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DOTTORATO DI RICERCA IN BIOMEDICINA TRASLAZIONALE

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DIAPHRAGMATIC ULTRASOUND AND RESPIRATORY MUSCLES EVALUATION IN PATIENTS WITH ATAXIA-TELANGIECTASIA

Tesi di Dottorato

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Abstract

Ataxia Telangiectasia (AT) (OMIM # 208900), is a rare, autosomal recessive, multisystemic, human genomic instability syndrome, caused by biallelic mutations in the *ATM* (*Ataxia Telangiectasia Mutated*) gene, which encodes the ATM protein [1-4].

Lung disease is a significant cause of morbidity and mortality in ataxia telangiectasia (AT).

In A-T lung disease three different, but related, phenotypes can be recognised: immune dysfunction leading to recurrent upper and lower respiratory tract infections, resulting in bronchiectasis; lung disease associated with dysfunctional swallow and inefficient cough due to neurological decline, which are linked to the risk for aspiration; ILD/pulmonary fibrosis.

The aim of the study was to evaluate the diaphragmatic functionality and the strength of the respiratory muscles in four genetically proven AT individuals [1F, 3M] at different stages of their disease. All subjects underwent pulmonary function tests (spirometry, MIP and MEP), nocturnal respiratory monitoring (PSG), and US examination with diaphragmatic thickness and excursion measurement. The B-mode was applied for diaphragmatic identification, and the M-mode was employed for the recording of the amplitude of diaphragm contraction during quiet breathing, deep breathing. Results were compared with reference values in the literature. MIP, MEP, QB and DB were low compared to healthy adults and children. The patients sample in the study is small and it is not possibile to obtain definitive results but this is only the first data and the work is moving forward.

Keywords: ataxia-telangiectasia; lung disease; diaphragmatic ultrasound; lung ultrasound.

SUMMARY

1. BACKGORUND

Ataxia Telangiectasia (AT) (OMIM # 208900), is a rare, autosomal recessive, multisystemic, human genomic instability syndrome, caused by biallelic mutations in the *ATM* (*Ataxia-Telangiectasia Mutated*) gene, which encodes the ATM protein [1-4]. Consistent with the rarity of the disease, the incidence worldwide is estimated to span from 1:40,000 to 1:100,000 individuals and 1:300,000 in the West Midlands population. Carrier frequency is estimated at 0.5–2.0% in the general population [5,6].

1.1 Clinical features

Clinically, the disease is characterized by early onset, progressive, neurodegeneration, which affects mainly the cerebellum and develops into severe neuromotor dysfunction, telangiectasia (i.e. the dilation of small blood vessels observed primarily in the eyes but also in the skin), immunodeficiency that spans the B cell and T cell systems leading to recurrent sinopulmonary infections, thymus and gonadal atrophy, marked predisposition to malignancies (primarily lymphoreticular), increased serum levels of α-fetoprotein and carcinoembryonic antigen, acute sensitivity to ionizing radiation, and, often, cardiovascular defects, growth retardation, premature aging and insulin resistance [1-3]. The ***classic AT phenotype*** is caused by homozygosity or compound heterozygosity for *ATM*-null alleles, which typically truncate *ATM* or inactivate it. Patients with the classical form of AT survive until their second-third decade of life; the major causes of death are lung disease, due to recurrent sinopulmonary infections, and cancer (mainly T-cell leukemia and lymphomas). Overall, the severity and variability of the clinical picture displays a consistent inter- and intra-familiar variation, mostly related to the underlying mutations and residual ATM kinase activity. *Milder forms* of the disease [***mild AT***], mainly characterized by later onset or slower progression of symptoms, are associated with mutations that leave residual amounts of functional ATM protein [1,4,7,8].

Another disorder, ***ataxia-telangiectasia-like disorder*** (**ATLD**), is similar to mild AT, with a later age of onset and slower progression than the classic condition and is caused by hypomorphic mutations in the *MRE11A* gene, which encodes a component of the MRN (MRE11-RAD50-NBS1) complex. ATM haploinsufficiency increases the risk of cancer and heart disease and decreases the lifespan [8,9].

1.2 Treatments

No established and definitive treatment is currently available for AT: despite that, the lifespan of affected patients has been successfully improved over the last years by the use of immunoglobulins

and antibiotics to prevent infectious complications. Current treatments are still symptomatic and supportive [1,10, 11, 12]. Dexamethasone and ibuprofen received positive assessment for (clinical) neurological and (experimental) inflammatory disturbances; deep brain stimulation ameliorated gait and myoclonic disturbances; drugs imported from trials on hereditary cerebellar ataxias (e.g., varenicline, riluzole, and N-acetyl-L-Leucine) are under study in AT patients. Gene and stem cell therapies are also in progress [1,13,14].

