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Review

The Possible Impact of COVID-19 on Respiratory Muscles Structure and Functions: A Literature Review

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Abstract: The impact of SARS-CoV-2 infection on respiratory muscle functions is an important area of recent enquiry. COVID-19 has effects on the respiratory muscles. The diaphragm muscle is perturbed indirectly due to the mechanical-ventilation-induced-disuse, but also by direct mechanisms linked with SARS-CoV-2 viral infection. In this sense, a deeper understanding of the possible links between COVID-19 and alterations in structure and functions of the respiratory muscles may increase the success rate of preventive and supportive strategies. Ultrasound imaging alongside respiratory muscle strength tests and pulmonary function assessment are valid approaches to the screening and monitoring of disease, for mild to severe patients. The aim of the present review is to highlight the current literature regarding the links between COVID-19 and respiratory muscle functions. We examine from the pathophysiological aspects of disease, up to approaches taken to monitor and rehabilitate diseased muscle. We hope this work will add to a greater understanding of the pathophysiology and disease management of respiratory muscle pathology subsequent to SARS-CoV-2 infection.

Keywords: diaphragm muscle; respiratory functions; COVID-19; rehabilitation; SARS-CoV-2

Citation: Cesanelli, L.; Satkunskiene, D.; Bileviciute-Ljungar, I.; Kubilius, R.; Repečkaite, G.; Cesanelli, F.; Iovane, A.; Messina, G. The Possible Impact of COVID-19 on Respiratory Muscles Structure and Functions: A Literature Review. *Sustainability* **2022**, *14*, 7446. https://doi.org/10.3390/su14127446

Academic Editor: Gianpiero Greco

Received: 1 May 2022
Accepted: 14 June 2022
Published: 17 June 2022

1. Introduction

The coronavirus disease 2019 (COVID-19) was firstly reported and traced in December 2019 in the city of Wuhan, China, as a cluster of novel human pneumonia cases were spreading [1,2]. Following this initial incident, SARS-CoV-2 infections became globally widespread, and the scientific community undertook a huge effort to characterize the epidemiological and pathobiological features of COVID-19 [3]. The subsequent study of alterations in structure and function of respiratory muscles (especially the diaphragm), in patients with SARS-CoV-2 infection became a prominent focus of research. This is reflected in a simple search of the PubMed MEDLINE® database, where more than 80 studies may be found when running a search using the following keywords: (COVID-19 [Title/Abstract] AND (Respiratory muscles [Title/Abstract] OR Diaphragm [Title/Abstract])).

The effects of SARS-CoV-2 infection and prolonged mechanical ventilation in ICU (intensive care unit) represent a potential detrimental combination for the respiratory

muscles' health of infected patients [4–6]. Indeed, mechanical ventilation partially or completely unloads the respiratory muscles, as well as altering the respiratory control centers in the brain stem. Among the respiratory muscles, the diaphragm is impacted the greatest. One study found for example that weakness, reduced motion, changes to thickness (and/or thickness fraction), and its capacity to generate pressures in response to phrenic nerves stimulation, were among the complications reported, occurring after less than 4 days in the ICU [4].

Although the cellular mechanisms are not completely understood, such changes seem to be the result of diaphragm atrophy and reduced contractile functions, triggered by muscle inactivity. Diaphragm inactivity under mechanical ventilation has been associated with excessive reactive oxygen species production, caspase-3 expression and apoptotic processes activation, and upregulation of mRNAs coding for ligands related to the proteolytic ubiquitin-proteasome pathway [4]. Furthermore, age seems to play a role in determining the alterations in diaphragm muscle due to mechanical ventilation. For example, Lyu and collaborators [7] observed how the changes in diaphragm muscle of young rats differed compared to older rats when exposed to ICU conditions (5 days of complete immobilization and mechanical ventilation). The authors found that in the elderly cohort of rats, biological processes linked to inflammation were significantly upregulated, suggesting an age-related molecular alteration in the diaphragm muscle in response to inactivity [7]. Other studies [8,9] have reported altered gene expression and structure of the diaphragm from the analysis of muscle specimens obtained from autopsies of critically ill patients who died of COVID-19, compared to that of critically ill patients without COVID-19. Additionally, Shi and colleagues [9] provided evidence for angiotensin-converting enzyme 2 (ACE-2) expression in the diaphragm (predominantly localized at the myofiber membrane), and of SARS-CoV-2 viral infiltration in the diaphragm specimens of COVID-19 ICU patients, further linked with increased expression of genes involved in fibrosis.

In this regard, it has been established that SARS-CoV-2 uses the ACE-2 enzyme as a functional receptor, through which the virus induces acute respiratory distress syndrome [4]. As a membrane protein, ACE-2 has been identified in the diaphragm [9], but also in other muscles [10]. As the largest organ of the human body, skeletal muscle has the ability to produce and release a wide number of cytokines, known as myokines, in response to perturbed physiological conditions, acting in an endocrine, paracrine, or autocrine manner and contributing to immune response initiation and/or perseverance [11]. Mittal and collaborators [5] have suggested that the cytokine storm following the SARS-CoV-2 infection enters the circulatory system, interacts with skeletal muscle, and may cause damage to the diaphragm and other respiratory muscles [5]. This may lead to muscle dysfunction, atrophy, as well as injury, as reported by evidence, both directly and indirectly [5].

The respiratory muscles, and in particular the diaphragm muscle, seem to suffer profoundly from severe COVID-19, and the detrimental impact may arise indirectly from the muscle inactivity induced by mechanical ventilation in ICU conditions, but also by direct mechanisms linked with SARS-CoV-2 viral infection (Figure 1). Due to the fact that muscle perturbation may arise from both direct and indirect mechanisms, screening and monitoring, as well as supporting critically ill patients who suffer from respiratory muscle impairment with COVID-19 has become of essential importance for clinicians.

Therefore, the aim of the present review is to highlight the current literature regarding the links between COVID-19 and respiratory muscle functions, starting from the pathophysiological aspects described above, and also highlighting how such pathology may be monitored for, as well as examining rehabilitative approaches. In order to achieve this, we searched published articles focusing on the possible links between COVID-19 and respiratory muscle structure and function alterations. We used the PubMed/MEDLINE® and Web of Science databases, supplemented by additional external sources, such as using references from selected papers. We then used the results of our search to extract the main parameters that formed a framework for the structure of our literature review.

