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PNEUMON
Vol. 30, No 3
July - September 2017
The Fleischner Society diagnostic criteria for IPF
Clinical implications

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Key words:
- Idiopathic pulmonary fibrosis,
- IPF diagnosis,
- Fleischner Society

Idiopathic Pulmonary Fibrosis is a debilitating, relentlessly progressing disease with a median survival of about 3-5 years. The introduction of antifibrotic agents, pirfenidone and nintedanib marked the beginning of a new era in the management of IPF. These two agents have been tested only in the context of IPF. This means that the precise diagnosis of IPF is not just an academic exercise but has direct clinical implications.

The latest guidelines for the diagnosis of IPF by ATS/ERS/JRS/ALAT date back to 2011. According to them, the presence of a definite UIP pattern (presence of honeycombing in a predominantly peripheral bibasilar distribution) after exclusion of alternative diagnoses, is considered diagnostic of UIP/IPF obviating the need for surgical lung biopsy (SLB). Thus, honeycombing was a prerequisite in order to avoid tissue based diagnosis. When the patient presented with a possible UIP pattern (presence of traction bronchiectasis/bronchiolectasis in a predominantly peripheral bibasilar distribution but without honeycombing) and inconsistent with UIP pattern, surgical lung biopsy was advised in the diagnostic algorithm in order to establish diagnosis.

It is important to note that back then; there was no approved therapy for IPF. The main focus was to create clinical trial based guidelines in order to ensure the formation of a well characterized population of patients to enroll in clinical trials. The arrival of pirfenidone and nintedanib has created the need for new guidelines. The recently published diagnostic guidelines by the Fleischner Society represent a major step forward. They incorporate findings during the last decade and are clinical practice oriented. Several points are worth mentioning that have direct implications for clinical practice.

1. INTRODUCTION OF PRETEST PROBABILITY

An important new feature is the introduction in the diagnostic algorithm of pretest clinical probability. According to the Fleischner Society guidelines, a clinical context of IPF includes all of the following: age >60, absence of significant exposure and no evidence of collagen vascular disease. Male sex is another factor that has been linked to an increased clinical likelihood of IPF. When the clinical context is indeterminate for
IPF, a diagnostic biopsy is required to make a confident diagnosis, regardless of the pattern on HRCT.

2. UPGRADE OF POSSIBLE UIP PATTERN TO PROBABLE

The possible UIP pattern according to the 2011 guidelines (ie reticular pattern with bibasilar and peripheral traction bronchiolectasis/bronchiolectasis) has been upgraded to probable. This is not just a formality but has direct clinical implications. The presence of a probable UIP pattern within the clinical context of IPF obviates the need for tissue based diagnosis. This is a major breakthrough as honeycombing is no longer a prerequisite to avoid surgical lung biopsy. Peripheral distribution of fibrotic findings plays a pivotal role12.

3. INTRODUCTION OF INDETERMINATE FOR UIP PATTERN

A new CT category is introduced, indeterminate for UIP. It would be extremely convenient to easily categorize everything in boxes but this is not possible and IPF is no exception to this. IPF is actually a great mimic from a radiology point of view13. In almost one third of cases with UIP there is evidence of fibrosis that does not follow a predominantly peripheral and bibasilar distribution. In these patients even if in the clinical context of IPF, SLB is necessary in order to secure diagnosis.

4. INTRODUCTION OF SUBPLEURAL SPARING IN THE CT FEATURES MOST CONSISTENT WITH NON-IPF DIAGNOSIS

Subpleural sparing is considered a strong predictor of NSIP pathology14. Until know it was not included in diagnostic guidelines. Fleischner Society gives an important diagnostic role to subpleural sparing. Alongside upper or mid-lung predominant fibrosis and peribronchovascular predominance are considered as most consistent with non-IPF diagnosis.

5. FORMAL INTRODUCTION OF “WORKING DIAGNOSIS OF IPF”

As mentioned earlier, there are cases where SLB is necessary to secure diagnosis. However, in clinical practice this is not always feasible for a variety of reasons,
i.e. poor performance status or refusal of the patient. It is also important to keep in mind that in patients with Interstitial Lung Diseases (ILDs), SLB can trigger an acute exacerbation. Worryingly, the clinical context of IPF (male sex, increased age) actually increases the risk for acute exacerbation\textsuperscript{15,16}. Thus the decision on a surgical biopsy should not be taken lightly but after careful examination of the clinical benefit versus clinical risk for each patient.

The Fleischner Society diagnostic criteria for IPF were much needed. The previous ATS/ERS/JRS/ALAT guidelines were outdated and actually impossible to follow in every day clinical practice given the significant new findings in the field of IPF. Refining the role of HRCT regarding the diagnostic certainty of underlying UIP pathology gives us the ability to confidently establish a diagnosis of IPF without subjecting the patient to potentially life threatening diagnostic procedures. Also, defining pretest probability and embedding it into the diagnostic algorithm makes common clinical sense (unfortunately not always common practice) a central pillar of the diagnostic algorithm. Finally, taking consideration of real-life difficulties into account rationalize the term “working diagnosis" and made it a part of these diagnostic criteria. However, the practical application of these guidelines is not an easy task. The identification of the probable UIP pattern, the differentiation between probable UIP and indeterminate for UIP pattern, establishing a “working diagnosis” of IPF and in general applying differential diagnostics within the spectrum of ILDs, requires expert knowledge from multiple specialties in the context of a reference center\textsuperscript{17}. The upcoming ATS/ERS/JRS/ALAT guidelines for IPF are greatly anticipated.

\textbf{REFERENCES}


\textbf{TABLE 1.} Fleischner Society diagnostic criteria for IPF. Clinical implications

- It is mandatory to define the clinical likelihood for IPF before interpreting other diagnostic tests (pretest probability)
- Appropriate clinical context for IPF requires all of the following: age >60, absence of significant exposure and no evidence of collagen vascular disease
- Honeycombing is no longer required for a non invasive diagnosis of IPF
- In the appropriate clinical context, the presence of fibrosis (traction bronchiectasis) in a predominantly basal and subpleural distribution (probable UIP pattern) leads to a confident diagnosis of IPF
- The presence of fibrotic changes in the absence of predominantly peripheral distribution is indeterminate for UIP and mandates tissue based diagnosis
- Subpleural sparing points to a non IPF diagnosis
- Tissue based diagnosis is not feasible in every patient. In such cases, experts can establish a “working diagnosis" of IPF based on history, clinical examination, HRCT findings, pulmonary function tests, progression over time

\textbf{FIGURE 3.} Fine reticulation and areas of increased attenuation (ground glass) following a peribronchial distribution. There is mild bronchial distortion (white arrows). Characteristically, the abnormal findings spare the subpleural area (subpleural sparing). This is a pattern most consistent with a non-IPF diagnosis. Surgical lung biopsy demonstrated findings compatible with hypersensitivity pneumonitis.
Recurrence in patients that underwent surgery for lung cancer

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INTRODUCTION

It is well known that Lung Cancer is now the leading cause of cancer death for both males and females being responsible for 20% of cancer related deaths worldwide¹. The total number of patients dying from lung cancer outranges the number of patients dying from breast, prostate and bowel malignancies altogether². Every year 1.800.000 new cases of lung cancer are diagnosed, causing 1.180.000 deaths yearly. In the U.S.A. 210.000 new patients with lung cancer are diagnosed every year causing 157.000 deaths whereas in Europe there are 400.000 new cases each year³. According to unofficial records released by the Oncology Department of 3rd University Medical Clinic of”Sotiria”Hospital, 2000 new cases of lung cancer are grossly diagnosed in Greece yearly with 10.000 doctors from various specialties involved with their care such as Chest Physicians, Oncologists, Thoracic
Surgeons, Radiotherapists, Pathologists etc.

Only a 15-20% of those patients are eligible to undergo surgical therapy with the vast majority of them receiving chemotherapy and radiotherapy. Surgical resection is the only type of treatment with curative intent and can without any doubt, prolong survival and improve quality of life for these patients. One of the few weaknesses of surgical treatment for lung cancer is the possibility of cancer recurrence, appearing either locally at the area of operation or distally to the initial primary lesion.

All stages of lung cancer should be considered for surgical treatment if certain criteria are fulfilled. Stages I and II have absolute indication for resection, stage III can sometimes be operated, whereas stage IV is rarely operable. Patients that are initially subjected to surgical resection of cancer are always under regular follow-up for at least 5 years because of the following reasons:

1. Identifying any early or late complications that can occur postoperatively.
2. Determine the disease free interval in case of disease recurrence.
3. Determine the overall survival postoperatively.
4. Identify any disease recurrence.
5. Diagnose any second or metachronous primary cancer.

AIM

A brief review of the literature on this topic reveals no studies reporting lung cancer recurrence post-surgery in Greece. Main goal of this study was to report and analyse results of data collected from lung cancer recurrences postoperatively through a systematic and thorough follow-up of patients with primary Non-Small Cell Lung Cancer who were initially operated with curative intent from the same surgeon at “Sotiria” Athens Chest Diseases Hospital.

This study was mainly conducted to evaluate current data on lung cancer recurrences postoperatively and investigate the biological pathways thought to be responsible for cancer recurrence. Secondary this study identifies methods for early detection of tumor recurrence and suggests possible prognostic factors for resected lung cancer recurrence. Finally all the necessary clinical tests for the evaluation of fitness for surgery and the most common surgical methods are mentioned.

MATERIALS AND METHODS

Three Hundred and fifty patients (350) in total were enrolled in this study. All of them were operated by the same surgeon at the Thoracic Surgery Department of “Sotiria Regional Chest Diseases Hospital”, Athens, Greece, during a time period of 15 years (1995-2010). Eligible patients were initially treated with surgery for primary Non-Small Cell Lung Cancer and were systematically followed-up for at least 5 years postoperatively. Depending on the decision of a Multi-Disciplinary Oncology Meeting following surgery, doses of adjuvant chemotherapy or radiotherapy were administered to the patients who would benefit from adjuvant therapy.

In the present study only major lung resection procedures were included, meaning lobectomies and pneumonectomies. Mini resections such as wedge excisions and segmentectomies were excluded from this paper. Patients did not have a routine PET scan prior to surgery, because Patient recruitment started in 1995 and ended in 2010.

All patients were operated under general anaesthesia and intubated with double lumen tube to achieve isolation of the operated lung. Patients with central tumors underwent rigid bronchoscopy at the beginning of the operation for re-evaluation of the endobronchial extend of their disease. A typical posterolateral thoracotomy approach was applied with resection of the lung parenchyma involved by the tumor (lobectomy, bilobectomy or pneumonectomy), followed by systematic mediastinal lymph node sampling.

The systematic follow-up was conducted by the operating surgeon following guidelines from EACTS and ACCP, taking under consideration the characteristics of the Greek National Health System, demographics and location of each individual patient.

Each follow-up appointment included documentation of patients medical history and a complete physical examination of each one of them. A routine chest X-ray was done 15 days, 1 month, 4 months, 9 months and 12 months post surgery. At the time of 1 year postoperatively, a CT scan of the Thorax was performed, a full set of blood tests including cancer serum markers, as well as a flexible bronchoscopy for visual examination of the bronchial stump and cytology specimen examination of bronchial washing and brushing.

During 2nd and 3rd year of follow-up, patients medical history, complete physical examination and chest X-ray was conducted every 4 months while CT scan of chest
and upper abdomen was repeated every 6 months. For the 4th and 5th year post surgery patients medical history, complete physical examination and chest X-ray was conducted every 6 months while CT scan of chest and abdomen repeated yearly.

Between patients follow-up appointments if any symptom or radiographic study was suspicious for recurrence, a complete focal workup was done trying to confirm the diagnosis\textsuperscript{21}. If cancer was detected and proven after tissue diagnosis then the patient was referred to a Multi Disciplinary Oncology Meeting. The MDT decided on whether it was lung cancer recurrence or a second primary and which should be the optimal treatment plan. According to the MDT decision, patient received chemotherapy with or without radiotherapy or were referred back to the surgeon\textsuperscript{22}. The surgical options were completion pneumonectomy of the remaining lung parenchyma or metastatectomy of the tumor wherever that was technically feasible.