1.3 Outcome

Neurological degeneration is the major determinant to the quality of life of AT patients as it has a severe impact on the outcome of the disease. Survival is markedly determined by the occurrence of infections and malignancies. Over the past 20 years, the expected lifespan of individuals with AT has improved considerably: many patients now live beyond the age of 25 years and some have survived into their forties and fifties [1,2,3,15]. The increased lifespan has generated new challenges as now AT individuals face a number of problems related to involvement of organ and tissues previously "spared" because of the early death and/or relatively poor survival rates [e.g., growth failure, heart and vessels degeneration, liver degeneration/disease, inflammatory cutaneous and bone diseases]. Thus, there is now increased need for specific follow-up protocols devoted to the transition towards adulthood and special treatment strategies targeted against these emerging organ-related complications [1,2].

1.4 Pathophysiology

The ATM protein is a 350 kDa Ser/Thr protein kinase of the phosphatidylinositol 3-kinase-related kinase (**PIKK**) family, which include AT and RAD3-related, DNA-dependent protein kinase catalytic subunit (**DNA-PKcs**) and mammalian/mechanistic target of rapamycin (**mTOR**). The ATM kinase is best known as the chief mobilizer and sensor responsible for the initiation and propagation, in mitotic/proliferating cells, of nuclear signals to cell cycle check-points, which in turn activate the cellular machinery responsible to repair severe DNA lesion (i.e. double-strand break, **DSB**). DNA DSBs can be generated by DNA damaging agents and are also obligate intermediates in meiotic recombination and in the assembly of the genes encoding antigen receptors during lymphocyte maturation through V(D)J and class switch recombination. In fact, the hallmarks of the cellular phenotype of AT are increased chromosomal breakage, premature senescence of cultured primary fibroblasts and sensitivity to DNA-damaging agents. Additional roles (other-than DNA damage response, **DDR**) for the ATM kinase include cytoplasmic activation of many cell-signaling pathways regulating cell homeostasis and autophagy, prevalently occurring in post-

mitotic, non-proliferating cells. ATM associates with organelles including mitochondria and peroxisomes and with cytoplasmic and synaptic vesicles.

ATM is predominantly a **nuclear protein** in developing (e.g. in embryonic and fetal) and/or proliferating (i.e. in developing lymphocytes) cells, which replicate at higher rates: in these cells the ATM kinase is involved in the early recognition of DSBs and activation of cell cycle check- points leading to DDR (DNA damage response) and DSBs repair (canonical ATM role).

However, ATM also plays many important **cytoplasmic roles** in non-developing, non-proliferating cells (i.e. in post-mitotic cells: e.g. neurons), which replicate at lower rates or no longer replicate: in these circumstances, it phosphorylates hundreds of protein substrates that activate and coordinate cell-signaling pathways involved in cell-cycle checkpoints, nuclear localization, gene transcription and expression, response to oxidative stress, apoptosis and autophagy, nonsense-mediated decay, and others (non-canonical ATM role).

Non-canonical roles include:

(**1**) Phosphorylation and consequent inhibition of the translation repressor 4EBP1 (eIF4E-binding protein 1), which promotes protein synthesis;

(**2**) Activation of **AKT** to enhance glucose uptake;

(**3**) Regulation of histone deacetylase 4 (HDAC4)-mediated gene expression;

(**4**) In response to reactive oxygen species (ROS: i.e. the products generated in DNA either by exposure to physical sources [UV light and ionizing radiation], or via the physiological functions of numerous intracellular enzymes), activation of tuberous sclerosis complex 2 (**TSC2**) protein, which acts as negative regulator (down regulator) of the mammalian target of rapamycin (mTOR) protein (via the mTOR complex 1, mTORC1), thus increasing autophagy (which is usually responsible for recycling damaged organelles and for the retrieval of cellular nutrients) and promoting cell growth (this can also occur in response to hypoxia through phosphorylation of the transcription regulator HIF1α [hypoxia-inducible factor 1 α]); interestingly, the mTORC1 deregulation is associated with the onset of different disease phenotypes, including cancer and diabetes, which are among the most debilitating AT complications;

(**5**) In response to genotoxic stress, enhancement of the pentose phosphate cycle, which is a source of the anti-oxidant NADPH;

