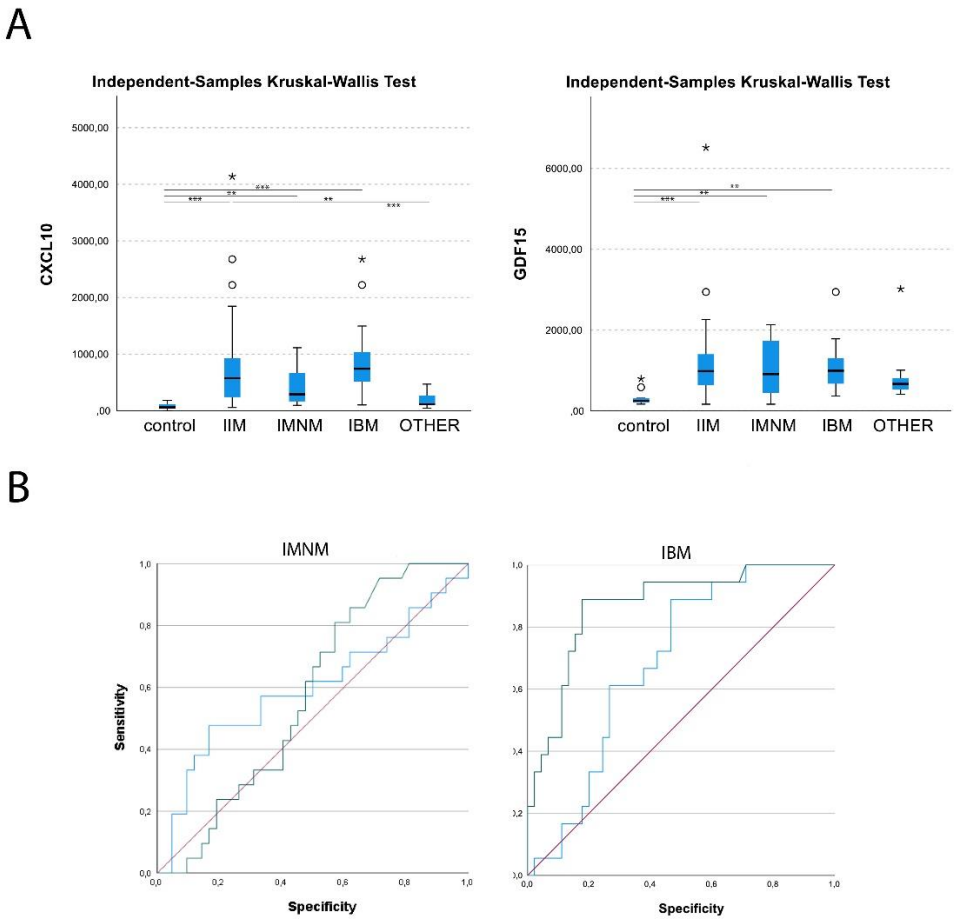

Figure 1. Statistical analysis of circulating levels of CXCL10 and GDF15 in myositis patients (A) Box plot of circulating levels of CXCL10 and GDF15 in controls, in idiopathic inflammatory myopathies combined (IIM), immune-mediated necrotizing myopathy (IMNM), sporadic inclusion body myositis (IBM) and patients with different hereditary muscle disorders (OTHER). Kruskal Wallis test by ranks for multiple groups of independent values, with Bonferroni correction for multiple tests determined significant differences: $p<0.05$*, $p<0.01$**, $p<0.001$***. (B) ROC analysis for CXCL10 (green) and GDF15 (blue) serum levels in IMNM and IBM patients, with reference line (red). Graphics were generated with SPSS software.