The systematic review of literature was conducted using data from U.S. National Library of Medicine through pub med website. Key words that were used for this search were: recurrence in patients with NSCLC, recurrence in patients with NSCLC after surgery, local and distant failure lung cancer, recurrence patterns NSCLC. All studies were reviewed and studies non relevant to the topic were excluded from review. A simple statistical analysis was performed, mainly using mean and median values of documented data. This study was approved by the Scientific and Ethical Committee of this Hospital for the collection and analysis of patients data operated in this department.

RESULTS

All 350 patients in total that were enrolled in this study were initially operated with curative intend for Non-Small Cell Lung Cancer in Thoracic Surgery Department of “Sotiria Regional Chest Diseases Hospital”, between the years 1995-2010. The same surgeon performed all surgical procedures and conducted the patients systematic follow-up and documentation of relevant data. Patients that required adjuvant therapy\textsuperscript{23} were treated at the Oncology Department of 3rd University Medical Clinic of “Sotiria Athens Chest Diseases Hospital”. In case of cancer recurrence patients were discussed at the Multi-Disciplinary Oncology Meeting with chest physicians, oncologists and the operating surgeon to determine optimal treatment strategy\textsuperscript{22}. Lung cancer staging was based on the 6th revision of the TNM staging system that was in use until the beginning of 2010\textsuperscript{24}.

Two Hundred and eight patients (280) out of the 350 in total were male with ages ranging from 42 to 82 (mean age 59). Male to female ratio was 7 to 1. Tumor histology was: 175 lung adenocarcinoma (50%), 140 squamous cell lung carcinomas (40%), 28 undifferentiated large cell lung carcinomas (8%) and 7 mixed type of adenosquamous lung carcinomas (2%).

Disease staging was found as follows:
- Stage I 118 patients: IA (T1N0) 38 patients, IB (T2N0) 80 patients
- Stage II 146 patients: IIA(T1N1) 18 patients, IIB(T2N1) 85 patients
- Stage IIIA 86 patients: (T3N1) 21 patients, (T2N2) 45 patients, (T3N2) 20 patients

Most common Lung cancer stage was IIB (T2N1) with 85 patients (24.3% of total number pf Pt), followed by IB (T2N0) with 80 patients (23%) and IIIA (T2N2) with 45 patients (13%).

Three Hundred and fifty (350) operations in total were conducted as follows:
- 158 Lobectomies
- 42 Bilobectomies
- 150 Pneumonectomies.

Of the 150 pneumonectomies 92 were done at the right side and 58 at the left while 120 of them were typical and 30 intrapericardial.

Most common procedure was the right upper lobectomy, whereas 22 out of 42 bilobectomies were upper (upper and middle lobes) and the other 20 were lower (middle and lower lobes)

All procedures included a systematic mediastinal lymph node sampling. This procedure was preferred because of the similar oncological results according to current literature (ACOSOG Z0030 study)\textsuperscript{25} as it is reported to have similar overall survival and seems to be superior than mediastinal nodal dissection in terms of surgical time and complications\textsuperscript{26}.

One hundred and thirty (130) out of the 350 patients (37%) were diagnosed with disease recurrence during the first 5 years of follow-up\textsuperscript{27}. Type of recurrence varied between local, distal metastases or both. The vast majority of recurrences (110 patients-90%) occurred in the time period of 18 to 36 months post-surgery. Out of the 130 patients found with recurrence: 31 (24%) had local recurrence, 65 (50%) had distant metastases and 34 (26%)
had both. Twelve patients were diagnosed with a second metachronous lung primary cancer thus all of them were excluded from this study.

350 patients → 130 recurrences (37%)

Among all cases of local recurrences, 17 patients had mediastinal disease recurrence, 10 within lung parenchyma and 4 on the parietal pleura.

Distant metastases were located in the brain for 20 of the cases, in the bones for 11 cases, in the liver for 8 cases, in adrenal glands for 6 of the cases and for 5 of the cases in soft tissues.

Local: 31 (24%)
Distal: 65 (50%)
Both Local and distal: 34 (26%)

Thirty four patients developed both local and distance recurrences of disease. Out of those there were 13 with combined lung and brain secondaries or combined lung and adrenal gland lesions with mediastinal involvement in some of those cases.

**DISCUSSION**

Despite the fact that surgical treatment of early stages I and II is done with curative intent, it is well known that a considerable number of patients (30% to 50%) develop disease recurrence postoperatively and eventually die of this. Therefore a question arises why someone with early stage lung cancer that underwent radical surgical resection with both macroscopically and microscopically free resection margins (R0 Resection), develop disease recurrence.

The development of post-surgery recurrence could physiologically be explained through the following 2 pathways:

1. **Existence of micro metastatic disease at the time of surgery**

This is disease that is not clinically evident (lesions with diameter of 0.2-2mm with an optical microscope of 5x) that is caused by solitary cancer cells (occult micro metastatic cells <0.2mm), which travel from the primary lesion through the blood stream to a distal site. If those cells manage to survive at the microenvironment of that distal site, they divide and grow forming a metastatic tumor. On the other hand any lesion identified at optical microscopical examination with size larger than 2mm is consider as a satellite tumor.

It is very likely that those occult metastases existed from the time of surgery but all available classic imaging tests (CT, MRI, Bone scan) as well as the biological imaging tests (PET Scan, PET CT) for staging of lung cancer were not able to identify those lesions due to their size. Tumor cells that could have been identified at the time of surgery are those circulating in peripheral blood stream (CTC – circulating cancer cells) or tumor cell deposits within the bone marrow or locoregional lymph node stations. CTC existence is still a field of debate and is still not clear if they represent a bad prognostic factor of tumor recurrence. Bone marrow microscopical tumor deposits is also not clear if can be considered as true bone metastasis. In current literature there is no clear evidence of correlation between CTC presence and reduced Lung cancer survival.

2. **Dissemination of tumor cells from primary tumor (DTC)**

During manipulations upon lung parenchyma that are necessary for lung resection some tumor cells might disseminate from primary lesion and spread through the blood stream. This is the main reason why some surgeons suggest that division of the pulmonary vein related to the tumor lobe (vessel that drains blood from the tumor) should be done first and prior to division of pulmonary artery (vessel that perfuses lung parenchyma with the tumor) when operating on lung cancer so that the spread of DTC risk is reduced. It is easy to conclude that DTC can cause disease recurrence at a later time than CTC.

Most of lung cancer recurrences post-surgically (90%) appear during the first two years postoperatively. They can occur either locally or distantly to primary tumor and most of the times are lethal within 1 year from the time of their diagnosis. The term local metastasis or local recurrence is defined as tumor growth at the surgical resection margins (bronchial stump, fissure, vessel stumps), at lung hilum or mediastinum. All other metastasis are considered as
distal metastases or recurrences. It is of great importance to define the type of metastasis (local or distal) in all studies investigating post-surgical recurrences of primary lung cancer and when this event was diagnosed if both types coexist and which one appeared first. On top of that it is important to exclude cases of second metachronous primary lung cancers.

One interesting question that arises is whether prognosticators for tumor recurrence of patients operated for primary lung cancer with curative intent exist. A review in relevant literature reveals that those prognosticators can be classified into two categories:

1. Factors of classical determination that are related with TNM staging system of primary tumor

Tumor diameter (>3cm and >5cm), presence of N1 or N2 involvement, advanced TNM stage, presence of symptoms, bad patients performance status, cessation or not of smoking, high SUV values on PET /CT, low tumor differentiation on pathology examination, less than lobectomy resection, invasion of blood vessels and lymphatics within tumor, invasion of visceral pleura, microscopically positive resection margins (R1) and neglect ion of typical lymph node dissection or systematic sampling as well as adjuvant chemotherapy or radiotherapy administration postoperatively.

2. Factors that can be identified with molecular biology techniques

It is very well known that lung cancer is an aggressive type of neoplasm that has many histological subtypes, each one of them with different molecular biology characteristics. The clinical behaviour of the tumor is related to many genetic alterations related to cell cycle and cell division, apoptosis, mutations, chromosomal dislocations and angiogenesis.

Another logical question arises next: Is there a way to prevent recurrences post primary lung cancer surgery from happening, delay them or reduce their extend?

Means that we have so far are well-established: Induction chemotherapy (doses of chemotherapy administered prior to surgery), adjuvant chemotherapy (doses administered days or weeks post-surgical treatment), adjuvant radiotherapy and quite recently developed targeted molecular drug therapy. The role of chemotherapy either in form of induction or adjuvant treatment has been thoroughly investigated over the last years so that we are now aware of pros and cons of each one of those, and the overall survival benefit to the patient which is around 5% in 5 years’ time.

Today, there is no question that chemotherapy is of great importance for the patient, regardless if it is in form of induction or adjuvant treatment, as long as patient receives it. The fact is that almost 90% patients could undergo induction chemotherapy, but only a 60% of them could receive adjuvant chemotherapy because of various reasons. Someone could conclude that if adjuvant chemotherapy could be administered in larger proportion of patients then survival benefit would outrange survival benefit from induction chemotherapy.

It is of general agreement that adjuvant radiotherapy, usually received by patients with N2 disease, also known as “mediastinal lymph node sterilization” is important for local disease control but there are no evidences of increasing overall survival.

In the near future it is more than clear that individualized molecular targeted drugs will play a very important role in prevention of metastases development post surgically and will change the natural history of disease recurrences. Targeted treatment in lung cancer is mainly classified into two categories: inhibitors of intracellular Epidermal Growth Factor Receptor (EGFR) and monoclonal antibodies targeting extracellular receptor of Vascular Endothelium (VEGF).

Tyrosine Kinase Inhibitors (Erlotinib, Gefitinib, Giotrif etc.) are targeting cancer cell intracellular receptor by blocking receptor phosphorylation, thus blocking cell cycle and cellular division of cancer cells and inducing apoptosis.

Bevacizumab is a monoclonal antibody that binds on VEGF and inhibits VEGF biological pathway, blocking angiogenesis and stops tumor growth.

Immune therapy in lung cancer is based on principles of immune reaction against an unknown antigen to the human body. More specifically, when a foreign antigen like a cancer cell for instance, is detected, two main defence systems are activated: natural and acquired immunity. Natural immunity is the first defence line after a bacterial od cancer cell invasion. There are many types of cells involved in natural immune system like macrophages, natural killer cells that bind to the cancer cells and destroy them and dendritic cells. Dendritic cells are antigen – presenting cells that detect foreign antigens and present their peptides to other cells of the immune system. At a later stage dendritic cells present antigen peptides to the lymph cell system (T and B lymphocytes) and this is how acquired immunity is developed. This
type of immunity if very target-specific because of the T and B receptors that identify specific antigen peptides.

Once T lymphocytes are activated through dendritic cells inside a lymph node, they travel through blood stream to detect and destroy any intruder like a cancer cell. T lymphocyte activation from dendritic cells is achieved through 2 steps (signal 1 and 2). During those steps surface antigens of T lymphocyte and dendritic cell come in contact and identify each other.

According to current practice lung cancer patients will receive cisplatin based chemotherapy as indicated. Adjuvant chemotherapy is mainly administered to the subgroup of patients that have lymph node involvement or tumor greater than 4 cm in diameter. At the moment the use of targeted molecular treatment and immune therapy as an adjuvant to surgery with curative intent for early stage lung cancer is under investigation, as a very high proportion (up to 50%) of patients receiving radical lung cancer resection eventually develops disease recurrence.

A recent Phase II trial that investigated the benefit of adding bevacizumab to adjuvant chemotherapy in early stage lung cancer patients that received chemotherapy post-surgical treatment had disappointing results. According to the Eastern Cooperative Oncology Group 1505 study, 1500 patients that were operated because of lung cancer stage IB, II, IIIA were randomized post-surgery and underwent 4 cycles of platinum based chemotherapy with or without adding bevacizumab. Final result of study is that there was no significant difference observed in overall survival in the two groups of this study (Hazard ratio 0.98, p value: 0.75).

RADIANT study has evaluated the role of Erlotinib in adjuvant treatment of patients with EGFR mutation that were operated for early stage non-small Cell lung cancer. Final results showed no improvement in disease free survival between the Erlotinib group and the placebo group (hazard ratio 0.9, p 0.324). Similar studies are running at the moment in Asia for such patients positive for EGFR mutations.