(**6**) A role in mitochondrial homeostasis and the secretion of ROS by modulating mitophagy (this pathway, together with the enhanced pentose phosphate cycle and inhibition of mTOR/mTORC1, all contribute to maintaining redox homeostasis: BID [BH3-interacting domain death agonist] is an ATM substrate that has emerged as an important mediator of stress responses, including the regulation of mitochondrial metabolism and of hematopoietic stem cell [HSC] quiescence);

(**7**) Interplays with specific membrane-bound tyrosine kinases receptors (e.g. platelet-derived growth factor receptor β, PDGFRB), might protect neurons in response to excessive excitation (e.g. glutamatergic excitotoxicity) by signaling to cytoplasmic ATM-dependent autophagy processes to initiate the removal of dysfunctional organelles, thus promoting neuronal survival (in this respect, PDGFRB plays a role only in the ATM activation of the oxidation pathway but not the DDR pathway).

Appreciating these different roles and interplays (linked to the different cellular compartments: i.e. nuclear vs. cytoplasmic/organelles), helps to provide new insights into the different clinical manifestations and variants exhibited by AT individuals. It is also important to consider that the consequences of organs' disruption (e.g. neurological deterioration, respiratory failure and endocrine imbalance) could interact but also tower the basic molecular mechanisms, thus driving the cascade of most systemic and debilitating AT manifestations and complications [1-3,16].

2. LUNG DISEASE IN A-T

Lung disease is a significant cause of morbidity and mortality in ataxia telangiectasia (AT). Immune dysregulation is in part responsible for recurrent lung infections and bronchiectasis and for developing interstitial lung disease (ILD) [17-20]. In fact, dyscoordination of cough, dysfunctional swallow and impaired mucociliary clearance potentially increase the risk of pneumonia.

Phenotypes of lung disease in AT is variable in relation to disease progression. For this reason, it is difficult to predict the natural course of lung decline in people with AT and evaluate correctly who may be at increased risk for developing lung disease. In McGrath-Morrow et al., in a subgroup of individuals with AT who were followed longitudinally, lung function was stable over a period of several years suggesting that recognition of groups who may be higher risk for lower pulmonary function may help direct care and improve clinical outcomes in AT patients.

Besides, McGrath-Morrow and collegues found several potential factors associated with lung function: females who were 11 years of age and older had lower FVC % predicted compared to males and younger females with AT, that has been seen in other chronic lung diseases; the use of supplemental gamma globulin was significantly associated with lower FVC % predicted; and a modest correlation between higher chromosomal breakage and lower FVC % predicted in males with A-T.

Recurrent infections and aspiration, which are common problems in AT, impaire mucociliary clearance resulting in mucosal damage and secondary ciliary dyskinesia with not fully functional secretion clearance. According to evidence from other chronic respiratory conditions, airway

clearance measures (chest physiotherapy, flutter valve therapy and cough assist devices) are likely to be beneficial in AT patients. This aspect is very important to be considered when neurological decline advances and during episodes of acute illness, as seen in different neuromuscular diseases [23-25].

As reported in Vilozni et al. which studied cough flow volume profile in A-T patientes, the yearly rate of increase of peak cough was significantly lower than normal but it increases anyway. Thus, AT patients had a weak cough compared to healthy controls of similar ages and cough worsened with age. Inhalation therapy (nebulised hypertonic saline, rhDNase, or inhaled mannitol) may be potentially beneficial in AT patients but there are no clear evidences.

In AT poor growth is a common feature and is associated with a general decline in overall health, poor caloric intake and endocrine abnormalities. Increased morbidity and mortality directly correlate with cachexia and impaired growth. Worsening nutritional status increases infection-related morbidity and mortality. In children, malnutrition is of particular concern since it adversely affects not only statural growth but may also impact lung development [26-30].

Simple measurements, such as weight and height, may be insufficient to identify changes in body composition in AT patients and more accurate assessment of body cell mass (BCM) by methods such as bioimpedance assessment could be used. In Ross et al., the prevalence of malnutrition in AT patients was found to be high (69%) [31].

In AT bulbar muscle function is impaired and is linked to swallowing dysfunction and chronic aspiration, causing and exacerbating lung disease. In particular, dysphagic problems commonly emerge in the second decade of life. Interestingly, children with AT are observed to have problems with thin consistencies, and this has been shown by videofluoroscopic swallow study (VFSS) evaluation, which reported that aspiration occurred most frequently when older patients were drinking thin liquids through a straw. This is in contrast with other neuromuscular disorders where weak swallow determines problems with thick consistencies.