Mean circulating levels of CXCL10 were 79±53 pg/ml for healthy controls, 180±123 pg/ml for patients with hereditary muscle disorders and 755±783 pg/ml for IIM patients. In IMNM, values were increased 5.4-fold compared to healthy controls, and 2.4-fold compared to disease controls. In IBM, CXCL10 levels were increased further 11.7-fold compared to healthy and 5.2-fold compared to disease controls. Only weak correlations between CXCL10 serum levels and clinical characteristics could be observed (supplementary Table S3), yet at times in different directions. Weak negative correlation with BMI was observed in IIM, while in hereditary muscle disorders weak positive correlation was found (r=0.22). Weak positive correlation with cardiac disease was observed in hereditary muscle disorders (r=0.20), while weak negative correlation was present in IIM and IBM (r=-0.24).

Mean circulating GDF15 levels were 326±204 pg/ml for healthy controls, 831±656 pg/ml for patients with hereditary muscle disorders and 1201±1017 pg/ml for IIM patients. Values were comparably increased in subgroups to 3.2-fold (IMNM) and 3.4-fold (IBM) compared to healthy controls, and 1.3-fold compared to disease controls. GDF15 levels were moderately correlated with age at sampling in IMNM and OTHER (r=0.53) (supplementary Table S3). When the IIM were

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combined, the correlation with age was only weak (r=0.26). In IMNM, a weak correlation of GDF15 with blood CK values was noted (r=0.22). Weak correlation was observed with cardiac disease in the IIM (r=0.27) and its subgroup of IBM (r=0.36).

Levels of CXCL10 and GDF15 were not correlated in any of the sera from all diagnostic groups. ROC analysis found AUCs for CXCL10 were 0.573 for IMNM and 0.870 for IBM, and 0.879 for the whole group of IIM. With the threshold set to 180 pg/ml of CXCL10, myositis patients could be differentiated from healthy and disease controls with a sensitivity of 0.80 and a specificity of 0.71. For GDF15, AUC were 0.596 for IMNM and 0.688 for IBM, and 0.772 in IIM combined.

3.2. Localization of CXCL10 to muscle fibers and actively invading inflammatory cells

To allow evaluation of CXCL10 expression alongside pathological changes to the muscle tissue, immunohistochemical staining was performed in sequential muscle sections. Muscle biopsies with normal histology were largely CXCL10 negative. In contrast, subsets of small muscle fibers in IIM tissues displayed granular staining pattern in necrotic muscle fibers and in SQSTM1 positive muscle fibers (Figure 2A–D).