As for the role of immune therapy in adjuvant treatment of patients operated for early stage non-small Cell lung cancer, various studies have been conducted worldwide. MAGRIT study, that was an international double blind randomized study, has recently announced its results, where it’s been shown that Adjuvant treatment with MAGE-A3 adjuvant immune therapy did not manage to increase disease free survival, compared to placebo treatment in patients that express gene 3 – MAGE family gene.

National Clinical Trials Network located in U.S.A. is conducting a clinical trial (ALCHEMIST STUDY) investigating molecular targeted drugs as adjuvant therapy in stage IB, II, IIIA lung cancer patients that received surgical treatment with curative intent. Postoperatively, patients have a full molecular profile test done, including EGFR mutation and ALK translocation presence. Following the positive patients for the above molecular tests the proper target inhibitor is administered, or a placebo drug. For the patients without those mutations (EGFR-, ALK-) after giving systematic chemotherapy, Nivolumab immune therapy follows or just observation.

This study is offering dep gene analysis on all patients enrolled, in order to clarify the molecular parameter that favours disease recurrence or sensitivity on specific anti-neoplastic agents of adjuvant chemotherapy.

Finally, Phase II and III clinical studies are running, comparing combination of surgical treatment after induction chemotherapy or before adjuvant therapy with molecular targeted drugs instead of conventional chemotherapy. The Western Japan Oncology Group phase III randomized trial is such a study that compares surgical treatment of stage II, IIA non-small Cell Lung Cancer, combined either with adjuvant chemotherapy (Gefitinib) or with tyrosine kinase inhibitors (TKI’s) if patients are positive for EGFR mutation. It is obvious that more studies are needed on targeted therapies before or after surgical treatment, so that there will be solid evidence on their effectiveness.

DISCUSSION

Unfortunately, it is a very unpleasant reality that lung cancer patients that underwent surgical resection have a high recurrence rate. It is still a fact that surgical resection is the only treatment strategy with curative intent, as chemotherapy and radiotherapy are as it is well known, adjuvant or/palliative treatment strategies. Surgical therapy of early stage lung cancer appears to correlate with very low recurrence rates, and on the other hand, the more advanced lung cancer is, the recurrence rate is higher, although not proportionally.

On top of all that, the well-known and established prognostic factors are playing important role in disease recurrence and overall survival. Factors that are related to molecular biology cancer pathways are a relatively new weapon in diagnosing and can clarify many aspects of biological tumor behaviour and define, up to a certain level, response to treatment. As for the role of targeted molecular therapy and immune therapy in adjuvant
treatment of early stage Non-Small Cell Lung Cancer, the international scientific community is investigating their effectiveness in increasing overall survival as the recurrence rate of lung cancer, post complete surgical tumor resection, is reportedly up to 50%.

Fighting lung cancer seems to be a problem for many years to come, especially if smoking still remains a bad human hobby. This fight that involves many different medical specialties with various treatment strategies that can be followed, has to be fought with great caution and alignment to the international guidelines with reassurance that this practice will be individualized to each every patient. Undoubtedly a complete clinical investigation of such a complex biological procedure as lung cancer recurrence, demands a multi-disciplinary approach of doctors with various specialties such as oncology, pulmonology, radiology and thoracic surgery.

Finally a special interest has to be shown on strict and regular follow-up of patients with lung cancer, that has to be systematic and thorough, since it is a sine-qua-non feedback factor, of every treatment method and practice for the clinician doctor.

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INTRODUCTION: Tuberculosis remains a serious threat to public health and one of the leading causes of death among infectious diseases. Monitoring treatment outcome is necessary in order to assess the effectiveness of the therapeutic intervention and to identify possible obstacles to disease control. In that setting outcome is an important indicator of the effectiveness of TB control programs.

MATERIAL AND METHODS: This is a retrospective study of TB patients registered at the Department of Pulmonary Medicine Aristotle University of Thessaloniki (AUTH), in the period from January 1st, 2012 to December 31st, 2014.

RESULTS: 89 patients aged 49.5±19.20 years were recorded. 67.5% were men. 68.5% of patients were of Greek origin and 91% suffered from pulmonary TB. In 78.7% of patients the diagnosis was confirmed microbiologically. 11.2% presented with monoresistance or polydrug resistance to anti-TB drugs. The positive outcome rate of the TB treatment was 67.5%, of which 38.2% were cured and 29.3% completed the treatment. The outcome was negative in 32.5% of patients and rates that corresponded specifically to death, treatment failure, loss to follow-up and lack of evaluation were 4.5%, 1.1%, 2.2% and 24.7% respectively.

CONCLUSION: The positive outcome rate of TB among patients was lower than the WHO global target of 85%. There is a clear need for a comprehensive management of problems in TB monitoring in Greece, both in terms of its impact and its outcome.

Pneumon 2017, 30(3):141-150.

INTRODUCTION

Despite the ongoing medical progress, tuberculosis (TB) is still a serious threat to public health and one of the leading causes of death among infectious diseases\(^1\). It is estimated that in 2015, the incidence of TB was
10.4 million cases worldwide, with 60% of them occurring in six countries (India, Indonesia, China, Nigeria, Pakistan and South Africa). In addition, about 480,000 new cases of multidrug-resistant TB and 100,000 cases with rifampicin resistance were recorded.

In the European Region of the World Health Organization (WHO) it is estimated that in 2015 there were 323,000 cases of TB that correspond to 35.5 cases per 100,000 people, and this accounts for about 3.0% of the global burden of TB, with 85% of all cases occurring in 18 countries.

In 2015, 482 cases were reported in Greece and as a result, the average incidence of the disease is approximately 4.4 per 100,000 people. Over the four years 2010-2014, Greece was one of the few countries in Europe that showed an increasing trend in TB incidence, with this increase being mainly related to the native population and not to foreign patients, while 2015 a small decrease in the incidence of the disease was observed. In Greece the disease is underreported and it has been shown that only 1/3 of treated TB cases are recorded.

In 1994 the WHO declared TB a “global emergency” because of the concern over the disease’s extent in most of the developing world. Over the last 22 years, strategies for the control of TB have been proposed at a global level. In 1995, the implementation of the Directly Observed Therapy (DOTS) program was proposed. In 2001-2005 the “first global plan to stop TB” was launched and in 2002 a global fund was created favoring access to international funding. In 2006 the WHO launched an enhanced strategy called “Stop TB Strategy” in order to achieve universal access to health services for people with TB, while the second “global plan to stop TB” covered the period 2006-2015. The new “global TB strategy after 2015” adopted by the 67th World Health Assembly (WHA) in May 2014 has set itself the objective of ending by 2035 the TB epidemic, “the end TB strategy”, by decreasing deaths from TB by 95% compared to 2015 and the incidence of the disease by 90% compared to 2015.

All those measures led to a slow and steady decrease in the incidence of the disease from 1997 to 2001, with an increase in 2001, when the number of cases among HIV-infected patients in Africa increased. Since then, TB incidence has been decreasing. TB mortality has been diminished by 47% between 1990 and 2015 and it is estimated that 43 million lives have been saved from 2000 to 2014.

Treatment outcome is an important indicator of the effectiveness of TB control programmes. Monitoring the outcome of treatment is necessary to assess the effectiveness of treatment but also to identify possible obstacles to disease control. Sadly, there is no systematic recording of the treatment’s outcome in Greece. Greece was not included in the study on the outcome of TB treatment in the European Union and the European Economic Area for the period from 2002 to 2011 because it was one of the countries that did not provide any data for any year of that decade.

In this context, this study aims to record the outcome of TB treatment at the Department of Pulmonary Medicine, AUTH, at “G. Papanikolaou” General Hospital of Thessaloniki and to identify the factors potentially associated with a negative outcome.

**MATERIAL AND METHODS**

This is a retrospective study of patients with TB registered at the Department of Pulmonary Medicine, AUTH, between January 1st, 2012 and December 31st, 2014. Data were collected from patients’ records and include social and demographic factors and data related to both the disease itself and treatment outcome.

The Department of Pulmonary Medicine, AUTH started its operation in 1966. The TB out-patient clinic has been operating in its current form since July 2011, twice a week.

In the present study patients’ gender, age, somatometric characteristics, country of origin, smoking habits, and co-morbidities were recorded.

TB cases were classified according to WHO’s 2013 definitions that were revised in December 2014 as follows:

- based on the method of diagnosis a) bacteriologically confirmed TB cases - cases in which a biological specimen is positive by smear microscopy, culture or nucleic acid amplification tests-NAATs and b) clinically diagnosed TB cases - cases that have been diagnosed with active TB by a clinician who has decided to initiate a full course of TB treatment. This definition includes cases diagnosed on the basis of X-ray abnormalities or suggestive histology and extrapulmonary cases without laboratory confirmation.
- based on anatomical site of disease a) pulmonary TB - any bacteriologically confirmed or clinically diagnosed case of TB involving the lung parenchyma or the tracheobronchial tree as well as miliary TB and b) extrapulmonary TB- any bacteriologically confirmed or clinically diagnosed case of TB involving organs other than the lungs, e.g. pleura, lymph nodes, ab-
domen, genitourinary tract, skin, joints and bones, meninges. In case of coexistence of pulmonary and extrapulmonary localization, the case is classified as pulmonary TB.

• based on the history of previous TB treatment, cases are classified as (a) new patients - patients who have never been treated for TB or have taken anti-TB drugs for less than 1 month and (b) Previously treated patients - patients who have received 1 month or more of anti-TB drugs in the past.

• based on the resistance to anti-TB drugs, cases are classified as: a) monoresistance - resistance to one first-line anti-TB drug only, b) polydrug resistance - resistance to more than one first-line anti-TB drug (other than both isoniazid and rifampicin), c) multidrug resistant TB, MDRTB - resistance to at least both isoniazid and rifampicin and e) extensively drug resistant TB (XDRTB) - resistance to any fluoroquinolone and to at least one of three second-line injectable drugs (capreomycin, kanamycin and amikacin), in addition to multidrug resistance.

Regarding TB outcome, WHO definitions were used:

Cured - A pulmonary TB patient with bacteriologically confirmed TB at the beginning of treatment who was smear- or culture-negative in the last month of treatment and on at least one previous occasion.

Treatment completed - A TB patient who completed treatment without evidence of failure but with no record to show that sputum smear or culture results in the last month of treatment and on at least one previous occasion were negative, either because tests were not done or because results are unavailable.

Treatment failure - A TB patient whose sputum smear or culture is positive at fifth month or later during treatment.

Death - A TB patient who dies for any reason at starting or during the course of treatment.

Lost to follow-up - A TB patient who did not start treatment or whose treatment was interrupted for 2 consecutive months or more.

Not evaluated - Refers to patients for whom an exact outcome of treatment has not been defined, for example because they were transferred to another center and the result is unknown.

This study includes bacteriologically confirmed cases but also clinically diagnosed cases, cases of pulmonary and extrapulmonary TB, newly diagnosed cases but also patients with a history of TB and cases of monoresistance and poly resistance. The study excluded patients with multidrug-resistant TB (MDRTB) and those with extended resistant TB (XDRTB). Favorable-positive outcome of the anti-TB therapy means the cure and completion of treatment. In contrast, negative outcome was considered that of patients whose treatment failed, those who died, patients lost to follow-up and the group of patients who were not evaluated.

An Excel spreadsheet was used to process and study the data, and all the data gathered were recorded therein. Then, the \( \chi^2 \) test was applied by the use of the Chi-Square Calculator freeware. (http://www.socscistatistics.com/tests/chisquare/Default2.aspx).

**RESULTS**

As evidenced by this study’s data, 89 patients aged 49.5 ±19.20 years were recorded in the TB clinic in the three-year period 2012-2014. The distribution of patients per year was 21 patients (23.6%) in 2012, 34 (38.2%) in 2013 and 34 (34.8%) in 2014. Most were men (67.5%) and of Greek origin (68.5%). 31.5% of all patients were foreigners, specifically: 8 from Georgia, 6 from Albania, 4 from Pakistan, 2 from Bulgaria and 1 patient from each of the following countries: Armenia, China, Moldova, Bangladesh, Romania, Russia, Ghana and one of unknown origin. The demographic characteristics are presented in detail in Table 1.