The onset of dysphagia appears to coincide with a decrease in nutritional status although in a cross-sectional study it was not possible to distinguish between nutritional deficiency as a cause or effect of the dysphagia. Neither the presence nor absence of coughing during mealtimes was found to be a reliable indicator of airway contamination during swallowing, as silent aspiration occurred in 71% of the patients whose aspiration was detected on videofluoroscopy.

Regular reviews by a speech and language therapist are vital so that appropriate early advice for changes in feeding routine.

Gastrostomy may be required to improve the quality and quantity of nutrition, reduce aspiration-related risks and improve the quality of life related to mealtimes in people with A-T [32-36].

Scoliosis, common problem in AT patients, could also contribute to impaire lung function. It seems to be more common in adolescents as neurological disease progresses and patients spend prolonged time in a wheelchair. Maintaining assisted ambulation as long as possible (e.g. use of walkers instead of wheelchairs) might prevent scoliosis and further deterioration of lung function, but further studies are needed to sustain this clinical observation [37].
Fatigue, while not measurable or well delineated in clinical research, is characteristic for AT patients and may further contribute to aspiration and inefficient cough [32].

2.1 Phenotypes of respiratory disease in A-T

In A-T lung disease three different, but related, phenotypes can be recognised:

- immune dysfunction leading to recurrent upper and lower respiratory tract infections, resulting in bronchiectasis;

- lung disease associated with dysfunctional swallow and inefficient cough due to neurological decline, which are linked to the risk for aspiration;

- ILD/pulmonary fibrosis.

As already said, the phenotypes are related and overlap in the natural history of AT patients. The aetiology and pathogenesis of ILD and pulmonary fibrosis in AT patients is unknown. Recurrent infections and chronic aspiration may trigger fibrosis along with immune dysregulation.
In fact, ILS is well described in immune deficiency conditions but no correlation was found between the development of ILD and abnormalities in Ig levels [32, 38].
Diffuse lung diseases, mimicking ILD in AT, may be secondary to lymphoma and to chemotherapy for the treatment of a malignancy, due to an underlying defect in injury repair [39-41].

2.2 Respiratory monitoring in A-T

2.2.1 Functional pulmonary tests

Reproducible dynamic lung function tests require effort and coordination, difficult to obtain in AT people with relevant neurological involvement. However, pulmonary function testing with certain modifications may be used to track the rate of decline in lung function over time. Stabilising the patient's head and holding the cheeks while the patient performs the forced expiratory manoeuvre increase the reproducibility of testing.

It is important to underline that flow–volume curves could meet recommended reproducibility criteria but often fail to achieve other standard recommendations for forced spirometry underestimating respiratory function [42-45].

Vilozni and collegues reported that spirometry was reproducible in AT and that hyper-reactive airway disease was associated with a greater decline in lung function per year in their population.

On the other hand, data from different studies demonstrated that lung function in AT was more consistent with a restrictive pattern due in part to underlying neuromuscular abnormalities [46].

McGrath-Morrow et al. tested AT adolescents and found that most of them had normal functional residual capacity (FRC), near-normal total lung capacity, high residual volume (RV), decreased vital capacity and low maximal expiratory pressure. In this cases, both expiratory muscle weakness and incoordination and difficulty expiring to RV could be significant. FRC was considered the most reliable lung measurement in individuals who may not be able to perform maximal inspiratory and expiratory manoeuvres, requiring only passive cooperation.

Since FVC predicted values in AT are often low, repeated and sequential spirometry in the same patient may be more useful to evaluate lung function along time rather than to compare to normal predicted values. Berkun et al. studied a younger cohort of children with AT (median (range) age 8.8 (3.7–19.3) years). They found a delay in initiation of the breath with a longer rise time to peak flow compared to healthy values. They suggested that this may be linked to both obstructive lung disease and neurological dysfunction. Besides young AT patients often do not exhale for more than 1 s, for this reason forced expiratory volume in 1 s (FEV1) may not be an accurate parameter for describing bronchial obstruction in this particular group and FEV0.5 may be a better index.

Cerebellar coordination is fundamental for breathing and coughing involving cyclic motor acts. In this way both impaired muscle control and muscle weakness exacerbate the decline in respiratory function leading to decreased tidal volumes and ineffective cough. A *decline* in *maximal respiratory muscle function* has been found to be **a marker for disease progression** and respiratory

failure in AT patients. Chronic cough and prolonged or difficult recovery from respiratory illnesses are clinical indicators of lung disease.