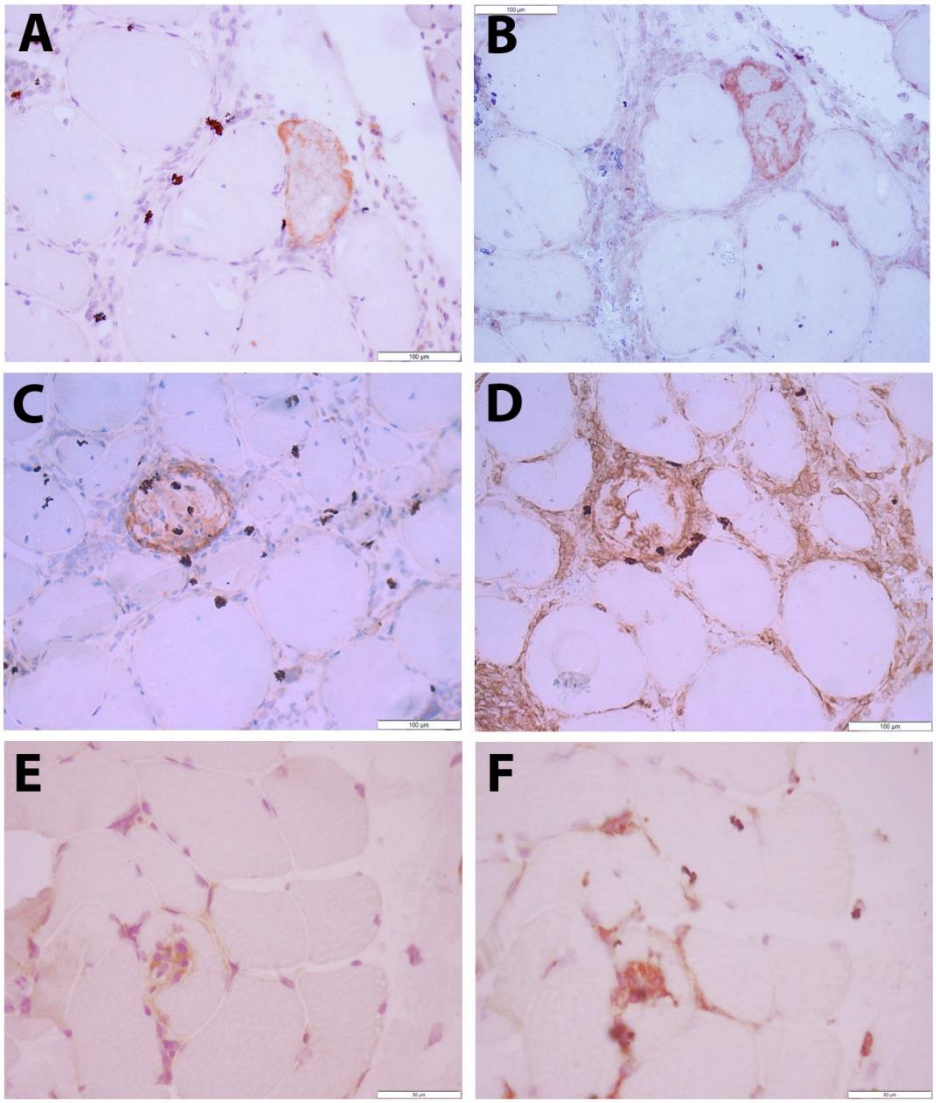

Figure 2. Immunolocalization of CXCL10 in skeletal muscle tissue (A-D) Immune-mediated necrotizing myopathy (IMNM21): A muscle fiber stains for CXCL10 (brown in A). In a sequential

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section, this fiber is shown to be SQSTM1 positive (brown in B). CXCL10 staining (brown in C) is observed in a necrotic muscle fiber. From a sequential section stained with macrophage marker CD68 (brown in D), it can be observed that invading and non-invading macrophages are mostly CXCL10 negative. (E-F) Sporadic inclusion body myositis (IBM15): Immune cells actively invading a nonnecrotic muscle fiber are partly CXCL10 positive (brown in E). A sequential section stained with macrophage marker CD68 (brown in F) shows partial colocalization. Scale bar=50μm.

The pattern of myopathological changes differed between IMNM and IBM patients (supplementary Table S4). IMNM was associated with muscle fiber necrosis and less severe inflammatory damage, while IBM was strongly associated with endomysial buildup of inflammation and active invasion of non-necrotic muscle fibers by auto-aggressive immune cells. In IBM tissues, a subset of inflammatory cells was CXCL10 positive, notably immune cells invading non-necrotic muscle fibers of which most were CD68 positive (Figure 2E,F). Severity of inflammatory changes in individual IIM patients tended to associate with circulating levels of CXCL10 (Figure 3), though no significance was shown in this smaller patient sample.

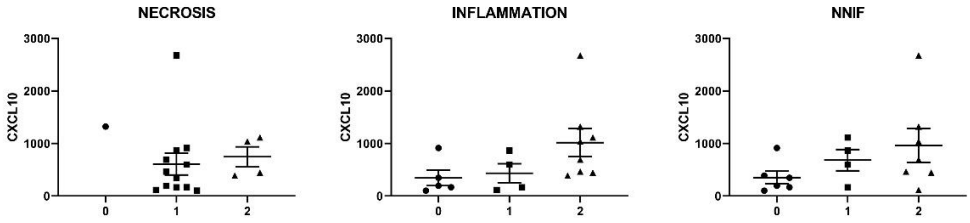

Figure 3. Relation between serum CXCL10 levels and scoring of myopathological changes in skeletal muscle tissues of a selection of IMNM (n=9) and IBM (n=8) patients CXCL10 values are given in pg/ml. Muscle fiber necrosis, buildup of intramuscular inflammation and presence of non-necrotic invaded muscle fibers (NNIF) were scored absent (0) intermediate (1) or severe (2). For detailed scoring results, consult supplementary Table S4.