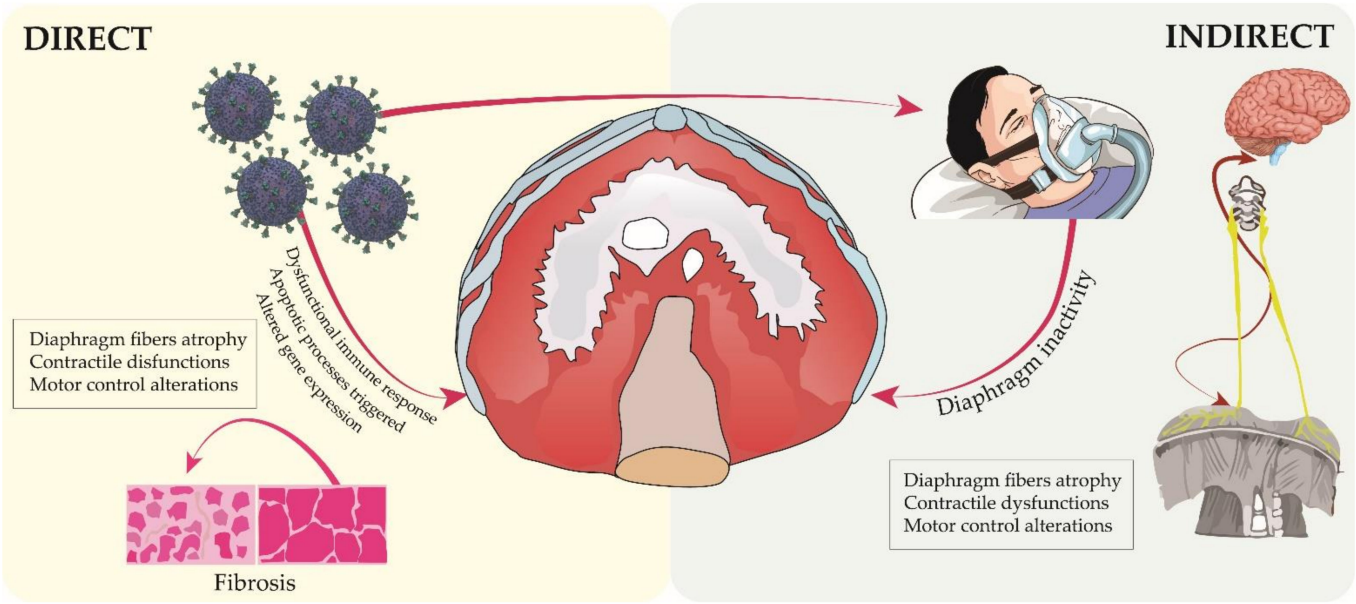

Figure 1. Graphical representation of the direct and indirect influence of SARS-CoV-2 infection on diaphragm structure and functions. Viral infiltrations and infection (direct mechanism) may trigger a dysfunctional immune response and myokines release from diaphragm and adjacent muscles leading to a rise in localized inflammation. The viral infection may further induce alterations in gene expression, with upregulation of genes involved in tissue fibrosis. Further, apoptotic mechanisms are upregulated, culminating in an altered diaphragmatic structure. Additionally, severe COVID-19 may lead to mechanical ventilation (indirect mechanism) triggering all the dysfunctional processes associated with muscle inactivity. This leads to further alterations to diaphragm structure and function.

2. Ultrasound Imaging of the Respiratory Muscles in COVID-19 Patients

2.1. Ultrasound Imaging Techniques

Considering the respiratory muscles, and in particular the diaphragm, the application of ultrasound imaging (US) techniques to assess muscular dysfunction, morphological changes and/or responses to rehabilitation, are a valid strategy [12–29]. Two main ultrasound approaches to image the diaphragm have been consistently reported in the literature: the mid-axillary intercostal approach at the zone of apposition, and the subcostal approach using the liver or spleen as an acoustic window [28,30] (Figure 2). Diaphragm thickness (measured perpendicular to its fiber direction between the pleural and peritoneal membrane) and thickening fraction [calculated from B-mode or M-mode images and as the percentage inspiratory increase in diaphragm thickness relative to end-expiratory thickness during tidal breaths (TFdi) or maximal inspiratory efforts (TFdimax)], have been described as reliable indicators of diaphragm muscle morphology and functions [28,30].

Using the subcostal approach, it is possible to measure diaphragmatic excursion in M-mode US settings, as during the inspiration the diaphragm should move toward the probe and its excursion quantified by the M-mode [28,30]. This is indicated during a maximum inspiratory effort by the patient and from unassisted breathing only, since active contraction of the diaphragm cannot be distinguished from passive displacement due to the ventilator inspiratory pressures [28,30]. Other muscles have also been imaged using US, which allows for further understanding of the functioning and health of respiratory muscles [30]. These muscles include: the parasternal intercostals, rectus abdominis, transversus abdominis, and the external and internal obliques.