Wheelchair use may contribute to the deterioration in lung function because of lack of conditioning. Evaluation of maximum inspiratory and expiratory pressures (**MIP** and **MEP**, respectively) is a well-established marker of *respiratory muscle strength and coordination* and can be used to monitor lung function prospectly.

In Felix et al., it was shown that inspiratory muscle training was effective in improving ventilatory pattern, lung volume, respiratory muscle strength and the of quality of life in patients with AT. Inspiratory muscle training may be an effective adjunct therapy to drug treatment for patients with AT. Respiratory muscle strength training (RMST) focuses on increasing the force-generating capacity of the inspiratory and expiratory muscles [47-49].

In children who are unable to perform spirometry, impulse oscillometry is an option. This test is noninvasive and can measure airway resistance.

Body plethysmography is often not a practical option considering that many patients are dependent on wheelchairs and may be unable to perform the required respiratory manoeuvre [50].

Gas exchange (DLCO) measurements are sometimes considered, particularly to provide evidence of impaired gas exchange when evaluating a patient for ILD. However, the single-breath DLCO measurement may simply not be feasible because of severe neurological impairment in AT.

2.2.2 Sleep-related breathing disorder

Sleep-related breathing problems may contribute to respiratory morbidity in late teens with decreased pulmonary reserve. McGrath-Morrow et al. [45] performed overnight polysomnography on 12 adolescents with AT at a median (range) age of 16 (13–20) years. All subjects in the study were wheelchair-bound and the median (range) FVC was 44 (16–82)% pred. Infrequent partial or complete upper airway obstructions during sleep and minimal night-time hypoxaemia were noted in the majority of AT adolescents. Subjects did have decreased sleep efficiency. No correlation was found between FVC and sleep efficiency in the AT subjects, unlike subjects with cystic fibrosis where the magnitude of sleep disruption is associated with severity of lung disease (low FEV1).

There are no studies on the use of noninvasive ventilation in AT for sleep-related breathing disorder, but extrapolating from other neuromuscular diseases, if there is evidence of abnormal gas exchange or sleep fragmentation then supplemental oxygen or noninvasive ventilation support should be considered [51-53].

3. DIAPHRAGMATIC DYSFUNCTION

In AT patients cerebellar dysfuntion, muscle weakness and recurrent pulmonary infections may contribute to impaired diaphragm function. In the literature, diaphragmatic dysfunction is not yet well defined lacking noninvasive reference diagnostic tool. Diaphragmatic dysfunction (DD) may be defined as a loss of muscle force that may be partial (weakness) or complete (paralysis) with reduced inspiratory capacity and impaired respiratory muscle endurance [54].

A diagnostic approach can be based on the motion and thickness changes of both hemidiaphragms during respiratory acts.

Diaphragmatic ultrasound is a useful technique to evaluate the anatomy and function of the diaphragm, specifically diaphragmatic excursion and thickening [55-56].

Diaphragmatic ultrasound can be easily performed as an outpatient procedure or at the bedside. The ultrasound system needs a 2.5-5.0 MHz convex transducer and a 7.5-10.0 MHz linear transducer.

Diaphragm ultrasound involves two acoustic windows: over the subcostal area (SCA) and over the zone of apposition (ZOA) at the ninth intercostal space, between the anterior axillary and midaxillary lines. Through the SCA window, the diaphragm appear as a deeply located curved structure that separates the thorax from the abdomen, and, during diaphragmatic contraction in healthy individuals, the SCA window shows the diaphragm descending in the craniocaudal direction. Through the ZOA window, the diaphragm is identifiable as a three-layer structure (one hypoechoic inner muscle layer surrounded by two hyperechoic outer membranes,the peritoneum and pleura) and ultrasound shows the shortening and thickening of the muscle.

For this reasons, ultrasound allows the measurement of diaphragmatic mobility and thickness. To quantify diaphragmatic mobility and thickening in an objective manner, at least three images should be evaluated and the values should be averaged [57-59].

Diaphragmatic mobility is measured by visualizing the hemidiaphragms via the anterior subcostal view (the preferred method), posterior subcostal view, or subxiphoid view, in the two-dimensional (B) mode or in the one-dimensional (M) mode.

Diaphragmatic mobility is measured at three time points: during *quiet breathing*; during *deep breathing at maximal inspiration*, and during *voluntary sniffing*.