3.3. Co-localization of GDF15 with markers of autophagy and regeneration in muscle fibers

The low constitutive sarcoplasmic GDF15 staining observed in healthy controls was notably increased in IIM muscle biopsies, mostly in small regenerating muscle fibers (Figure 4A–C). A granular staining pattern was observed in other subsets of muscle fibers, co-localizing with autophagic markers (Figure 4D–I). The vast majority of inflammatory cells were GDF15 negative (data not shown).

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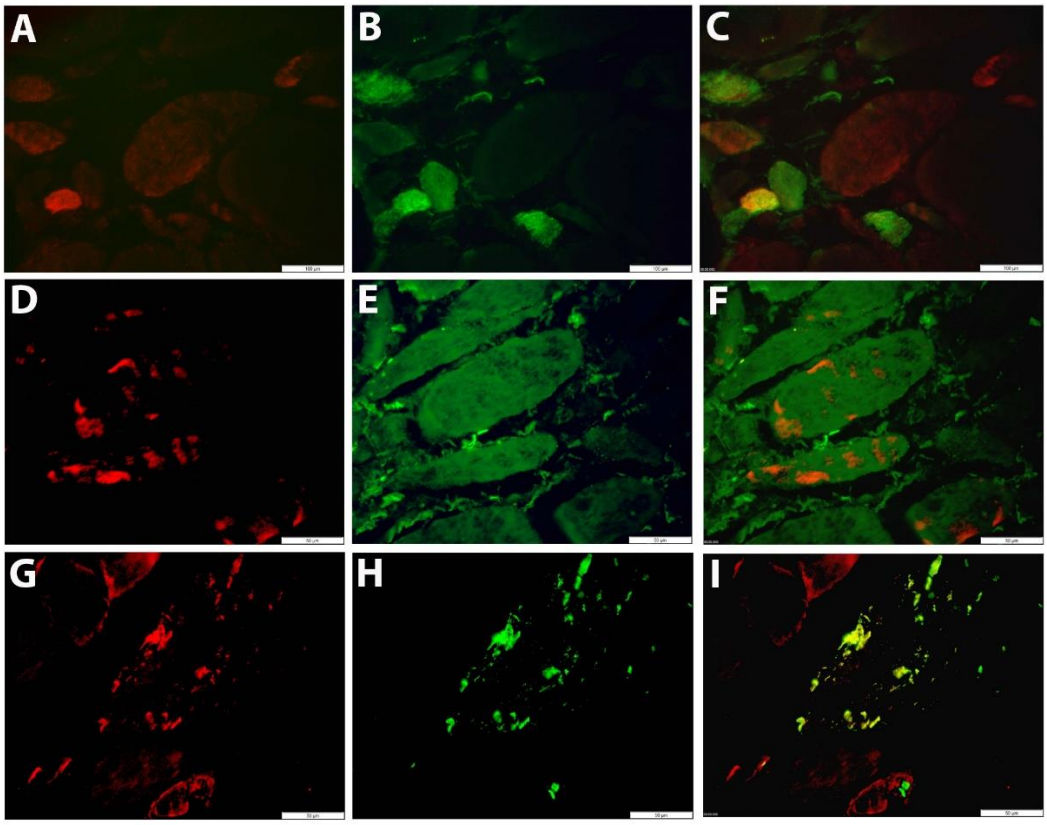

Figure 4. Fluorescent immunolocalization of GDF15 in skeletal muscle tissue (A-C) Immune-mediated necrotizing myopathy (IMNM17): A small fiber stains strongly for GDF15 (red in A), which is CD56 positive (green in B). The double stain (C) shows lower or absent expression in other regenerating muscle fibers. (D-I) Sporadic inclusion body myositis (IBM01): Granular GDF15 staining is observed in muscle fibers (red in D and G), co-localizing with LC3 (green in E) and SQSTM1 (green in H). Double staining shows GDF15 and SQSTM1 immunostaining overlaps in protein aggregates (yellow in I). Scale bar=50µm.