91% of the patients suffered from pulmonary TB and only 9% from extrapulmonary TB, especially of the

<table>
<thead>
<tr>
<th>TABLE 1. Social and demographic data</th>
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<tbody>
<tr>
<td>Patients characteristics</td>
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<tr>
<td>---------------------------</td>
</tr>
<tr>
<td>Number of patients</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Greek</td>
</tr>
<tr>
<td>Foreigners</td>
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</table>
pleura (Table 2). Most (86.5%) of the cases were new. In 78.7% of patients (70 persons) the diagnosis was microbiologically confirmed, of which 46 patients showed positive AFB (acid-fast bacilli) smears, and 14 were NAATs positive and AFB-negative. 11.2% had monoresistance or polydrug resistance to anti-TB drugs, of which 50% were of Greek origin (Table 3). 58.4% were negative for HIV co-infection, while in 41.6% testing was not recorded. 62.9% of all patients presented with co-morbidities (Table 4). Regarding smoking, 35.9% were active smokers, 4.5% were ex-smokers, 22.5% were non-smokers, and 37.1% provided no data on their smoking habits.

The positive outcome rate of the TB treatment was 67.5% (60 patients), of whom 34 patients (38.2%) were cured and 26 patients (29.3%) completed their treatment. Negative outcome was recorded in 32.5% of patients (29 patients). More specifically, the rates that correspond to death, treatment failure, lost to follow up and not evaluated were 4.5% (4 patients), 1.1% (1 patient), 2.2% (2 patients) and 24.7% (22 patients) respectively (Figure 1). The annual outcome of patient treatment is shown in Figure 2.

As shown in Table 5, no statistically significant factors were found that could be implicated in the negative treatment outcome. Patients who died during TB treatment were two men and two women aged over 70 years, of Greek origin with pulmonary drug-susceptible TB and with co-morbidities (three patients with cardiovascular problems and one with chronic obstructive pulmonary disease). Death in the first three patients occurred on the 13th, 30th, 42nd day of treatment and was attributed to TB. The fourth patient died of an unrelated to TB cause in the 9th month of treatment while showing significant clinical and radiological improvement.

**DISCUSSION**

Based on the results of the present study, the positive outcome rate in TB patients at the Department of Pulmonary Medicine, AUTH, during the three-year period 2012-2014 was 67.5% in total, and specifically 76.2%, 61.7% and 67.6% in the years 2012, 2013 and 2014 respectively. This rate is certainly lower than the recommended WHO

<table>
<thead>
<tr>
<th>TABLE 2. Anatomical site of disease</th>
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</thead>
<tbody>
<tr>
<td><strong>Anatomical site</strong></td>
</tr>
<tr>
<td>Pulmonary</td>
</tr>
<tr>
<td>Extrapulmonary</td>
</tr>
<tr>
<td>Pleura</td>
</tr>
<tr>
<td>Lymph nodes</td>
</tr>
<tr>
<td>Spine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 3. Number of patients with drug resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patients with resistance (n,%)</strong></td>
</tr>
<tr>
<td>Isoniazid</td>
</tr>
<tr>
<td>Rifampicin</td>
</tr>
<tr>
<td>Streptomycin</td>
</tr>
<tr>
<td>Ethambutol</td>
</tr>
<tr>
<td>Pyrazinamide</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 4. Co-morbidities of patients with tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of patients (%)</strong></td>
</tr>
<tr>
<td>Number of patients with co-morbidities</td>
</tr>
<tr>
<td>Patients with one concomitant disease</td>
</tr>
<tr>
<td>Patients with more than one concomitant disease</td>
</tr>
<tr>
<td>Central nervous system diseases</td>
</tr>
<tr>
<td>Cardiovascular system diseases</td>
</tr>
<tr>
<td>Respiratory diseases</td>
</tr>
<tr>
<td>Gastrointestinal disorders</td>
</tr>
<tr>
<td>Diseases of the genitourinary system</td>
</tr>
<tr>
<td>Diseases of the musculoskeletal system</td>
</tr>
<tr>
<td>Diabetes mellitus and metabolic diseases</td>
</tr>
<tr>
<td>Patients with mental disorders</td>
</tr>
<tr>
<td>Autoimmune Diseases</td>
</tr>
<tr>
<td>Patients with immunosuppression</td>
</tr>
<tr>
<td>Patients with neoplastic disease</td>
</tr>
<tr>
<td>Viral hepatitis</td>
</tr>
<tr>
<td>Alcoholism</td>
</tr>
<tr>
<td>Users of intravenous substances</td>
</tr>
</tbody>
</table>
FIGURE 1. Tuberculosis treatment outcome for the three year period 2012-2014.

FIGURE 2. Annual and total tuberculosis treatment outcome.
target rate of 85%\(^1\). It is also evident that: 1) the highest number of patients with negative outcome corresponds to patients who were not evaluated, 2) TB remains a cause of mortality and 3) for a large number of patients testing for HIV co-infection was not recorded. No statistically significant factors that could be implicated in the negative treatment outcome were found. It is worth noting that the rate of microbiological confirmation of the disease was high (78.7% of all patients).

As described above, according to our data, 38.2% of patients were cured and 29.3% completed treatment, thus resulting in a total positive outcome of 67.5%. According to the WHO report published in 2017 regarding the surveillance and monitoring of TB in Europe, the positive outcome of TB treatment in our clinic was higher than in Cyprus (58.8% in 2014), Denmark (58.1% in 2014) and Germany (60.1% in 2014)\(^1\) and lower than other European countries such as Albania (88.2% in 2014), Austria (73.1% in 2014), Belgium (79.7% in 2014), Bulgaria (84.9% in 2014), the Former Yugoslav Republic of Macedonia (86.8% in 2014) and Turkey (86.9% in 2014)\(^1\). Moreover, the overall positive treatment outcome rate in our clinic was lower than the overall success rate of treatment in the European Union and the European Economic Area for the years 2002 to 2011, which amounted to 78.2%\(^1\), and 76% for the year 2014\(^2\). Higher success rates were reported in the US in 2013 (89%)\(^9\) and in Canada in 2012 (86%)\(^2\). Finally, at a global level the success rate of treatment for patients newly diagnosed with TB was 86% in 2013\(^1\) and 83% in 2014\(^4\).

Unfortunately, no data were found in the literature concerning the outcome of treatment in other TB clinics in Greece. The outcome is unknown in Greece, since its report is not mandatory. Ideally, in the context of an anti-TB program, the TB clinics across Greece could be integrated into a national network where it would be compulsory to report the course of the disease and the outcome of each patient’s treatment. This of course requires adequate medical and paramedical staff as well as the appropriate electronic equipment. It is worth mentioning that our clinic was staffed by a specialized nurse only in June 2016, almost five years after the initiation of its operation.

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Based on the present data, the percentage of patients whose treatment failed, died, lost to follow-up and those who cannot be evaluated were 1.1%, 4.5%, 2.2% and 24.7%, respectively. Therefore, it is clear that the negative outcome of treatment is mainly caused by the group of not evaluated patients for whom the outcome

### Table 5. Clinical, social and demographic characteristics of patients and tuberculosis treatment outcome

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of patients (n, %)</th>
<th>Treatment success (n, %)</th>
<th>Treatment failure (n, %)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>60 (67.5%)</td>
<td>39 (65%)</td>
<td>21 (35%)</td>
<td>0.4843</td>
</tr>
<tr>
<td>Women</td>
<td>29 (32.5%)</td>
<td>21 (72.4%)</td>
<td>8 (27.6%)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤64</td>
<td>69 (77.5%)</td>
<td>45 (65.2%)</td>
<td>24 (34.8%)</td>
<td>0.4111</td>
</tr>
<tr>
<td>≥65</td>
<td>20 (22.5%)</td>
<td>15 (75%)</td>
<td>5 (25%)</td>
<td></td>
</tr>
<tr>
<td>Country of origin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greece</td>
<td>61 (68.5%)</td>
<td>45 (73.8%)</td>
<td>16 (26.2%)</td>
<td>0.0590</td>
</tr>
<tr>
<td>Foreigners</td>
<td>28 (31.5%)</td>
<td>15 (53.6%)</td>
<td>13 (46.4%)</td>
<td></td>
</tr>
<tr>
<td>Anatomical site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>81 (91%)</td>
<td>53 (65.4%)</td>
<td>28 (34.6%)</td>
<td>0.2039</td>
</tr>
<tr>
<td>Extrapulmonary</td>
<td>8 (9%)</td>
<td>7 (87.5%)</td>
<td>1 (12.5%)</td>
<td></td>
</tr>
<tr>
<td>History of previous TB treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>77 (86.5%)</td>
<td>51 (66.2%)</td>
<td>26 (33.8%)</td>
<td>0.5467</td>
</tr>
<tr>
<td>Positive</td>
<td>12 (13.5%)</td>
<td>9 (75%)</td>
<td>3 (23%)</td>
<td></td>
</tr>
<tr>
<td>Co-morbidities</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>56 (62.9%)</td>
<td>40 (71.4%)</td>
<td>16 (28.6%)</td>
<td>0.2927</td>
</tr>
<tr>
<td>Present</td>
<td>33 (37.1%)</td>
<td>20 (60.6%)</td>
<td>13 (39.4%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non smokers</td>
<td>20 (22.5%)</td>
<td>15 (75%)</td>
<td>5 (25%)</td>
<td>0.8279</td>
</tr>
<tr>
<td>Active smokers</td>
<td>32 (35.9%)</td>
<td>21 (65.6%)</td>
<td>11 (34.4%)</td>
<td></td>
</tr>
<tr>
<td>Ex smokers</td>
<td>4 (4.5%)</td>
<td>3 (75%)</td>
<td>1 (25%)</td>
<td></td>
</tr>
<tr>
<td>No data</td>
<td>33 (37.1%)</td>
<td>21 (63.6%)</td>
<td>12 (36.4%)</td>
<td></td>
</tr>
<tr>
<td>Resistance to antituberculosis drugs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>79 (88.8%)</td>
<td>52 (65.8%)</td>
<td>27 (34.2%)</td>
<td>0.3674</td>
</tr>
<tr>
<td>Present</td>
<td>10 (11.2%)</td>
<td>8 (80%)</td>
<td>2 (20%)</td>
<td></td>
</tr>
</tbody>
</table>
is unknown. However, an unknown treatment outcome does not necessarily mean a negative outcome. In any case, the large number of the patients who do not show up for their follow up may be associated with the gap in the legislative framework and also the lack of incentives that would encourage them to complete their treatment under medical supervision. It is a fact that many of the patients do not come to the scheduled appointment, despite the systematic communication effort by the clinic's staff. Again, the lack of an appropriate legal framework and the lack of incentives (such as free tickets or financial support for the less well-off patients) make the control of attendance extremely difficult.

Death caused by TB remains a reality. As already mentioned, there were 1.8 million deaths from the disease in 2015. Patients in this study who died were of Greek origin, with pulmonary TB, without history of previous treatment, or resistance to anti-TB drugs, but all of them were elderly patients with co-morbidities. Increased mortality in coexisting concomitant diseases was also noticed in other studies. Many studies also suggested that elderly people in general show an increased death rate. A study in South Africa suggested that older age is the most important independent factor associated with increased mortality. The elderly are at risk for receiving a wrong diagnosis mainly because disease at advanced age is manifested by non-specific symptoms and diagnosis may be confused by concomitant diseases. Therefore, the diagnosis is made at a more advanced stage leading to increased mortality. The significance of the clinical suspicion of TB in the elderly is therefore evident. In Japan, early diagnosis is considered to be the most important measure for controlling TB in the elderly. The history of previous TB and the gender of patients did not seem to have led to increased mortality as opposed to other studies.