To assess atrophy and contraction of the diaphragm, it is necessary to evaluate *diaphragm thickness* (Tdi) and the *thickening fraction* (TF), respectively.

In the literature, data about the potential use of diaphragm ultrasound in AT patients lack but it is possible to deduce data from different studies in other neuromuscular diseases (amyotrophic lateral sclerosis, Duchenne muscular dystrophy and myotonic dystrophy type 1).

Main findings are reduced Tdi-exp and thickening in patients with ALS with vital capacity < 80% predicted and in those with bulbar-onset ALS; thickening with inspiration correlated with SNIP and MEP and lung function; maximal excursion correlated with FVC; the Tdi at VT/Tdi at TLC ratio is indicative of weakness and may predict NIV initiation in ALS; in Duchenne dystrophy and myotonic dystrophy type 1 reduced mobility during DB and DB excursion correlated with FVC values [60-66]. For this reason, it is possible to assert that diaphragm thickness and excursion are reduced and correlate with lung function in ALS; diaphragm thickening may be related to respiratory muscle strength in ALS; the Tdi at VT/Tdi at TLC ratio may suggest weakness and predict the initiation of NIV in ALS; DB diaphragmatic mobility may be related to SNIP and lung function in Duchenne dystrophy and myotonic dystrophy type 1 [54].

Diaphragm ultrasound may be performed together with spirometry and volitional assessment of global respiratory muscle strength-MIP, MEP, and (sniff nasal inspiratory pressure) SNIP.

Because spirometry and volitional assessment may have limitations due to reduced patient motivation, cognitive decline, and orofacial muscle weakness, diaphragm ultrasound could play a very significant role in the evaluation of A-T patients.

Maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), and sniff nasal inspiratory pressure (SNIP) are the most useful tests to assess muscle strength.

It is important to consider these tests if respiratory muscle weakness is suspected (e.g.reduced vital capacity) or to monitor improvement, stability, or deterioration.

The strength of the diaphragm and other inspiratory muscles (eg, sternocleidomastoid) is linked to the maximal inspiratory pressure (MIP) while the maximal expiratory pressure (MEP) reflects the strength of the abdominal muscles and other expiratory muscles.

According to MIP and MEP evaluation, it is possible to recognize different inspiratory or expiratory muscle involvement: a low MIP and SNIP but a normal MEP suggest isolated inspiratory muscle weakness (usually diaphragmatic); a low MIP, SNIP, and MEP suggest generalized skeletal muscle weakness (eg, ALS); a normal MIP and SNIP and low MEP suggest isolated expiratory muscle weakness (rare).

In neuromuscular diseases, respiratory and skeletal muscle weakness are often present at the same time showing mixed patterns.

Besides, the evaluation of the severity of muscle weakness through MIP, MEP and SNIP allow a risk stratification to better understand prognosis as supported by literature observations in

neuromuscular disorders: a MIP below one-third of normal predicts hypercapnic respiratory failure (PaCO2 >45 mmHg); a SNIP that is 35 percent of normal predicts likely ventilatory failure in patients with ALS; a MEP less than 60 cm H2O predicts a weak cough with difficulty clearing secretions; a reduced MIP is associated with increased mortality [Rafferty et al. 2023] [67-71].

Respiratory muscle weakness is often subclinical and MIP, MEP and SNIP usually decline before the symptoms or signs because a large reserve of respiratory muscle strength exists. These tests can be repeated in a serial way to evaluate how the respiratory muscle weakness changes.

Tests of respiratory muscle strength need to be interpreted according to normal reference ranges and signs and symptoms.

Spiesshoefer et al. proposed a standardized and comprehensive protocol for diaphragm ultrasound in order to determine lower limits of normal (LLN) for both diaphragm excursion and thickness in healthy subjects and explored the association between volitional tests of respiratory muscle strength and diaphragm ultrasound parameters.

LLNs (defined as the 5th percentile) for diaphragm excursion were as follows: (a) during tidal breathing: 1.2 cm (males; M) and 1.2 cm (females; F) for amplitude, and 0.8 cm/s (M) and 0.8 cm/s (F) for velocity, (b) during maximum voluntary sniff: 2.0 cm (M) and 1.5 cm (F) for amplitude, and 6.7 (M) cm/s and 5.2 cm/s (F) for velocity, and (c) at TLC: 7.9 cm (M) and 6.4 cm (F) for amplitude. LLN for diaphragm thickness was 0.17 cm (M) and 0.15 cm (F) at FRC, and 0.46 cm (M) and 0.35 cm (F) at TLC. Values for males were consistently higher than for females, independent of age. LLN for diaphragmatic thickening ratio was 2.2 with no difference between genders [72].