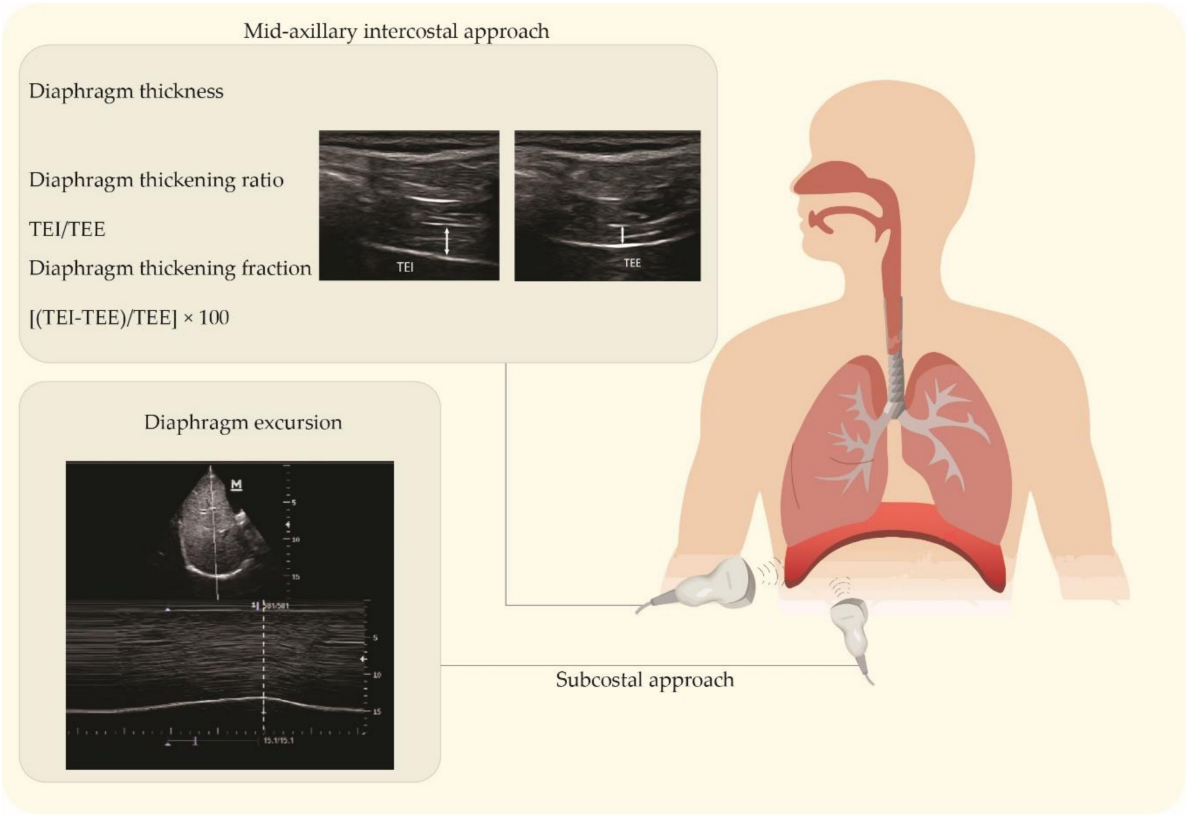

Figure 2. Graphical representation of the mid-axillary intercostal approach, and subcostal approach to image diaphragm muscle through ultrasonography. Notes: TEE: thickness at end expiration; TEI: thickness at end inspiration.

2.2. COVID-19 Context

There are a range of studies on the respiratory muscles of COVID-19 patients that have used the techniques described to indicate severity of pathology. Farr and colleagues [17] observed a B-mode sonographic abnormality (76%) of the diaphragm muscle (reduction of the thickening ratio) in a cohort of severe post-COVID-19 survivors, compared to non-infected control subjects. Pivetta and collaborators [23] reported the application of diaphragm ultrasonography as a feasible and reliable approach in order to measure diaphragmatic motion and thickness in COVID-19 patients, to monitor the impact of the infection on respiratory functions and respiratory muscle health. In a case report, van Steveninck and Imming [13] observed the diaphragm dysfunction (reduced diaphragm thickness and thickening fraction) in a severe COVID-19 patient prior to intubation was observed, with respiratory failure not yet evident. After two days of controlled mechanical ventilation the authors reported recovery of diaphragm contractility, switching the patient to a pressure support mode [13]. Therefore, US imaging of diaphragm muscle allowed the assessment of diaphragm dysfunction in a spontaneously breathing severe COVID-19 patient and supported the decision making through phase-by-phase monitoring and in order to enhance patient recovery.

Further studies also highlight the impact of COVID-19 on diaphragm dysfunction and disease. Corradi and colleagues [16] described patients who developed adverse outcomes from COVID-19 infection having thinner diaphragms compared to those who did not have complications. The authors also reported that diaphragm thickness represented a significant predictor of COVID-19 adverse outcomes, especially end-expiratory diaphragm thickness). Similarly, Helmy and co-workers [14] observed how diaphragmatic excursion, evaluated within 12 h after ICU admission, can accurately predict the need for ventilatory support and mortality in patients with severe COVID-19. Interestingly, Umbrello and colleagues [26]

observed significant decreases in the sizes of both diaphragm and rectus femoris muscles, at a time of 7 days from ICU admission in COVID-19 patients. The reduction was significantly greater in non-survivors compared to survivors [26]. In addition, the authors observed an increase in both diaphragm and rectus femoris echo intensity (a potential index of "muscle quality", see [31]), especially in non-survivors. Such changes were additionally influenced by nutritional (cumulative protein deficit) and hydration (cumulative fluid balance) management strategies. Similar data have also been reported in a study by Formenti and collaborators [27], in which the US images of the diaphragm, parasternal intercostal, and rectus femoris muscles of patients with acute respiratory distress syndrome arising from COVID-19, were collected at the admission of the ICU. Survivors showed a significantly lower echogenicity compared to non-survivors in all of the diaphragm, parasternal intercostal, and rectus femoris muscles.

The ultrasonography of respiratory muscles, and of the diaphragm, has also been used to evaluate the differences between the awake prone position compared to the supine position as a non-invasive ventilation setting rescue therapy [15]. It has been observed that despite peripheral oxygen saturation improvement, turning from the supine to prone position can induce discomfort and increase in diaphragmatic thickening fraction of COVID-19 critically ill patients [15]. Satici and co-workers [25] proposed a joint approach through electromyographic and sonographic assessment of the diaphragm muscle dysfunction in patients recovering from COVID-19. The authors observed both compatible outcomes from US and electromyographic measures (i.e., reductions in diaphragm excursion accompanied by altered distal motor latency and more in general altered electromyographic activity, after phrenic nerve stimulation) [25]. An alteration in electrophysiologic muscle activity despite a normal diaphragm excursion was also reported [25].

Taken together, the US morphological assessment of respiratory muscles, in particular the diaphragm muscle, has been revealed to be a feasible and valid technique in the evaluation of muscular dysfunctions, which might support decision making in the different phases of treatment of patients affected by COVID-19, and with applications ranging from mild to severe cases. Reduction in diaphragm thickness and thickening ratio (or fraction) is associated with the severity of the disease, as indicated by several studies [13,14,16,17]. Additionally, worsening of these morpho-functional variables has been associated with a deterioration in patient conditions [26,27].