According to this study, testing for HIV co-infection was not recorded for a significant number of patients (41.6%), although it is systematically pursued in our clinic. In 2014, it is estimated that there were about 1.2 million new cases of TB among HIV-positive people, of whom 74% lived in Africa and about 0.4 million people died of HIV and Mycobacterium tuberculosis co-infection. People with HIV infection are 20-30 times more likely to develop active TB than the rest of the population due to immunodeficiency and the coexistence of degraded social conditions. All patients newly diagnosed with TB should know if they have HIV co-infection, therefore diagnosis of TB is an indication for HIV testing. Early diagnosis of TB/HIV infection reduces morbidity and mortality, costs of hospitalization and provides an opportunity for a better quality of life. This study did not reveal any statistically significant factors that may have adversely affected treatment outcome, although the small total number of patients does not allow safe conclusions. The origin of patients seems to have a marginal effect on the outcome of the disease, the Greek patients showing a higher positive outcome than foreigners (73.8% and 53.6%, respectively). The rate of successful treatment was higher for natives than for foreigners in other studies as well. In Italy and Switzerland treatment discontinuation has been associated with foreign patients. On the other hand, it has been reported that migrants in the European Union do not receive the same level of healthcare at the level of prevention, diagnosis and treatment, possibly because of their social exclusion and financial situation. The gender of patients does not seem to have affected the outcome of the treatment as opposed to other studies that reported lower rates of TB treatment success in male patients, a fact that was attributed to social and environmental factors. A study conducted in South Africa suggested that male sex was an independent risk factor for treatment discontinuation. However, biological factors may also play a role as shown by a study in mice, where male mice developed a more severe form of TB. Patients with resistance to anti-TB drugs seemed to have a successful treatment rate equivalent to the success rates of drug sensitive TB regardless of whether they had mono or poly-drug resistance. Resistance to pyrazinamide did not seem to adversely affect the therapeutic effects in a California study. In contrast, other studies, pretreatment resistance, acquired resistance (new drug resistance during or at the end of treatment), but also any resistance to isoniazid other than MDR-TB, rifampicin resistance and streptomycin resistance were associated with therapeutic failure. Increased mortality was found with rifampicin resistance in a Peruvian study. The presence of concomitant diseases does not seem to have affected the outcome of the treatment in the present study. In contrast, other studies have shown that the presence of any concomitant disease is associated with an adverse outcome. In China diabetes mellitus seemed to result in therapeutic failure. In some studies therapeutic failure seemed to be caused by the intravenous use of illicit drugs as well as by alcohol dependence. This study also shows that 34.4% of active smokers, 25% of ex-smokers and 36.4% of patients that provided no
data on their smoking habits had a negative outcome. Smoking has been associated with adverse treatment outcome\textsuperscript{34}; it significantly increases the risk of TB, and in particular more than 20% of TB cases worldwide are caused by smoking\textsuperscript{14}.

The rate of bacteriologically confirmed diagnosis of the disease in this study amounted to 78.7%. In 2015 the rate of bacteriological diagnosis of TB in Greece was 86.9\%\textsuperscript{3}. However, it should be taken into account that this rate probably does not reflect the reality because of the significant lack of reporting, since it is more likely that there is no microbiological confirmation in unreported cases. In Europe, the disease’s bacteriological confirmation varies considerably between countries, from 34\% to 96\% in Uzbekistan and Slovenia respectively, with four countries having a bacteriologically confirmed diagnosis rate of less than 50\%, thus highlighting the need to improve diagnostic methods\textsuperscript{3}. The microbiological diagnosis of TB is lower in general in countries outside the European Union and the European Economic Area, (EU/EEA) compared to that of the EU/EEA countries (57\% and 79.5\%, respectively)\textsuperscript{3}.

The most significant restriction of the study is the small number of patients that did not allow conclusions about the factors that may have adversely affected the outcome of the treatment. At the same time, the large number of patients who cannot be evaluated raises numerous questions about the actual outcome of TB.

In conclusion, this study shows that the positive outcome rate of TB patients at the Department of Pulmonary Medicine, AUTH, from 2012 to 2014 was lower than the WHO global target of 85\%, whereas death from TB is still a reality. Since the outcome of TB is an indicator of the effectiveness of healthcare services, there is a clear need for a comprehensive response to the problems of recording and monitoring TB in Greece in terms of both its impact and its outcome. This could be achieved by developing, implementing and continuously evaluating an integrated anti-TB program and a clear underlying legislative framework for the safe management, and the precise description of the obligations and rights of the TB patients.

There are no conflicts of interest of all authors.

Without any potential funding or grant support of the work described.

REFERENCES

Is Vitamin C supplementation beneficial in asthmatic patients?

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SUMMARY
As asthma prevails worldwide, nutritional hypotheses appear to suggest that vitamin C supplementation may have a positive effect in asthma management. However, scientific community has not reached an agreement concerning the association between vitamin C and asthma. In this review, we provide evidence of several in vitro and in vivo studies in mouse, rat and pig models that implicate vitamin C in immune response and also as an anti-inflammatory agent with antiviral properties. Finally, whether vitamin C supplementation in asthmatics is beneficial or not, a number of systematic reviews and meta-analyses have tried to shed more light in this controversy, but no robust assessment concerning the use of vitamin C in the management of asthma is available.

Pneumon 2017, 30(3):151-156.

INTRODUCTION
Asthma is one of the most prevalent diseases worldwide with high economic burden, causing a major public health concern. According to the Global Initiative for Asthma, bronchial asthma is a chronic inflammatory disorder that is related to airways hyperresponsiveness and leads to repeated episodes of wheezing, dyspnea, chest tightness, and cough.1-6 As asthma is a complex and heterogeneous syndrome, risk factors contributing to its etiology are multiple genetic and environmental or lifestyle factors, including dietary intake. Indeed, a “dietary inflammatory index” (DII) was associated with asthma, reduced lung function and increased IL-6 plasma level in a case-control study of adults.7

Among nutritional hypotheses, it has been reported that a reduction in fruit intake in “western diet” could be associated with the increase in prevalence and severity of asthma in the developed countries.8,9 This association comes from the reduction of dietary antioxidants such as vitamin C. In specific, western diet, which is low in fruits and vegetables, and high in refined grains and saturated fat, has been suggested to increase the risk of asthma, whereas a “Mediterranean diet”, which is rich in fruits and...
vegetables, and low in refined grains and saturated fat, could protect against the development of asthma or asthma symptoms.\textsuperscript{16,17} However, this topic remains still controversial as several studies have evaluated the association between antioxidant vitamins (A, C and E) and asthma (1-10) have not reached an agreement.\textsuperscript{12-16}

This report aims to demonstrate evidence of studies of meta-analysis of vitamin C supplementation that may or may not have a positive effect in asthma management.

**VITAMIN C OVERVIEW**

Vitamin C is an exogenous nonenzymatic antioxidant. Some mammals including humans, some primates and guinea pigs cannot produce vitamin C by themselves, while most other mammals can. As a result, humans have to be supplemented with exogenous vitamin C by taking fresh fruits and vegetables, and/or supplementary pills, because deficit of vitamin C leads to the scurvy.\textsuperscript{14}

Lungs are exposed to a range of substances, which directly or indirectly might cause production of oxidants. Vitamin C is abundant in the extracellular and intracellular lining fluid of the lungs, participates in the primary lung defense against the reduction of oxidative damage\textsuperscript{17,18} and also helps to reduce airway hyperresponsiveness.\textsuperscript{19} Studies have shown that exogenous vitamin C supplementation may restore the imbalance of oxidative damage.\textsuperscript{17,20} In specific, oxidative stress in asthma may be initiated by many aerial hazardous substances, such as cigarettes, pollutant particles, or respiratory viruses, resulting in asthma morbidity and pathogenic exacerbations as some patients have a deficiency in anti-oxidative mechanisms.\textsuperscript{21} Moreover, due to generation of oxidative stress when heavy physical exertion occurs, it has been suggested that the effects of vitamin C might be more manifest in people who exercise regularly.\textsuperscript{22,23}

Other mechanisms of the protective effects of vitamin C concerning asthma have been proposed, as an effect on the arachidonic acid pathway\textsuperscript{24} as well as antiviral properties.\textsuperscript{25} Since studies have established that asthmatic patients suffer from oxidative stress due to abnormal airway inflammation\textsuperscript{26,27}, Jeong et al hypothesized that vitamin C could be used for the treatment and/or management of asthma as an antioxidant and/or as a Th1-shifting agent, when considering asthma pathophysiology. In their experimental mouse model, they concluded that vitamin C did not change Th1/Th2 balance in asthma, but it exerted some anti-inflammatory effects against lung inflammation.\textsuperscript{28} The anti-inflammatory effect of vitamin C has been ascribed not only to its anti-oxidant property, but also to direct inhibition of IkB kinase phosphorylation leading to eventual inhibition of NF-kB activation, which plays a critical role in inflammation.\textsuperscript{29}

Recently, Bansal et al hypothesized that supplementation of might have a therapeutic effect. In their study of a mouse model of airway disease, the combination of vitamin C with both choline chloride and selenium had the strongest effect against allergic airway disease\textsuperscript{30} as intranasal treatment with vitamin C (alone or in combination with choline chloride and selenium) decreased oxidative stress, AHR, airway inflammation, IL-4 and IL-5, IL-10, IgE, and NF-kB. Moreover, Nounou et al in a rat model of bronchial asthma suggested that a combination of antioxidant vitamins may be effective in the treatment of asthma, considering their reported effects on lowering malondialdehyde, IL-4, and IgE levels.\textsuperscript{31} Several studies have also suggested vitamin C implication in inflammation\textsuperscript{12-34}, however, some authors denied its anti-inflammatory role.\textsuperscript{35-37}

Furthermore, it has been reported that vitamin C affects lung function by influencing various prostanoids in lung tissues and that vitamin C deficiency increases the level of bronchoconstrictor Prostaglandin F2α (PGF2α).\textsuperscript{38-40} In guinea pigs on a diet deficient in vitamin C, an increase in airway hyper-responsiveness to histamine was observed\textsuperscript{46} and in isolated guinea pig trachea smooth muscle, vitamin C decreased the contractions caused by PGF2α, histamine and carbamylcholine.\textsuperscript{22,41,42} The role of vitamin C in prostaglandin metabolism has also been strengthened by another study in humans, where a 2-week vitamin C diet reduced post-exercise increase in the urinary markers for the bronchoconstricators leukotriene C4–E4, 9a and 11b-PGF2 as well as exhaled nitric oxide when compared to placebo and usual diet.\textsuperscript{43}

Furthermore, another study reported that in vivo administration of vitamin C modulates T cell proliferation and cytokine secretion\textsuperscript{44}, associating vitamin C with the immune response. Khassaf et al concluded that vitamin C-supplemented subjects, showed attenuation of adaptive responses to oxidants, however suggesting that this may only reflect an increased baseline expression of potential protective systems against oxidative stress.\textsuperscript{45} Another study concluded that mega-dose vitamin C administration in mice shifted immune responses toward Th1 lineage with concomitant suppression of the Th2 responses.\textsuperscript{46} Conversely, Lee et al showed that inadequate vitamin C supplementation in L-gulono-γ-lactone oxidase deficient
mice which cannot synthesize vitamin C by themselves resulted in a decreased Th1 response against *H. pylori* infection.47 Considering all the above as well as asthma pathophysiology, some authors have suggested vitamin C as an antioxidant and/or as a Th1-shifting agent for the treatment and/or management of asthma.27 However, the beneficial effect of vitamin C in asthma remains a subject in controversy.30,48,49 Recently, an interesting clinical trial on pregnant smokers (n=179) suggested that vitamin C supplementation (500 mg/d) may be an inexpensive and simple approach to decrease the effects of smoking in pregnancy on newborn, based on the improvement of pulmonary function and decreased risk of wheeze in their newborns, up to age 1 year.50 Besides, another recent study on the same group of pregnant smokers, demonstrated that vitamin C prevents offspring DNA methylation changes associated with maternal smoking in pregnancy.51

Besides asthma, there has been evidence that vitamin C may have a treatment effect on some cardiovascular disorders.52-56 Furthermore, in a recent meta-analysis, vitamin C decreased the risk of contrast-induced acute kidney injury in patients undergoing coronary angiography.57 Also, concerning common cold, upper and lower respiratory tract infections58,59 and pneumonia60, several controlled trials have indicated potential physiological effects for vitamin C as a non-scorbutic effect, still further evidence is needed.

**VITAMIN C SUPPLEMENTATION IN ASTHMATICS, SYSTEMATIC REVIEWS AND META-ANALYSES**

Several epidemiological studies and meta-analysis in adults and children have focused on the role of vitamin C supplementation in asthma or asthma-related symptoms with inconsistent results49,61-63 (Table 1).