In Zumpano Cardenas et al. diaphragm ultrasound (DUS) has been used to identify diaphragm dysfunction. In the study a total of 64 healthy patients (30 males) had lung function and inspiratory strength (maximal inspiratory pressure and sniff nasal inspiratory pressure) measured. Gastric and oesophageal pressures were measured in a subgroup (n = 40). DUS was characterized by mobility (quiet breathing [QB] and deep breathing [DB]) and thickness (at functional residual capacity [ThFRC] and total lung capacity [ThTLC]). During QB, DUS was similar between sexes. However, during DB, females had lower mobility, thickness and TF than males. Mobility at DB, ThTLC and TF significantly correlated with lung function and inspiratory strength. These correlations were affected by sex. DUS correlated with inspiratory gastric pressure. In healthy patients, DUS correlated with lung function and inspiratory strength during DB. Significant differences between genders were noticeable when DUS was performed during DB [73].

In Vieira Santana et al. diaphragmatic mobility and thickness during quiet (QB) and deep breathing (DB) and calculated thickening fraction (TF) were measured in 30 fibrotic interstitial lung disease

(FLID) patients and 30 healthy controls. FILD cases' diaphragmatic findings were correlated with dyspnea, exercise tolerance (six-minute walk test), lung function and HRQoL. Diaphragmatic mobility was similar between groups during QB but was lower in FILD cases during DB when compared to healthy controls (3.99 cm vs 7.02 cm; $p < 0.01$). FILD cases showed higher diaphragm thickness during QB but TF was lower in FILD when compared to healthy controls (70% vs 188%, $p < 0.01$). During DB, diaphragmatic mobility and thickness correlated with lung function, exercise tolerance and HRQoL, but inversely correlated with dyspnea. Most FILD cases (70%) presented reduced TF, and these patients had higher dyspnea and exercise desaturation, lower HRQoL and lung function [74].

In Ishak et al. a case–control study was conducted on 130 patients with pediatric chronic pulmonary diseases (childhood interstitial lung diseases, cystic fibrosis, and non-cystic fibrosis bronchiectasis) and 100 control subjects. Ultrasound was used to detect diaphragmatic excursion and thickness, which were correlated with the severity of the disease, both clinically and functionally. The right and left diaphragmatic excursions were significantly lower in the patients (19.469 ± 9.984 and 18.5 ± 10.131, respectively) than in the control subjects (29.6 ± 14.131 and 25.6 ± 12.827, respectively) (p values of 0.002 and 0.019). In contrast, the difference in the right and left diaphragmatic thicknesses between the patients and the controls was statistically insignificant (p values of 0.884 and 0.344). The left diaphragmatic excursion was positively correlated with the patients' age and weight, while both the right and the left diaphragmatic excursion significantly correlated with the patients' height, FEV1/FVC ratio, and heart rate [75].

In Ho et al. diaphragm ultrasound (DUS) is a noninvasive method of evaluating the diaphragm's structure and function. This study explored the relationships between DUS, spirometry, and respiratory mouth pressures in 10 healthy children (median age: 11 [range: 7–14 years]; 5 females, 5 males). Thickening fraction correlated with maximal inspiratory pressure (MIP) (Spearman's rho [rs] = 0.64, p = 0.05). During quiet breaths, excursion time correlated with MIP (rs = 0.78, p = 0.01) while velocity correlated with maximal expiratory pressure (rs = −0.82, p = 0.01). During deep breaths, MIP correlated with excursion (rs = 0.64, p = 0.05) and time (rs = 0.87, p = 0.01). Excursion time during deep breaths also correlated with forced vital capacity (rs = 0.65, p = 0.04). Our findings suggest that DUS parameters are closely related to spirometry and respiratory mouth pressures in healthy children and further support the use of DUS as a noninvasive method of respiratory assessment [76].