Alterations in motor control of the diaphragm have also been reported in COVID-19 patients, in the absence of morphology changes [25]. Additional approaches may potentially bring additional insights and facilitate decision making and monitoring of the patient. These approaches may include: imaging of the phrenic nerves to identify possible nerve pathologies prior to end-organ damages (in this case prior to the detection of the diaphragm muscle atrophy or dysfunction) [28], or application of US elastographic techniques (i.e., shear wave and/or strain elastography) [28,32], although not widely reported in the context of COVID-19 currently. Animal experiments have already suggested how structural changes in diaphragm muscle are linked to changes in its mechanical properties and how this may further result in altered respiratory functions [33]. In the context of COVID-19, and given SARS-CoV-2 may directly impact diaphragm structural changes (e.g., tissue fibrosis [8,9]), non-invasive evaluations of diaphragm mechanical properties (e.g., US elastography) may provide additional insights into disease etiology and related muscle functioning [32].

3. Mechanical Properties of the Respiratory Muscles and Its Potential Significance in COVID-19 Patients

The mechanical properties of skeletal muscle have significant functional significance, as both basic and complex movements and exercises are dependent on them [34]. These actions occur through correct range of motion at a minimal metabolic cost, and have a perceived effort and low risk of injury [34]. Therefore, healthy muscles are essential for executing a range of functional activities. Correct muscle functioning is particularly

important in the respiratory muscle context, where stiffness changes of the diaphragm may have profound changes on the work of breathing. This is caused by reductions in the muscle mobility, and changes in breathing patterns, or by placing the muscle at a mechanical disadvantage (i.e., an overstretched diaphragm), leading to decreased inspiratory strength and efficiency [35,36].

The mechanical properties of skeletal muscles are profoundly connected with the composite anatomical structures which form them. Intramuscular connective tissue, organized in three levels (epimysium, surrounding the whole muscle; perimysium, surrounding the individual fascicles; and endomysium, surrounding individual muscle fibers) and its conformation, represent a primary determinant of mechanical properties [37–40]. Intramuscular (collagen but also adipose tissue) and sarcolemmal connective tissue contribute to the mechanical properties of relaxed and active muscle [37,40,41]. Additionally, intrinsic components of the muscle cells, or parts of the muscle cells (e.g., sarcolemma, myofilaments interactions, intracellular proteins such as connectin, sarcoplasmic reticulum) [37], as well as fiber type composition of the muscle [42,43], may determine the mechanical properties of relaxed and active muscles.

There are a few studies that demonstrate how either diaphragm denervation or diet induced obesity in rats have produced a strong morphological remodeling with increased deposition of connective tissue, alteration of the muscle contractile properties and breathing patterns [33,44,45]. An additional primary determinant of skeletal muscle mechanical properties is the sarcomeric giant protein titin, and its interactions with external stimuli, that may consequently induce changes on its mechanical properties. Indeed, it has been suggested how titin stiffness can be modulated, among different other mechanisms [46] by oxidative stress-induced modification. In this sense, two oxidative mechanisms have been reported that can alter muscle cell mechanical properties, impacting the sarcomere protein functions, and in particular titin.

The first mechanism that can alter muscle cell properties is S-glutathiolation of cysteines that become exposed when an immunoglobulin (Ig) domain of titin unfolds, and with glutathione forming a mixed disulfide with titin, blocking the refolding of the Ig domain [46]. The second mechanism involves oxidant-induced formation of disulfide bridges within the mechanically unfolded titin Ig domain, reducing its length and consequently the titin-based spring stiffness [46]. Other studies suggest how stress conditions or inherited myopathies may trigger hyper- or hypo- phosphorylation of titin regions of skeletal muscle sarcomeres, enhancing proteolytic degradation (contributing to muscle atrophy) or changes in titin-based spring force, including diaphragm muscle [46,47].

Despite the importance of such observations, there have been few studies currently that have investigated whether muscle stretching under oxidative stress (e.g., COVID-19) may potentially increase Ig domain oxidation and alter the sarcomere mechanical properties. Considering this, the degenerative [8,9] and inflammatory [5] processes affecting the respiratory muscles and resulting from COVID-19, may induce alterations also to mechanical properties. Moreover, the possible links between changes to respiratory muscles as a result of COVID-19, and functional implications, have not been explored.

3.1. Historical Studies

Research interest in respiratory muscles, in particular the diaphragm, increased from the second half of the 20th century (e.g., [48–51]). We have summarised pertinent findings from the literature in Figure 4, and this summary is used as a basis for discussion herein. Studies have covered a wide range of aspects linked to morphological analyses [33,44,45,52–57], passive and active mechanical [52,56,58–67] and contractile [33,45,49–52,54,62,68–71] properties characterization, in response to different conditions and diseases. Due to the difficulties in analyzing in vivo the structural aspects of the respiratory muscles, most evidence has come from animal models. However, advances in imaging techniques, and in particular ultrasonography, have allowed for deeper early assessments of diaphragm structure-function relationships in humans [72]. Recent studies

have indicated that elastographic techniques are valid, non-invasive methodologies that can be used to assess mechanical properties of the diaphragm [67,73].

In 1986, Bellemare and colleagues [68] described the human diaphragm contractile properties as intermediate, between those of fast- and slow-twitch muscle fibers. This was discovered indirectly by analyzing the changes in transdiaphragmatic pressure in response to phrenic nerve stimulations [68]. These observations were confirmed by direct evidence through the work of Eddinger and Moss [74] who examined the fiber types from skinned rat diaphragm fibers.

Regarding the mechanical properties of respiratory muscles, Kelly and collaborators [60], in 1993, observed an age-dependent change in passive mechanical properties. They found that diaphragm muscles of adult rats (6–12 months) were more compliant than that that of young (3 months) or old (24 months) rats. In contrast, the intercostal muscles from old rats were stiffer than those of the younger or adult rats. In the same year, Müller-Felber and co-workers [72] pioneered the application of combined B- and M-mode ultrasonography and phrenic nerve magnetic stimulation, to study diaphragm functions, non-invasively.