4. Discussion

Subtyping of IIM is a necessary effort to design treatment strategies suited to the individual patient. While subgroups of patients react well to standard immunosuppressive therapies, others might require alternative immunomodulatory strategies. In IMNM, autoantibody status aids as an indicator whether the response to different treatment regimen would be favorable [23]. IBM is largely unresponsive to current immunomodulatory treatment. In addition to subclassification, it is imperative to differentiate IIM from muscular dystrophies to avoid inappropriate treatment with glucocorticoids in the latter. Circulating biomarkers have been in use for diagnosing myositis for decades, with blood samples routinely taken to evaluate CK and other muscle enzymes. However, this strategy has certainly not yet been developed to its full potential. In this respect, implementing the analysis of the expression of key pathogenic factors in patient sera is an attractive prospect. A good choice would be to analyze myokines, i.e. cytokines and other proteins produced and released by muscle cells which enable the skeletal muscle tissue to communicate with the body's other organs, as indicators of muscle dysfunction [24].

Circulating CXCL10 has already been described a reliable and sensitive biomarker for IIM subgroups. In a study of 125 patients diagnosed with juvenile DM, serum CXCL10 levels displayed 0.87 sensitivity and 1.00 specificity for active disease [17]. Our current study confirmed the association with IIM, and indicates higher levels in the subgroup of IBM in comparison to IMNM. Though CXCL10 is present in muscle fibers and subset of inflammatory cells, it remains enigmatic if the muscle tissue is an important source of the chemokine, or if intramuscular inflammation is more a

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consequence of systemic CXCL10 expression. CXCL10 elevation as an indicator of muscle disease severity goes beyond the IIM. In systemic sclerosis also, serum CXCL10 levels strongly correlate with clinical severity of muscle involvement and with CK serum concentration, suggesting a potential mechanistic involvement in muscle damage [25].

No single diagnostic feature can differentiate IIM, let alone reliably subtype the different subgroups. A threshold of 180 pg/ml of CXCL10 differentiates myositis patients from healthy and disease controls with a sensitivity of 0.80 and a specificity of 0.71. Importantly, we showed that CXCL10 levels aid to differentiate IIM from hereditary muscle disorders, the latter often display secondary inflammatory changes that can be confused with myositis. We found CXCL10 levels in hereditary muscle disorders to be no different than in healthy controls, however, another study has reported CXCL10 to be significantly elevated in serum and muscle samples of DMD patients, relative to age-matched healthy controls [26]. We speculate that adding CXCL10 to the diagnostic toolkit might be useful, but might not be able to boost diagnostic performance sufficiently. We propose circulating CXCL10 could, however, be part of a bigger strategy for evaluating clever combinations of biomarkers. In this respect, our results appoint GDF15 consideration as an additional, more general marker for muscle disorders [27]. GDF15 is currently explored as a biomarker in many disorders including cardiovascular disease [28], cancer [29] and mitochondrial myopathy [30].

When considering novel circulating biomarkers, it is imperative to determine normal value variations in the healthy population. Many factor may influence serum levels, including gender, age and physical activity. It is known that the complex mixture of myokines secreted into the bloodstream varies during muscle contraction [31]. In this respect, GDF15 and CXCL10 seem to be somewhat opposite poles. While GDF15 gene expression is induced in muscle tissues of mice when exercised [32] and in response to oxidative stress [33], in contrast, treadmill running significantly reduced CXCL10 gene expression in mice soleus muscle [34]. Either way, circulating GDF15 and CXCL10 both appear regulated by physical activity. Nonetheless, CXCL10 levels have been observed to remain stable among healthy controls [26], while GDF15 values appear more prone to changes in humans. In pregnant women, blood levels rise rapidly and stay high during the whole pregnancy[35]. In addition, GDF15 levels associate with aging and tend to increase across the lifespan. Elevated GDF15 has been observed to correlate with reduced muscle strength and extremity function in older patients with cardiometabolic disease [36] and to associate with lower muscle mass in men specifically [37], the latter a further indication of sex differences. A limitation of our study is the age variation between diagnostic groups, with average ages of healthy controls (34±12) and patients with hereditary muscle disorders (45±13) substantially lower than of IMNM (65±9) and IBM (71±7) patients. In IMNM patients and the group of patients with hereditary muscle disorders we found a moderate correlation of GDF15 serum levels with age at sampling. An effort to determine values that can be used as reference ranges has been published recently [38], with most notable increases in the aging population associated with heart disease and diabetes. Another characteristic described to associate with elevated circulating GDF15 levels is obesity [39,40]. In our IIM cohort, 51% of patients were overweight of which 13.3% were obese (defined by a BMI over 30), yet we did not find a correlation between BMI and serum GDF15 levels.