In Duyndam et al. diaphragmatic thickness (Tdi) and diaphragm thickening fraction (dTF) reference values were determined using ultrasound in healthy children aged 0–8 years old and it was assessed their reproducibility. In a prospective, observational cohort, Tdi and dTF were

measured on ultrasound images across four age groups comprising at least 30 children per group: group 1 (0–6 months), group 2 (7 months-1 year), group 3 (2–4 years) and group 4 (5–8 years). Ultrasound images of 137 healthy children were included. Mean Tdi at inspiration was 2.07 (SD 0.40), 2.09 (SD 0.40), 1.69 (SD 0.30) and 1.72 (SD 0.30) mm for groups 1, 2, 3 and 4, respectively. Mean Tdi at expiration was 1.64 (SD 0.30), 1.67 (SD 0.30), 1.38 (SD 0.20) and 1.42 (SD 0.20) mm for groups 1, 2, 3 and 4, respectively. Mean Tdi at inspiration and mean Tdi at expiration for groups 1 and 2 were significantly greater than those for groups 3 and 4 (both $p < 0.001$). Mean dTF was 25.4% (SD 10.4), 25.2% (SD 8.3), 22.8% (SD 10.9) and 21.3% (SD 7.1) for group 1, 2, 3 and 4, respectively. The intraclass correlation coefficients (ICC) representing the level of inter-rater reliability between two examiners performing the ultrasounds was 0.996 (95% CI 0.982–0.999). ICC of the inter-rater reliability between the raters in 11 paired assessments was 0.989 (95% CI 0.973–0.995) [77].

Fayssoil et al. study included 74 patients with DMD. The right diaphragm thickening fraction (TF) was significantly associated with age (P = .001), Walton score (P = .012), inspiratory capacity (IC) (P = .004), upright forced vital capacity (FVC) (P < .0001), supine FVC (P = .038), and maximal inspiratory pressure (MIP) (P = .002). Right diaphragm excursion was significantly associated with age (P < .0001), steroid use (P = .008), history of spinal fusion (P < .0001), body mass index (BMI) (P = .002), Walton score (P < .0001), IC (P < .0001), upright FVC (P < .0001), supine FVC (P < .0001), and MIP (P < .0001). A right diaphragm TF >28% and a right diaphragm excursion>25.4 mm were associated with an FVC >50% with, respectively, an area under the curve (AUC) of 0.95 (P = .001) and 0.93 (P < .001). A left diaphragm TF >26.8% and a left diaphragm excursion >21.5 mm were associated with an FVC >50% with, respectively, an AUC of 0.95 (P = .011) and 0.97 (P < .001).Diaphragm excursion and diaphragm TF can predict restrictive pulmonary insufficiency in DMD [78].

4. RESEARCH: DIAPHRAGM ULTRASOUND AND PULMONARY FUNCTIONAL TESTS IN A-T

4.1 Aim of the study

The aim of the study was to evaluate the diaphragmatic functionality and the strength of the respiratory muscles in four genetically proven AT individuals [1F, 3M] at different stages of their disease.

4.2 Methods

All subjects underwent pulmonary function tests (spirometry, MIP and MEP), nocturnal respiratory monitoring (PSG), and US examination with diaphragmatic thickness and excursion measurement. The B-mode was applied for diaphragmatic identification, and the M-mode was employed for the recording of the amplitude of diaphragm contraction during quiet breathing, deep breathing.

4.3 Results

Notably, all subjects suffered from recurrent respiratory infections from early childhood. Three were adults (mean age of 26 years, + 7,8 SD) vs. a child of 12 years. Mean values of MIP were 31,33 ± (SD 13,65) vs. 21 cmH2o in the pediatric patient, whereas the mean values of MEP were 41,66 (SD 10,21) vs. 26 cmH2o. The mean valuation of quiet breathing (QB) was 2 (SD ± 0,93) vs. 1,21 cm while the reported mean for deep breathing (DB) was of 3,19 (SD 2) vs. 4,21 cm. Concerning PSG, the AHI resulted in 5,7 (SD 0,7) vs. 7.

4.4 Discussion

Results were compared with reference values in the literature. MIP, MEP, QB and DB were low compared to healthy adults and children. These findings, along with different studies focusing on pulmonary functional tests in A-T, support the hypothesis that diaphragm dysfuntion is a relevant aspect of lung disease in A-T patients. Bearing in mind the sensitivity to ionizing radiation in A-T, the possibility of the use of lung and specially diaphragm ultrasound to evaluate the A-T patient in acute phase of lung disease may be relevant both to titrate therapies and to assess recovery but not only. In fact, data derived from pulmonary functional tests and diaphragm ultrasound could be related to genetics and immunological profiles in order to realize a risk stratification for severe lund disease and a targeted approach for each A-T patient.

4.5 Conclusions

The patients sample in the study is small and it is not possibile to obtain definitive results but this is only the first data and the work is moving forward.

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