In 1994, Farkas and colleagues [52] observed how diaphragm muscle in obese Zucker rats underwent structural remodeling with an increase in type I and type IIa muscle fibers and a consequently greater muscle thickness, describing it as a strategy to cope with the additional mass loading resulting from obesity. In contrast, Ameredes and co-workers [53] reported in 1998 how chronic undernutrition induced a decrease in type I (slow) and IIa myosin heavy chain profile, but an increase in type IIB and IIX (fast) in Fischer rats. The authors also found a concomitant increase in fatiguability and a disproportional increase in passive tension during fatigue [53]. Later studies reported also how undernutrition was able to deform the structure of the diaphragm and intercostal muscles, causing perturbations such as: atrophy, disarrangement of the myofibrils, and deposition of collagen type I fibers) [75].

In summary, the observations made in the second part of the 20th century suggested how the diaphragm may relatively "easily" adapt towards a slower or faster myosin heavy chain profile and undergo structural remodeling, in response to different stimuli [76]. Boriek and colleagues [56] described in 2001 the role of desmin, and thus extra-myofilament cytoskeletal structures, in determining the mechanical (i.e., stiffness and viscoelasticity) and contractile properties of the diaphragm muscle. In 2003, Jannapureddy and collaborators [62] reported similar findings by studying merosin, an extracellular matrix (ECM) protein, forming a mechanical junction between sarcolemma and collagen] -deficient mice diaphragm muscles. Furthermore, Patel and co-workers [77] investigated deficiency of α-sarcoglycan, while in 2005 Lopez and colleagues [78] described altered mechanical and contractile properties in $\alpha 7\beta 1$ integrin (transmembrane structural and receptor protein of skeletal muscle) -deficient mice diaphragm muscle. The relevance of such findings relies on the fact that diaphragm muscle fibers are exposed to biaxial loading (transverse and longitudinal loading) during each respiratory cycle, and thus, a different length-tension relationship is expected compared to a fiber loaded uniaxially [63,79]. In addition, cytoskeletal and transmembrane proteins play a role in mediating transverse loading.

Indeed, compared to other muscles such as the biceps femoris, when the diaphragm muscle has been exposed to longitudinal, and later transverse load (mechanical stretch), the latter has increased passive tension, suggesting an "easier" extensibility in the longitudinal direction (along the muscle fibers) compared to transverse [80]. Therefore, the experiments on mutant mice null proteins responsible for mechanical properties has allowed for a deeper understanding of the role of extra-myofilament cytoskeletal structures that are part of the costamers such as desmin-, integrins-, and the dystrophin- associated glycoprotein complex (Figure 3).

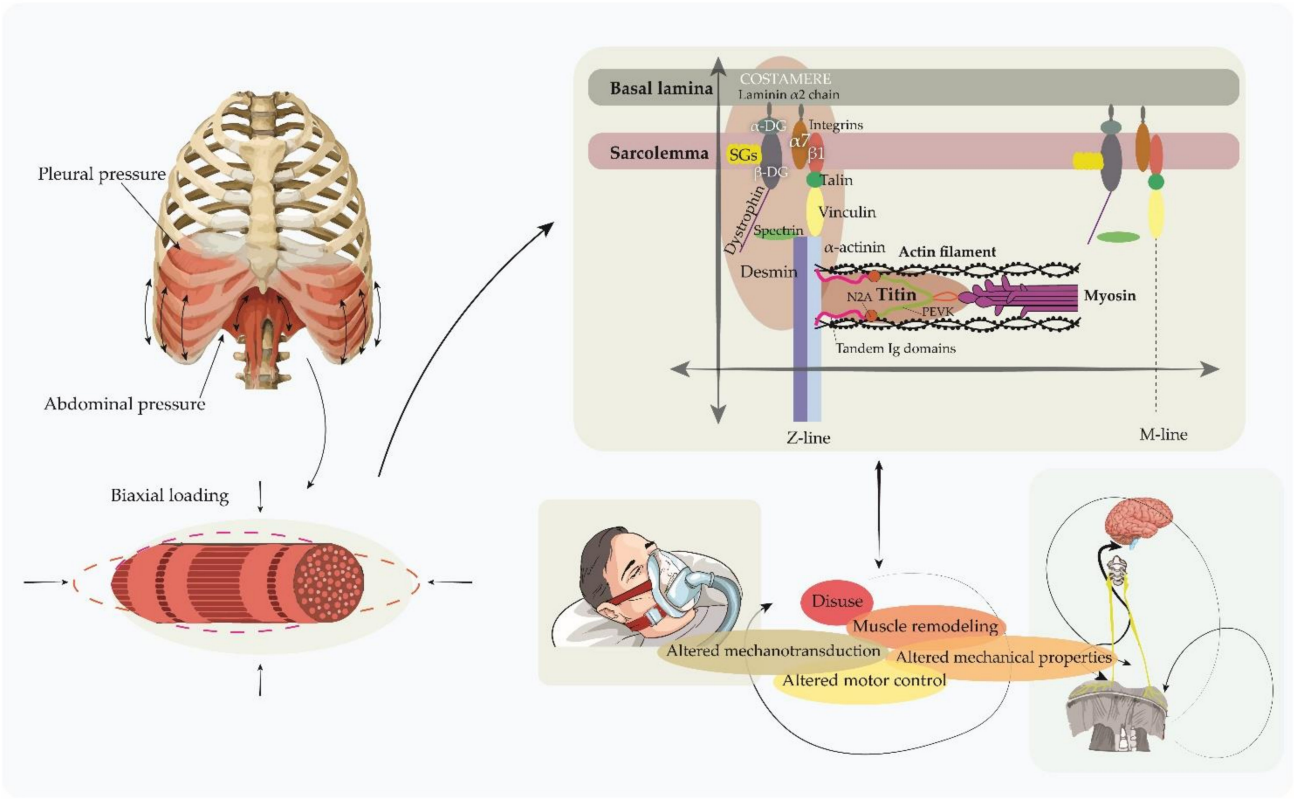

Figure 3. Diaphragm muscle mechanical properties functional implications: an overview. In the lung-apposed region of the diaphragm, the difference between abdominal pressure and pleural pressure results in an orthogonal pressure load to the surface of the diaphragm. In contrast to most of skeletal muscles that act by exerting an axial force, the diaphragm acts through a combination of muscle tension and curvature, to balance a pressure load in a direction perpendicular to the axis of the muscle, and the muscle fibers axial tension. Changes in the muscle composition due to tissue remodeling, and in response to disease (e.g., forced disuse during mechanical ventilation or direct oxidative stressors) or different external stimuli (e.g., training), may determine alterations in the behavior in one or both axes. The mechanotransduction response and signaling cascades may also be disrupted. This may cause a potential remodeling loop. Notes: SGs, Sarcoglycans; β-DG, beta-distroglycan; α-DG: alpha-distroglycan.