We propose our study may contribute to patient-friendly diagnostic innovation. Further minimization and multiplex immunoassays could allow expansion and analysis of combinations of biomakers. In this respect, blood spot analysis could be put forward as a convenient approach, as sampling can be done by nontrained persons and the material can be stored and transported at ambient temperature Studies evaluating spotted TNFα confirmed this methodology can detect cytokine concentrations commonly observed in patient samples, which range from 5 to 27pg/ml [41]. For CXCL10, high correlation of blood spot analysis with serum levels (r=0.96) have already been described [17]. Another innovation could be to attempt the least invasive sample collection available, which is to analyze a urine sample. The urine proteome as a possible source of biomarkers has been explored for the juvenile form of DM [42]. In chronic kidney disease, urine GDF15 levels have already been shown predictors of mortality with an AUC of 0.95 [43].

In addition to the diagnostic purposes of biomarker studies, serum biomarkers can be useful as follow-up therapeutic markers in clinical trials, with comparison of levels pre- and post-treatment as exploratory outcome measures in individual patients. Additionally, biomarker studies advance our understanding of pathogenic changes in IIM patients and may identify novel therapeutic targets. Targeted modulation of myokines involved in the immunopathological processes triggered by the immune system, aggravating or ameliorating inflammatory muscle disease, may become important therapeutic targets in their own right as an appropriate personalized therapeutic strategy [10]. Myokines evolving from biomarkers to therapeutic targets have been proposed for cancer cachexia [44].

5. Conclusions

Our study found significant elevation of serum CXCL10 and GDF15 levels in myositis patients. The skeletal muscle tissue is one of the possible sources, with localization to subsets of affected muscle fibers and inflammatory cells. CXCL10 expression was notably high in immune cells invading non-necrotic muscle fibers and correlated with muscle tissue inflammation grade. We propose circulating CXCL10 and GDF15 levels could be of aid to diagnose myositis. If our findings were to be confirmed, GDF15 could be developed into a more general biomarker for muscle disease and CXCL10 levels as an indicator toward IIM subtypes characterized by severe muscle inflammation and active invasion of muscle fibers by auto-aggressive immune cells. Further implementation of circulating biomarkers might reduce the need for taking a diagnostic muscle biopsy further, at least in part of the patients.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Healthy and disease controls; Table S2: Quantification of circulating CXCL10 and GDF15 in human sera using enzyme-linked immuno sorbent assays; Table S3: Pearson's correlation coefficients between variables; Table S4: Scoring myopathological changes in muscle biopsies from a selection of patients.

Author Contributions: Conceptualization, B.D.P.; methodology, B.D.P, K.B., J.D.B.; validation, B.D.P.; formal analysis, B.D.P.; investigation, B.D.P., J.D.B.; resources, K.B., J.D.B.; data curation, J.D.B.; writing—original draft preparation, B.D.P.; writing—review and editing, B.D.P., K.B., J.D.B.; visualization, B.D.P.; supervision, J.D.B.; project administration, B.D.P; funding acquisition, B.D.P., K.B., J.D.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Ghent University Hospital, protocol codes B670201836756 (2018-0820) and B670201938779 (2019-0046), dates of approval November 16th 2018 and April 1st 2019.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patient(s) to publish this paper.

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Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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