One of the first systematic reviews and meta-analysis was conducted by Allen et al in accordance with the MOOSE guidelines, in an effort to provide pooled quantitative estimates of the likely magnitude of the effect of dietary intake and serum levels of vitamins A, C and E to asthma development and severity.62 The authors concluded that even relatively low dietary intakes of vitamin C are associated with statistically significant increased odds of asthma and wheeze. However, a year earlier, Gao et al in their meta-analysis, including 10 studies on asthma, did not support the hypothesis of the effect of dietary intake of the antioxidant vitamins on a lower risk of asthma63 with the only positive association found concerning lung function being between vitamin C intake and an increase in FEV1. Similarly, a recent Cochrane review of eleven randomized controlled trials including 419 children and adults with asthma concluded that there is insufficient evidence to recommend vitamin C as a therapeutic agent in asthma.64 According to Hemila Harri, a Cochrane review on vitamin C and asthma that has been withdrawn recently, was misleading readers.

**TABLE 1. Studies of meta-analyses of vitamin C on asthma and asthma related symptoms**

<table>
<thead>
<tr>
<th>Authors of meta-analyses</th>
<th>Year</th>
<th>No of trials included in meta-analyses</th>
<th>Focus</th>
<th>Conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gao et al63</td>
<td>2008</td>
<td>10</td>
<td>Dietary antioxidants and risk of having asthma</td>
<td>No association</td>
</tr>
<tr>
<td>Allen et al62</td>
<td>2009</td>
<td>37</td>
<td>Serum levels of vitamin C association with asthma</td>
<td>Low dietary intakes of vitamin C are associated with increased odds of asthma and wheeze</td>
</tr>
<tr>
<td>Kaur et al49</td>
<td>2009</td>
<td>9</td>
<td>Efficacy of vitamin C in the treatment of asthma</td>
<td>Insufficient evidence to recommend vitamin C as a therapeutic agent in asthma</td>
</tr>
<tr>
<td>Hemila et al61</td>
<td>2013</td>
<td>3</td>
<td>Vitamin C benefit on FEV1 decline caused by exercise</td>
<td>Consistency of positive findings on vitamin C against EIB</td>
</tr>
<tr>
<td>Milan et al64</td>
<td>2013</td>
<td>11</td>
<td>Vitamin C supplementation on preventing exacerbations and improve HRQL</td>
<td>Insufficient evidence to recommend vitamin C as a therapeutic agent in asthma</td>
</tr>
</tbody>
</table>
for a decade reporting insufficient data to recommend a specific role for vitamin C in the treatment of asthma. 49 He reported that Kaur et al had substantial errors in the extraction of data and data analysis. 65 Thus, to determine the role of vitamin C in asthma management, the published data on vitamin C should be analyzed carefully and comprehensively. Most of the trials are clinically so heterogeneous in experimental settings such as patient selection criteria, recommended dosage of vitamin C, and parameters assessed. As a result, the positive findings cannot be generalized widely.

As atopic asthma includes the most common phenotype, a systematic review and meta-analysis investigated the potential role of nutrient and food intake in modifying the risk of children developing allergy. 66 Although the available epidemiologic evidence is weak, they found no link between vitamin C and allergic outcomes. Another common phenotype in asthmatics is exercise-induced bronchoconstriction (EIB). 67 EIB is a transient narrowing of the airways that occurs during or after exercise. Recently, Hemila et al in their meta-analysis concerning three randomized placebo controlled double-blind trials on patients with exercise-induced bronchoconstriction, found that vitamin C administration (0.5–2 g) before exercise reduced post-exercise decline in FEV1. 61 Moreover, despite the small size of these trials and given that vitamin C is safe and low cost, they suggested that physically active people could use vitamin C on an individual basis if they have respiratory symptoms such as cough associated with exercise.

CONCLUSIONS AND FUTURE PERSPECTIVES

Finally, is Vitamin C supplementation beneficial in asthmatics? In spite of the controversy reported, there is an indication that vitamin C as a chronic usage may positively affect asthmatics patients. However, currently, there is no available evidence on a robust assessment concerning the use of vitamin C in the management of asthma or exercise induced bronchoconstriction. Besides, there are still no data available to evaluate the effects of vitamin C supplementation on asthma exacerbations in adults. Larger well-designed trials are needed to focus on good quality clinical endpoints, such as exacerbation rates and health-related quality of life scores, in order to address the question of the potential therapeutic or preventive usage of vitamin C on asthma and asthma-related symptoms.

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Chest Elastofibroma – Elastofibroma Dorsi

SUMMARY
Chest wall primary tumors include a broad spectrum of benign and malignant tumors. Tumor biopsy is essential to set diagnosis. This rule does not apply to the elastofibroma dorsi. Elstofibroma is an unusual, well defined tumor of soft tissues typically located in the peri-scapular region, composed of characteristic abnormal elastinophilic fibers and adipose tissue. It affects mainly elderly people and especially women. In magnetic resonance imaging and computed tomography elastofibroma presents a characteristic layered pattern of fatty and fibrous tissue. The typical location and the characteristic imaging pattern set the diagnosis of elastofibroma without the need for biopsy. Biopsy is required in atypical cases to exclude malignancy, especially sarcoma. In asymptomatic cases, simple monitoring is sufficient. Surgical excision is recommended for serious symptoms and diagnostic doubt. The prognosis is excellent. No case of malignant transformation has been described.


INDRODUCTION
Chest wall is a site of location for primary and metastatic tumors. Chest wall primary tumors represent 1-2% of all primary tumors and include benign and malignant tumors originating from the bones, cartilage and soft tissues (muscles, vessels, nerves) of the chest wall, and some hematological diseases.

Chest wall benign tumors consist the 56% (23/41) of the cases and include enchondroma, fibrous dysplasia, neurilemmoma, osteochondroma, granular cell tumor, fibroma, lipoma, fibrolipoma, eosinophilic granuloma, aneurysmal bone cyst. Chest wall malignant primary tumors account for 46% (18/41) of the cases and include plasmacytoma, chondrosarcoma, osteosarcoma, fibrosarcoma, desmoid tumor, leiomyosarcoma, malignant fibrous histiocytoma, tendon sheath sarcoma, hemangiosarcoma, and neurofibrosarcoma. The most common are the cartilaginous tumors (chondroma and chondrosarcoma) and then those derivated of the soft tissue (fibromas, lipomas, neurogenic tumors).

Consequently, the presence of a tumor in the CWI is a diagnostic chal-
challenge for the clinician. The likelihood of malignancy and especially of sarcoma forces the clinician to consider all chest wall tumors as malignant until evidence of the opposite. In this context tumor biopsy becomes necessary for tumor identification.

This rule does not apply fully to elastofibroma. This benign tumor has in many cases typical clinical and imaging features that allow for safe diagnosis, avoiding further biopsy investigation or even surgical excision.

The presentation of the clinical and imaging characteristics of elastofibroma is the purpose of this review.

DEFINITION

Elastofibroma or elastofibroma dorsi (ED) is a benign, slowly growing tumour of soft tissue of subscapular area, composed of characteristic abnormal elastinophilic fibers and adipose tissue. It was first described by Järvi and Saxen in 1959 at the 12th Congress of Scandinavian Pathologists, who then published their work in 1961.

The 2002 World Health Organization (WHO) soft tissue tumor classification ranked the ED in the benign and myoinoblastic soft tissue tumors of the peri-scapular region.

Location

ED is typically located between the lower corner of the scapula and the posterior CW. It may be connected to the periosteum of the ribs but without invading it or other adjacent structures.

Elastofibroma may be unilateral or bilateral. Bilateral location is more frequent with a reported frequency in different series at 58.62% (17/29), 66.27% (112/169), 75% (6/8), and 100% (9/9) of cases.

Unilateral elastofibromas are more commonly located in the right chest wall with a reported frequency of 61% (35/57) of cases.

Size - Symmetry

The diameter of surgically excised tumours ranges from 2 cm to more than 15 cm, while in the computed tomography (CT), very small elastofibromas with diameter 1×1.5 cm and 2×0.5×2 cm have been described.

The diameter of the larger palpable tumours is similar to physical examination and CT. However, elastofibromas with diameter of less than 3 cm are difficult to be found in physical examination and CT.

Bilateral elastofibromas may be symmetrical in size and location, or asymmetric, and many times asynchronous.

UNUSUAL LOCATION OF ELASTOFIBROMAS

Elastofibromas are typically located at the periscapular region. However, a growing number of publications in the literature have described elastofibromas located at various anatomical sites, mainly the musculoskeletal system, and more rarely the visceral organs and the gastrointestinal tract.

Unusual locations of elastofibromas have been described in eye, face, intraspinal space, mediastinum, tricuspid valve, hand, foot, axilla and inguinal region, infraolecranons regions, ischial tuberosity, deltoid, greater trochanter, posterior elbow, pancreas, stomach, intestine, sigmoid colon, and greater omentum.

These locations may be single or multiple, coexisting with the typical location in the sub-scapular region. Nagamine N et al in a clinical-pathology study involving 170 cases of ED described 27 cases in which the tumour was located in both subscapular areas and in addition in the infraolecranon regions, a case with seven different anatomical locations (sub-scapular, bilateral thoracic wall, and both infraolecranon areas), a case with four different anatomical locations (sub-scapular on both sides, left infraolecranon, and right ischial tuberosity) and a case with tumour in both sub-scapular areas and in the right ischial tuberosity. One patient had a single tumor located only in the olecranon. Shimizu S et al described a case which presented 15 separate elastofibromas. The location of the masses included the classic sub-scapular sites as well as buttocks and upper limbs.

EPIDEMIOLOGY

Frequency

Although ED is considered as a rare entity, its exact frequency is not known. Significant differences exist in the described incidence of ED between clinical and pathology studies.

Pauline H. Go et al in a study based on data from 14 series of elastofibroma cases published between 1980 – 2009 described 330 symptomatic cases of ED. Brandser et al in a CT study involving 258 asymptomatic patients over 60 years of age found five elastofibromas in 4 patients (prevalence of 2%). Blumenkrantz et al described 29 cases of ED in a total of 1,751 patients subjected to PET-CT with 18F-fluorodeoxyglucose (FDG), (1.66%). Review of Ortho-
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pedic Oncology data from the Royal Orthopaedic Hospital in Birmingham, UK, revealed 15 cases of elastofibromas in a total of 17,500 cases (0.086%) in patients >20 years of age. Nagano S et al., in an analysis of the Japanese Soft Tissue Tumor Registry database, found 130 cases of ED in a total of 12,557 cases with soft tissue volume (1%).

In contrast, autopsy studies have described a much higher incidence of ED. Jarvi and Lansimies in a series of 235 autopsies, found changes in the subcapsular thoracic fascia similar to ED in 39 cases. In people over 55 years, the frequency was 24.4 per cent in females (29 of 119) and 11.2 per cent in males (10 of 89).

Giebel et al. in a study of 100 autopsies revealed 13 elderly patients with elastofibroma. Pre-elastofibroma-like morphological changes (e.g. few or many degenerated elastic fibres) were observed in 81% of the autopsies.

The differences in the reported incidence between clinical and autopsy studies suggest that the actual incidence of ED is higher than that described in the clinical studies. This is also supported by the fact of incidental discover of asymptomatic elastofibromas. The absence of symptoms and the small size are obvious causes for the escape of the elastofibromas.

Racial - Age Distribution

ED affects mainly elderly patients. The average age at diagnosis in different series has been described in 62 years (range 6-94 years), 63.7 years (range 46 - 79 years), 54.9 years (range 44 - 62 years), 61.1 years (range 38-78 years), 65 years (range 48-72 years) and 68.4 years (range 51-79 years). In contrast, ED is rare in children and adolescents.

Elastofibroma affects predominantly women. The female/male ratio has been described in 2.2:1 (11 women/5 men), 2.5:1 (5 women/2 men), 3.14:1 (22 women/7 men), 3.9:1 (263 women/67 men), 5.1 in 1 (7 women/1 men). However, Chandrasekar CR et al. described that in a series of 15 elastofibroma cases 12 patients (80%) were men and 3 were women (20%).

ETIOLOGY

The etiology of ED is not fully elucidated. A number of theories have been proposed for its development.

The location of ED between the scapula and the chest wall and the initial description of the tumour in people with hard manual labor constituted the basis for the theory of mechanical friction between the chest wall and the lower tip of scapula as a mechanism of ED development.

According to this theory, direct mechanical stress on the elastic tissue can cause hypertrophy and secondary degeneration of the elastic fibers, as well as diffuse growth of collagenous tissue alternating with the deposition of hyperplastic fat.