Some studies further highlight the role of costameric proteins. Across some studies, desmin intermediate filament [56] and α-sarcoglycan [77] deficiency have led to an increased diaphragm passive transverse extensibility, while in contrast, deficiency of α7-integrin [78] has resulted in decreased extensibility and contractile force production. The common feature among the three studies described is a disruption in the coupling of the passive transverse elements and contractile longitudinal element, due to deficiency in costameric proteins. In addition, the previously discussed findings from Jannapureddy and co-workers [62] demonstrated that merosin deficient diaphragm maintained the coupling between transverse and axial elements. In summary, costameric proteins seem to be responsible, at least partially, for transmitting axial contractile forces in the transverse direction to muscle fibers of the diaphragm.

Regarding axial sarcomeric elements, Lopez and colleagues described, in 2008 [81] how a titin protein mutation led to alterations in the morphological, contractile and passive mechanical properties of mouse diaphragm muscle. In the same year, Pardo and collaborators [82] evaluated the effects of diaphragm stretch on forkhead box class O (FOXO) protein isoforms, in normal mice of different ages. The authors observed a downregulation of FOXO DNA-binding activity triggered by muscle stretch in young mice but not in old

ones, and that alterations in the regulation of FOXO were linked to changes in the muscle mechanical properties. Such findings suggested age-related changes in diaphragm mechanical properties, linked to an altered regulation of the stretch-induced signaling pathways controlling FOXO activities.

An additional study published in 2010 by Rowe and collaborators [65] focused on the role of ECM alterations on diaphragm muscle passive mechanical properties. The authors induced collagen digestion through muscle immersions in clostridium histolyticum derived bacterial collagenase. The authors revealed linear relationships between passive muscle stiffness and collagen digestion level and associated ECM degradation, revealed by histological analysis that examined disruptions to epimysial tissue [65]. Taken together, studies performed in the first decade of the 21st century focused not only on the impact of sarcolemmal, but also cytoskeletal and ECM structures, on diaphragm and/or other respiratory muscles mechanical properties and contractility. The results seem to suggest a significant role of both titin and ECM components, as previously demonstrated by studies in other muscles or muscle groups [37], but with the biaxial loading to which diaphragm fibers are being exposed, representing an additional explanation of the possible role of the two components.

In the beginning of the second decade of the 21st century, a literature review by Klimathianaki and colleagues [83] described the different determinants "from muscle to cell" of chronic obstructive pulmonary diseases (COPD). Among other key aspects, the review highlighted the impact of COPD on the passive and contractile mechanical properties as alterations in titin structure, reduced myosin content and Ca++ sensitivity linked to oxidative status (e.g., rise in ROS concentration) and fiber type switch. Phrenic nerve denervation determined, after only 4 weeks, profound alterations in diaphragm muscle morphology [44], indicating how relatively short-term diaphragm muscle disuse may result in significant non-functional remodeling.

Departing from previous evidence, Smith and Barton [66] studied mice using an mdx model, which emulates conditions of Duchenne muscular dystrophy. The authors found that collagen content and organization correlated strongly with active force generation, but weakly with diaphragm muscle passive stiffness [66]. However, there was greater content and altered organization observed in mdx mice [66]. Greising and colleagues [84] and Tallis and collaborators [71] confirmed the role of ageing in decreasing mechanical integrity of diaphragm muscle tissue, caused mainly by the onset and progression of tissue fibrosis, not being solely related to a shift in fiber type.

More recently, Buras and colleagues demonstrated in a mice model study that the contractile properties of the diaphragm may be changed through increased adipose tissue and collagen that arises due to induced obesity [33]. Intra-diaphragmatic fibro adipogenic progenitor cells revealed to proliferate with long term high fat diet. Additionally, they represented the primary source of intramuscular adipocytes and collagen deposition, under the upregulation of thrombospondin 1 (a circulating adipokine) and depicting an obesity-associated diaphragm muscle remodeling path, with fibroadipogenic progenitor cells as the main mediators. Sahani [85] described in mdx mice also, ECM remodeling through collagen fibers structure and spatial conformation changes, in relation to muscle fibers). The authors predicted the diaphragm muscle mechanical behavior through finite element model (FEM) simulation and described alterations in the mechanical properties as age- and disease- dependent [85]. Pardo and co-workers [80] reviewed the biochemical pathways involved in remodeling of diaphragm mechanical properties, describing the importance of alterations of diaphragm mechanical properties also in terms of mechanotransduction disruption or abnormalities, and its consequences on respiratory functions. These studies are all important in enhancing our understanding of pathogenic changes to the mechanical properties of the diaphragm.

3.2. Recent Research

Recent research has applied ultrasound elastography techniques to evaluate human mechanical properties non-invasively [67]. Although the evidence is still in its early phases, such techniques may help our understanding of diaphragm mechanical properties and its functional importance, non-invasively and covering different field of application— for example, from looking at pathological muscle to assessing athletic performance. Taken together, alterations in diaphragm mechanical properties seem to occur as the consequence of remodeling processes associated with different conditions, including different diseases. This may occur through either, or both direct and indirect mechanisms. However, for some aspects the evidence is still in its early phases, and results from different studies have reported discordant findings. What seems to be clear is that the diaphragm, among the respiratory muscles, is highly responsive to changes to use or disuse, and alterations to its structure may have profound implications on respiratory functions. Indeed, such alterations may directly compromise respiration, but also alter the regulation of signaling pathways involved in mediating the response to mechanical load.

In other words, the impairment in mechanotransduction may arise from alterations in the biophysical process by which cells sense their physical environment through translating physical forces and deformations in biochemical signals. Together, this may represent a non-functional loop, in which a lack of change in mechanical load to the diaphragm (e.g., disuse) may trigger non-functional remodeling processes and drop of motor control, culminating in a different structure, altered mechanical properties and reduced mobility of the muscle. The same alteration may disrupt the physiological biaxial loading and consequently downregulate the mechanotransduction pathways arising from transverse and axial loading, fueling again the non-functional remodeling processes, and closing the loop (Figure 3). Therefore, in the context of COVID-19 pathophysiology, analysis and monitoring of the diaphragm and respiratory muscle changes (to motor control, morphology and mechanical properties) may help further understand disease. In addition, this can help in supporting decision making in both the acute phase (mechanical ventilation) and rehabilitation phase for patients (Figure 4).

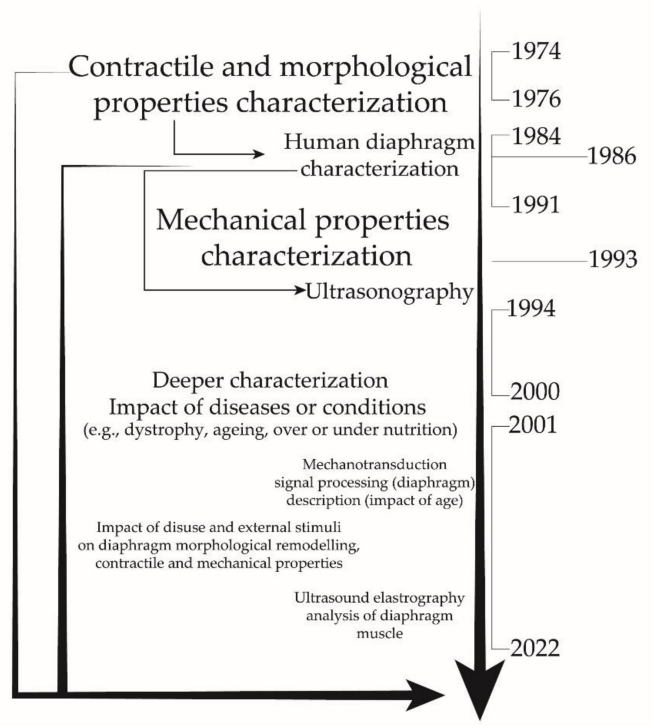

Figure 4. A representative timeline of research focused on respiratory muscles, and in particular the diaphragm muscle, morphology, contractility, and mechanical properties.

4. Pulmonary Functions and Respiratory Muscles Strength Assessment in COVID-19 Patients

While morphological changes to respiratory muscles have been an important focus of research, a recent systematic review by Torres-Castro and collaborators [6] suggested that changes to lung and respiratory function due to COVID-19 has also been an important focus. The main testing methods used in studies that have examined the impact of COVID-19 on respiratory function have been spirometry, lung volumes, and diffusion capacity analysis [6]. In this regard, it has been reported how these parameters have been found in percentages of patients as follows: altered diffusion capacity (39%), restrictive (15%), and obstructive (7%) patterns [6]. Subjects were tested after approximately 30 days from symptom onset or 30 days after discharge from the hospital [6].

In this context, the prevalence of alterations in diffusion capacity has ranged from 44–56% when assessed during the first month post-infection [86–88]. However, it has also dropped to 16% when assessed after months from the infection or after patients being discharged from the hospital [89]. Indeed, the time in which a pulmonary function test is performed is of primary importance, especially at follow-up with patients suspected of having an interstitial disease [6]. This is because it could be difficult to determine the contribution of the disease or of the inflammation arising from the acute event to the limitation, potentially leading to errors in functional diagnosis [6]. Despite these methodological issues, changes to respiratory function may be the result of alveolar damage and pulmonary interstitial fibrosis, as observed in autopsy specimens from COVID-19 patients [90].

Indeed, the highest prevalence of altered diffusion capacity has been seen to emerge in severe patients, and especially those with high inflammatory indicators, who are more likely to develop pulmonary fibrosis [6,91]. But this also pertains to the respiratory muscles' dysfunctions, with the diaphragm among the most affected muscles, associated with COVID-19 and as observed by different investigations [5,9,13,16]. Regarding the restrictive patterns, the prevalence that emerged from the studies included in the review of Torres-Castro [6] was of 15%, while 7% for the obstructive patterns. However, there may be significant confounders which include different criteria in different studies, methodological heterogeneity, and different evaluation timepoints.

Çelik and co-workers [92] compared cross-sectionally the respiratory muscles strength (maximal inspiratory and expiratory mouth pressures) and pulmonary functions (spirometry testing) in female volleyball players with COVID-19, compared with players without infection. The authors observed similar values in pulmonary function parameters but significantly lower values in respiratory muscles strength in COVID-19 volleyball players compared to the non-COVID-19 group [92]. Despite the limitations of a cross-sectional study design, this research is a good example of a methodological approach to assess respiratory function in patients (including athletes), with COVID-19. Furthermore, the study suggested a potential profound impact of the infection on respiratory muscle strength. Overall, all these assessment tools may help to improve the respiratory functions and respiratory muscle health characterization in COVID-19 patients, as well as providing a promising approach to support rehabilitation strategies. However, given the heterogenicity of the clinical presentation of COVID-19 and the large number of COVID-19 survivors who require follow-up, it is essential to approach the problem through a simple but reproducible, comprehensive strategy. Such a strategy should also allow for the identification of patients suffering from slow to incomplete recovery, and guide them to healing targets. In this sense, the investigation of the effects of rehabilitative strategies to recover respiratory functions and respiratory muscle strength after COVID-19 drew less attention.

5. Rehabilitation Strategies Targeting Respiratory Muscles in COVID-19 Patients

The benefits arising from physical activity and exercise linked to the cardiorespiratory system are well known [93–96]. In the context of COVID-19, physiotherapy treatments and breath training strategies have been extensively reviewed in other contexts such as

COPD [97–100], and to COVID-19 [101–104]. The rehabilitation strategy for patients healing from COVID-19 should be individually tailored, particularly according to the severity of the disease, comorbidities, and age of the subject. Abodonya and colleagues [103] reported significant improvements in pulmonary functions in patients weaned from mechanical ventilation after COVID-19, after two weeks of inspiratory muscle training protocol using a threshold inspiratory muscle trainer. Pancera and co-workers [101] observed beneficial outcomes from physiotherapy treatments in the subacute phase of COVID-19 in a case report involving a subject with severe respiratory symptoms and after prolonged ICU stay. In their review, Yang and Yang and collaborators [104] proposed a comprehensive rehabilitation approach involving respiratory muscles specific exercises, but also full-body physical exercise, physiotherapy treatments and nutritional support. Therefore, physical exercise by itself plays an important role in rehabilitation following COVID-19.