However, the higher incidence of ED in women and its location at anatomical sites not involved in mechanical overload cannot support the mechanism of mechanical friction, at least as the unique cause, for the development of ED. In addition, there have been described cases of ED with absence of this causative agent, such as in patients with no history of recurrent or previous trauma in the tumor area or in patients who were not hard workers.

Development of ED as a result of the normal age process rather than an abnormal elastogenesis or degeneration, was supported by Giebel et al. This view was based on finding "pre-elastofibroma changes" in their autopsy series. These changes are determined by a weakly eosinophilic material which does not express a definitive elastic tissue formation.

Nagamine et al. supported the familial predisposition for the formation of ED. This view was based on the finding that of 170 cases of ED 32% of cases occurred within a single family. Familial predisposition with an underlying enzymatic defect have also been proposed as possible etiologic factors.

Jarvi OH and Lansimies argued that in addition to direct mechanical stress on elastic tissue, nutritional deficiency due to failure of the vascular system against friction of the scapula and stretching movements of the upper extremities may play a major role in necrotic tissue changes.

The nature of the abnormal elastic fibers is questionable and controversial and can not substantiate their mechanism of formation. Abnormal elastogenesis or degeneration as a secondary process, even combination of both mechanisms may be responsible.

Di Vito A et al. in a study of extracellular matrix and histopathogenesis in ED, argued that a mechanical strain-dependent reactivation of periostrin and tenasin-C expression, as well as elastin deposition, could be responsible for the development of ED.

Kakudo N et al. in a histochemical, immunohistochemical, and ultrastructural study of an ED, argued that although fibroblasts can produce large amounts of elastin, microinid and collagen, elastin deposition on collagen fibers may be involved in the formation of
abnormal elastic fibers.

Although the mechanism of ED development is not fully elucidated, it is internationally accepted that it is more often in individuals performing repetitive manual labor involving the shoulder\textsuperscript{5,35}, perhaps in combination with genetic factors\textsuperscript{29}.

**CLINICAL PRESENTATION**

The presence and type of symptoms of ED depend on its location and size\textsuperscript{22}.

ED is usually asymptomatic\textsuperscript{22,29}, especially during the initial phases\textsuperscript{36}.

In these cases ED is accidentally detected by the patient himself, eg in the mirror, or by another person, such as a spouse or member of the family\textsuperscript{7,16,29}, or during physical examination\textsuperscript{5,7}, investigation for other disease\textsuperscript{12} or for symptomatic ED on the contralateral side\textsuperscript{5,7,10} or during surgery for unrelated chest injury\textsuperscript{7}.

In symptomatic cases, patients usually experience a long history of slowly growing swelling in the shoulder area for which the patient is not looking for medical attention either because the mass is asymptomatic or because the symptoms are mild.

The interval between the detection of swelling or the presence of symptoms until the search for medical assistance has been described in 3-60 months (mean duration of symptoms 20 months)\textsuperscript{16}, 4 months to 4 years\textsuperscript{22}, seven months\textsuperscript{13} and five years\textsuperscript{35}.

When symptoms are present, these are typical and include local swelling of the shoulder\textsuperscript{10}, limitation of movement of the upper limb, mainly in the upward movements requiring slipping of the shoulder with respect to the chest wall\textsuperscript{13,35}, and sometimes pain during the movement of the shoulder\textsuperscript{5,16,29,33-37}.

The pain is usually moderate, but cases of painful swelling of the shoulder have been described\textsuperscript{11,35}.

Other symptoms include a clunking sensation during abduction and adduction of the arm\textsuperscript{5,13} and snapping of the scapula\textsuperscript{29}.

**PHYSICAL EXAMINATION**

The clinical examination reveals a large, palpable, solid mass well circumscribed in the lower corner of the shoulder, usually painless. The mass may be mobile or immobile due to its attachment to the chest wall\textsuperscript{13,16,29,35}.

The tumour is more prominent on the forward flexion of the shoulder due to forward movement of the inferior angle of the scapula\textsuperscript{16,29,35}.

Small elastofibromas are not visible in the neutral position with the risk of escaping unless the patient is called to move his arm laterally or posteriorly\textsuperscript{29}.

Another mass may be present in the opposite shoulder, often smaller and clinically silent\textsuperscript{35}.

**IMAGING FINDINGS**

The typical diagnostic pattern of ED is characterized by alternating bundles of fibrous and adipose tissue.

**CHEST RADIOGRAPHY**

Chest X-ray is usually normal. Except a possible soft tissue signal intensity or elevation of the shoulder, simple X-rays do not show specific changes capable of diagnosing\textsuperscript{22,35,38}.

**COMPUTED TOMOGRAPHY (CT) AND MAGNETIC RESONANCE IMAGING (MRI)**

EDs have typical imaging findings in CT and MRI (Figures 1, 2a, b) which allow definitive diagnosis\textsuperscript{7,38}.

Typical imaging of ED in CT and MRI include a poorly circumscribed mass of soft tissues without wall. The mass is heterogeneous with a characteristic layered pattern of
fatty tissue (low-density by CT, high-signal on T1 images and intermediate signal on T2 images by MRI) and fibrous tissue (similar to muscle in terms of density by CT and signal intensity by MRI)\(^7,38\). The lesions are located anterior to the scapula (subscapular) or caudal to the inferior pole of the scapula (infrascapular), deep in relation to the latissimus dorsi, rhomboid, and serratus anterior muscles. There is no evidence of infiltration of neighboring tissues\(^7\).

MRI is the imaging modality of choice in diagnosing ED because it depicts better than CT the characteristic layered pattern. The mass is well-defined. Slight or moderate enhancement within elastofibromas can be observed after gadolinium administration\(^7,37,38\). However, in some cases of ED, strong enhancement can be observed after gadolinium administration which is characteristic of malignant tumors. In these cases biopsy and histopathological assessment will be required for definitive diagnosis\(^35,38\).

In computed tomography the ED displays a typical or a non typical imaging pattern.

The typical appearance of ED in the CT includes a subscapular lenticular mass of soft tissue, with no wall, of equal density to the adjacent muscles (fibrous tissue) and linear areas of low density (fatty tissue). Mass margins show poorer differentiation from surrounding muscles compared to ultrasound and MRI\(^7,13,22,35\). This CT pattern is diagnostic for ED.

The atypical appearance of ED in CT includes well-described soft tissue masses with a similar density to the adjacent skeletal muscle, relatively homogenous, with no defined interspersed areas of fat attenuation\(^7\). This atypical pattern is usually observed in cases of smaller masses\(^36\).

The fact that ED presents in the CT and MRI a density/signal intensity similar to that of the adjacent muscles interprets the escape of the EDs from the initial interpreting radiologists. Naylor MF et al\(^7\) described that of 21 fibroblastomas imaged, only four (19%) were perceived by the initial interpreting radiologist, and only one (5%) was correctly diagnosed\(^7\). Brandser EA\(^12\) described that none of 5 fibroblastomas in 4 asymptomatic patients had been described in the initial diagnosis of CT.

**PET-CT SCAN**

In PET-CT scan most tumors appear with mild or moderate diffuse metabolic activity with the SUVmax ranging from 1.4 to 3.2\(^9\) which should not be interpreted as a malignant finding\(^36\). However, hypermetabolic tumors have been described\(^39\).

**ULTRASOUND**

EDs present in the ultrasound a typical pattern of a well-defined soft tissue mass in the typical position of the ED, with a multilayered pattern of alternating layers of hypoechogenic fat and hyperechogenic elastic tissue, gently parallel to the chest wall\(^40-42\). Colour Doppler shows a vascular pattern similar to the surrounding muscles\(^42\).

Although ultrasound represents an inexpensive di-
agnostic tool\textsuperscript{22}, it is not an imaging technique of choice for diagnosing of ED. In addition, the examination is dependent on the examiner and for this reason it is not always diagnostic\textsuperscript{15}.

**DIAGNOSIS**

The diagnosis of ED can be definitively determined on the basis of its clinical and imaging characteristics. A history of long-term presence of a solid mass under the scapula\textsuperscript{43}, the bilateral location\textsuperscript{7} and the classical imaging characteristics described above in elderly patients and especially in women place the diagnosis of ED. In these cases, the biopsy is not necessary for diagnosis\textsuperscript{5,13,22,16,29,35}. Biopsy is necessary to confirm diagnosis in cases of unilateral EDs without the typical imaging pattern in MRI and CT scan\textsuperscript{5,7}.

Particularly unilateral mass of soft tissues larger than 5 cm in diameter in elderly patients, with MRI images of weakly defined, heterogeneous mass of soft tissue, sometimes enhanced by gadolinium, should be identified by biopsy because of the possibility of soft tissue sarcoma in this age group\textsuperscript{16,44}.

Fine needle aspiration biopsy (FNAB) is a commonly used technique to definitively diagnose the condition\textsuperscript{10,13}. However, the collagenous nature of the mass often results in a paucicellular FNA smear, resulting in a false-negative report\textsuperscript{5,10,22}. In these cases, a core or open biopsy is essential to get a representative tissue specimen to make a definitive diagnosis\textsuperscript{5,22} especially in case of suspicion of a soft tissue sarcoma\textsuperscript{15}.

The differential diagnosis of ED includes sarcoma, lipoma, fibroma, liposarcoma fibromuscular tumour, desmoid tumour, hemangioma, hematoma and aggressive fibromatosis\textsuperscript{5,35}.

**PATHOLOGY**

Macroscopically, the fibrolastoma is characterized by a solid, slightly elastic, irregular, weakly defined mass of fat and connective tissue without wall with a consistence that assimilates rubber. The cut-surface shows white and yellow tissue caused by fat trapping, similar to a checkerboard pattern\textsuperscript{22}.

Microscopically, the fibrolastoma is characterized by the presence of a large number of abnormal elastic fibers embedded in a matrix of dense eosinophilic collagen, benign fibroblasts, and mature adipocytes\textsuperscript{7,16,22}.

Elastic fibers are difficult to be detected by hematoxylin-eosin staining\textsuperscript{22}. They are best depicted by elastin staining\textsuperscript{22,22}, which reveals deep-stained branched and unbranched fibers\textsuperscript{7}. The elastic fibers are sometimes elongated and larger than regular ones, mostly round shaped, densely packed\textsuperscript{22}, simulating beads on the strings\textsuperscript{13}.

The fibroelastic fibers exhibit a central dense core and serrated margins\textsuperscript{7,46}. The central core represents a mature elastic tissue that appears to be secreted by active fibroblasts\textsuperscript{46}. The peripheral zones of elastic fibers consist of a variety of fuzzy, irregularly shaped amorphous components and compactly and randomly arranged large quantities of microfibers\textsuperscript{45}.

The lesions are predominantly subcellular with fibroblastic cells without atypia and mitotic activity\textsuperscript{22}. In the cytoplasm of the fibroblasts there are dense granular bodies, which are believed to represent elastin or elastin precursors\textsuperscript{46}. Between the benign fibroblasts there is a varied amount of fatty tissue\textsuperscript{5,16}.

**MANAGEMENT**

When diagnosis of ED is definitive, its treatment depends on the symptoms it causes to the patient. In asymptomatic patients, clinical monitoring is adequate\textsuperscript{4} due to the benign nature of the tumor and because malignant transformation has never been described\textsuperscript{13,22,25}.

Surgical excision is indicated when there is a diagnostic doubt\textsuperscript{10} and in severe symptomatic cases of snapping or blocking scapula, pain, or tumor-related discomfort and depending on the patient’s psychological and physical strain. A therapeutic marginal excision has been shown to be sufficient and it is preferred over broad or radical excision\textsuperscript{5,10,22,29,35,44}.

The most frequent complication observed postoperatively is hematoma due to the fact that the periscapular region is highly vascular\textsuperscript{13,29}.

**OUTCOME**

The prognosis after surgical excision is good\textsuperscript{13,37}. Tumor relapses have been described in 7% of the cases and are attributed to incomplete resection of the tumor\textsuperscript{10}.

**CONCLUSIONS**

ED is an unusual, well defined tumor of soft tissue typically located in the peri-scapular region, affecting mainly
elderly people and especially women. The typical location and the characteristic MRI and CT imaging pattern of the alternate bundles of fibrous and adipose tissue set the diagnosis without the need for biopsy. Biopsy is required in atypical cases to exclude malignancy, especially sarcoma. In asymptomatic cases, simple monitoring is sufficient. Surgical excision is recommended for serious symptoms and diagnostic doubt. The prognosis is excellent. No case of malignant transformation has been described.