Different studies have also focused on the application of traditional Chinese exercise protocols. In particular, Liuzijue Qigong [105,106], a kind of traditional Chinese exercise practice, has been a focus. Using this technique, breath pronunciation by performing the actions of inhaling and exhaling through different mouth patterns, has already been associated with positive effects on ventilatory functions, exercise endurance and health related quality of life of patients with stable COPD [107]. With regards to respiratory muscle morphological aspects and strength, Tang and colleagues [105] reported significant improvements in maximal inspiratory pressure and peak inspiratory flow in more than thirty COVID-19 patients. The cohort was enrolled after discharge in a 20 min Liuzijue Qigong exercise protocol performed every day for 4 consecutive weeks [105]. The improvements in markers of respiratory muscle strength were accompanied by increases in diaphragmatic excursion during deep breathing and diaphragm muscle thickness [105]. Taken together, this early evidence seems to suggest how the rehabilitative phases may cover a primary role in healing from mild to severe COVID-19 in patients. In particular, this constitutes a valid strategy from joint to respiratory functions, but also respiratory muscle strength and morphological aspects assessment and monitoring during rehabilitation.

6. Conclusions

COVID-19 impacts the respiratory muscles, and in particular the diaphragm, indirectly by forcing mechanical ventilation and the consequent muscle inactivity in ICU conditions. It also has a direct impact through mechanisms linked with SARS-CoV-2 viral infection. US imaging techniques coupled to respiratory muscle strength and pulmonary function assessment can represent focal components of a comprehensive screening and monitoring strategy. These can be used from the patient hospitalization up to the rehabilitation phase and can be targeted to mild to severe disease patients. In this sense, a successful rehabilitation approach should include such a comprehensive screening and monitoring strategy, targeting pulmonary functions but also structural evaluations such as muscle thickness analysis, and by identifying clear rehabilitation goals. Additional approaches as the inclusion of mechanical properties assessment through non-invasive techniques and/or surface electromyographic assessment coupled to phrenic nerve stimulation, may provide for a deeper understanding of the possible impact of SARS-CoV-2 infection on respiratory muscle structure and respiratory functioning. The evidence is still in its early stages, and further clarification is needed. However, a multifactorial approach, including the simultaneous utilization of the methodologies described through the present review, may represent a possible strategy in managing COVID-19 patients, helping to restore normal respiratory functioning (Figure 5). Accordingly, longitudinal studies including comprehensive approaches in the assessment of the respiratory muscles' structural aspects (e.g., diaphragm ultrasonography) and respiratory functions assessment from hospitalization to the rehabilitation phases may provide key information for patient management and for our understanding of the pathology. We hope this review will assist clinicians in the management of respiratory muscle pathology for patients with COVID-19.

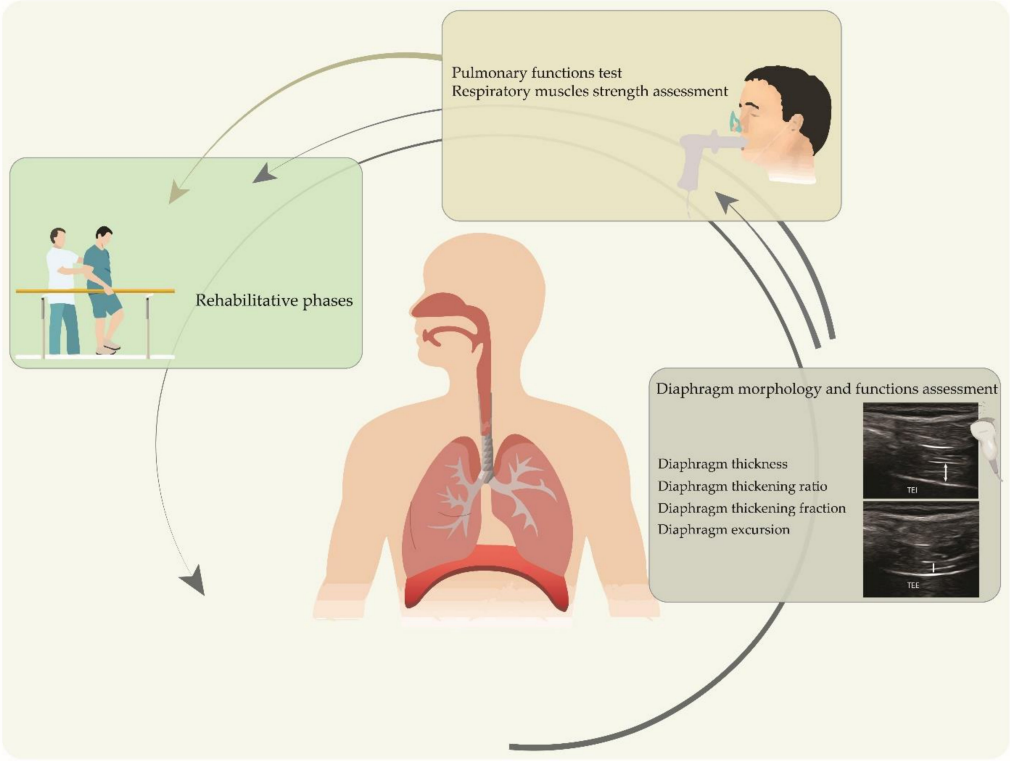

Figure 5. Graphical summary of the possible strategies to support and monitoring of COVID-patients' healing process.

Author Contributions: Conceptualization, L.C., D.S., A.I. and G.M.; methodology, L.C., D.S., I.B.-L. and R.K.; validation, L.C., D.S., I.B.-L., R.K., F.C., G.R., A.I., and G.M.; formal analysis, L.C., D.S., I.B.-L. and R.K.; writing—original draft preparation, L.C., D.S., and F.C.; writing—review and editing, L.C., D.S., I.B.-L., R.K., F.C., G.R., A.I., and G.M.; visualization, L.C.; supervision, D.S. and G.M.; project administration, L.C. and D.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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