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Lower Respiratory Tract Microbiome

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Key words:
- Lung Microbiota
- Lung Microbiome
- Lower Respiratory Tract Microbiome

INTRODUCTION

Microorganisms are well known to occupy all human body sites, most of them inhabiting the gastrointestinal tract1, though different communities were shown to also reside on skin2, vagina3, the oral cavity4 and recently lower respiratory tract5,6. To characterize microbial communities colonizing multiple body sites and highlight their disturbance in case of disease, National Institute of Health (NIH) funded the Human Microbiome Project. Surprisingly, lower respiratory tract was not included as a site of interest7. Due to the increasing attention shown by researchers, the National Heart Lung and Blood Institute currently supports the so-called Lung HIV Microbiome Project, a collaborative project, still in progress, aiming to outline lung microbiome and shed light to changes observed in lung disease8,9.

MODERN TECHNIQUES TO STUDY THE MICROBIOME

Terms and Definitions

To clarify the terms herein used it would be useful to provide readers with the following definitions. Microbiota makes reference to all the living...
microorganisms inhabiting a particular ecologic niche, such as the respiratory or gastrointestinal tract. The term **microbiome** refers to the genome of microorganisms entire population, including their metabolites. Since the beginnings of microbiology, in the 17th century, researchers compared human microbiota, of different habitats (oral-feces) and individuals to prove differences in health and disease state. Recently, it came as a surprise to them that the human microbiota numbers 10 to 100 trillion cells, more than 10 times the human somatic and germ cells.

Since the introduction of plating techniques by Robert Koch, in late 1880’s, and intensively during the past decades, **culture-based techniques** have been widely used for bacteria identification in a plethora of biological fluids samples, including sputum and bronchoalveolar lavage (BAL). Samples have been plated on specific media, shown to be suitable for each microorganism growth. Therefore, microbiota has been identified according to the medium allowing growth, the unique structural features of colonies and the metabolites produced or depleted accordingly.

Traditionally, phenotypic traits (phenotypic fingerprinting), such as optimal growth temperature or medium, have been used to identify different strains (strain-typing). These methods are highly dependent on the researcher’s ability to simulate specific growth conditions in vitro and consequently can be difficult to standardize. To overcome this, researchers introduced genotypic fingerprinting. Strains populations are recognized and distinguished by phenotypically similar ones, usually using special restriction enzymes to digest their DNA and genetic probes to label these unique for each strain fragments.

Taking into consideration that traditional culture-dependent techniques have been reported to identify microbiological etiology in only 25% of patients diagnosed with pneumonia in ward and ICU, the need for introduction of novel methods in pathogen identification was raised into a burning issue.

Over the last 20 years **culture-independent techniques** have been put into practice in order to identify microbial communities in different niches of human body (such as gut, lower respiratory tract, skin etc.). The introduction of Polymerase Chain Reaction (PCR), microarrays and metagenomics led to deeper knowledge of microbiota characteristics in healthy persons and patients suffering from diverse disorders (Figure 1).

Making a breakthrough in the 1980’s Woese and coworkers showed that the gene coding for 16S rRNA can depict phylogenetic relationships among bacterial strains, since it shows remarkably high conservation, a fact probably due to the significance of this component of the small subunit of ribosomes for proper cell function. 16S rRNA gene is around only 1.5kb long thus allowing for quick sequencing outcomes and consists of highly conserved regions alternating with 9 hypervariable regions (V1-V9). Small sequences within these hypervariable regions were shown to differentiate among bacterial species. Sequence analysis and comparison of accurate sequences with those available in verified databases have allowed identification of mycobacteria, who traditionally need a long time to be cultured, and unculturable bacteria, such as *Treponema pallidum*.

Until recently researchers have been widely using the Sanger sequencing technique, to accurately determine DNA sequences. This method is based on elongation of the primer of interest using a DNA polymerase, normal deoxynucleotides (dNTPs) and specially modified and flagged dideoxynucleotides (ddNTPs). The latter lack 3’-OH group, which is indispensable for the formation of the phosphodiester bond, holding the next nucleotide closely attached to the elongating chain. Each time such a modified molecule is attached DNA chain elongation ceases. After many rounds of DNA extention, using PCR, and separation through (gel or capillary) electrophoresis, sequence is determined identifying either the radio-labelled or dye-labelled chain terminators.

To overcome the major disadvantage of conventional Sanger method, which is poor throughput, next generation sequencing (NGS) techniques have been recently introduced. A plethora of different NGS platforms, based on various sequencing technologies, can carry out multiple sequencing reactions, thus sharply increasing output. Furthermore, these techniques provide many copies of shorter sequencing reads (less than 400 base pairs) coming from single DNA molecules, unlike Sanger sequencing.
technique which required PCR amplified samples while giving out single long reads. In this way not only accurate sequencing results are delivered but mutations in small cells subpopulations can be recognized25,26.

**Lung microbiome analysis**

In terms of lung microbiome research, the use of real-time quantitative PCR in sputum samples raised the percentage of successful microbiological agent identification up to 67% of pneumonia cases. This was further increased (87%) when full sampling was performed (sputum, blood and nasopharyngeal secretion samples)27.

Culture-independent microbiological techniques have been recently used to analyse lung microbiota in bronchoalveolar lavage samples obtained through bronchoscopy. According to Hogan and co-workers this technique gives the chance to examine differences in microbiota among separate lung regions, even though statistically important difference has not yet been reported28.

One of the main challenges shared among all the microbiome studies is the choice of primers used to recognize microbial diversity. Most studies use the V1-V3 and/or V3-V5 hypervariable regions of 16S rRNA gene to prove presence of bacterial taxa even though these regions were shown to sporadically give inconsequent results, especially as far as sub-genus operational taxonomic units levels are concerned29. Results were thus shown to be affected by the specific gene region targeted by pyrosequencing technique, thus making comparisons among studies insecure30.

**Procedure obstacles**

Studying lung microbiome particularly, researchers had to deal with samples contamination coming from upper respiratory tract, since bronchoscope can drift a plethora of microbial factors passing through oropharynx. Taking into consideration the continuity of microorganisms found along the respiratory tract30, a variety of methods have been proposed in order to reveal genuine lung microbiota. These include use of two equipments, with the first one being used to take supraglottic samples for comparison, and design of specific single-sided outlier tests, in order to identify bacteria replicating in lower respiratory tract notwithstanding oropharyngeal background30,31.

Due to very low levels of microbiota reported in lower respiratory tract and great sensitivity of PCR in positive detection, sterile bronchoscope washes samples as well as reagent controls must always be included in studies to support reliable results32.

It is also of great importance to mention that 16S rRNA gene sequencing technique, as well as other DNA-based methods, are proved to overrate lung bacterial burden, since they can discern no difference between viable and non-viable microorganisms. Further supporting our knowledge on the robust immune response of lower respiratory tract against bacteria33, Pezzulo and co-workers showed that in pigs BAL fluids 63% of bacterial DNA retrieved was DNAse I sensitive, meaning coming from dead bacteria. It is worth noting that even if bacterial burden was shown to drop in BAL samples treated with DNAse I, bacterial diversity remained unaffected34. To shed further light to this, Venkataraman and co-workers, reported opposing results since they managed to cultivate 61% of species, identified with 16s rRNA gene sequencing methods in healthy human BAL samples, applying a variety of media and incubation conditions35.

**HEALTHY LOWER RESPIRATORY TRACT MICROBIOME**

According to Dickson and Huffnagle it is the dynamic equilibrium of three main factors that affects the lung microbiome synthesis in health state or illness. The two opposing factors, playing the major role in healthy lung microbiome composition are microbial immigration from upper to lower respiratory tract parts and microbial excretion, usually achieved through cough, mucociliary clearance and host immune defense. Microbial movement along respiratory tract can be due to unintentional/subclinical aspiration of small quantities of fluids or secretions, bacteria carried by air flow and direct spreading due to airways mucosal continuity. Gastric reflux has also been suggested as a way to further repopulate the indigenous bacterial population of lower respiratory tract36. The third factor, reported to be altered in case of disease and thus to affect microbiome, are growth conditions as these are locally determined by temperature, ph, oxygen and nutrients abundance, host defense and inflammation activity37 (Figure 2).

**Bacterial populations**

A plethora of studies describing the pulmonary microbiota of healthy individuals have been published. Due to small size of samples, inconsistency of methodology and lack of longitude these should be considered with caution, though being indicative30,38,39. In terms of phylum rank-
ing Bacteroides, Firmicutes and Proteobacteria have been systematically identified in healthy lungs using culture-independent microbiological techniques. 

Streptococcus, Pseudomonas, Prevotella and Veillonella were the genera identified in most controls in several studies comparing lung microbiome in BAL samples between diseased and healthy lungs.

Upper and Lower Respiratory Tract Continuity

Same bacterial taxa were recognized in mouth washes and BAL samples retrieved from healthy individuals. It is worth noting that Morris and co-workers underline the presence of members of Enterobacteriaceae and Pasteurellaceae (mainly belonging to the genus Haemophilus) families. These particular populations were shown to exist in significantly larger quantities compared to neutral model, given the fact that they come from upper airways, thus showing that lung microbiota significantly differs from mouth bacteria. Further enhancing results of Lozupone et al. and Charlson et al., the aforementioned study identified Tropheryma whipplei in healthy individuals BAL samples, even though this was not detected in their oropharynx samples. To explain this phenomenon, Segal and Blaser suggested either the microaspirations scenario or this of hematogeneous spreading, thus implying that lung is an ecological niche for this specific bacterium. In the same study, differences were reported between healthy smokers and non-smokers lung microbiota.

All in all, differing from gastrointestinal tract, it seems that respiratory tract has largely homogenous microbiota, scaling down in biomass as moving forward the respiratory system.

Transplanted lungs

Transplants were inhabited by a greater variety of different bacteria, as proven by bacterial sequences identified, compared to healthy controls. These were in the majority Proteobacteria, whereas in healthy lungs Proteobacteria (class Gammaproteobacteria) and Firmicutes were predominant.

Variations:

a. Geographical

Given the fact that geographical differences have been reported in the case of gut microbiota in healthy controls and that as already mentioned temperature, oxygen, ph and nutrients presence play an important role in microbiota growth, it would be reasonable to expect that lung microbiome would be altered according to climate. Interestingly 19 pairs of sputum samples coming from patients diagnosed with cystic fibrosis from two centers (U.K. and U.S.) when analyzed using culture-independent techniques revealed significant heterogeneity between the groups as far as the bacterial populations inhabiting lower respiratory tract. No data is available comparing lung microbiome in healthy controls living across the world.
b. Spatial

Taking into consideration that microbial growth conditions (such as temperature, ph, oxygen tension) are well known to vary among different regions of healthy lungs, Dickson and co-workers examined whether lung microbiome varies accordingly. They showed that there is no significant spatial variation in healthy individual’s lung microbiota, thus proving that BAL results coming from a discrete lung segment can be representative of the individual’s microbiota, if healthy. In contrast, lung microbiota was shown to differentiate among segments in severe COPD and CF patients, thus raising the matter of unsuccessful infections treatment based in BAL samples in specific lung segments in such cases.

DISEASED LOWER RESPIRATORY TRACT MICROBIOME

a. COPD

Since early research times, COPD has been thought to be characterized by chronic inflammation, a situation partly induced by successive infections. This excessive inflammatory response is characterized by T-lymphocytes and macrophages penetration in the bronchial mucosa and has been proven to be precipitated by bacterial causes.

Leading the way in COPD lung microbiome research based on culture-independent techniques Hilty and co-workers showed that COPD patients lower airways exhibited a statistically important decrease in Bacteroidetes (specifically Prevotella spp.) and a reverse increase in Proteobacteria phylum (particularly Haemophilus spp.), thus proving for the first time an alteration in COPD lung microbiota. Interestingly, it was shown that no important differences exist in terms of quantitative results, though bacterial diversity was reported to be significantly diminished in patients diagnosed with moderate to severe COPD. Their BAL samples were highly abundant in Prevotella, Pseudomonas, Streptococcus and Haemophilus, genera which were though present in healthy controls too. Sze and co-workers highlighted an important increase of Firmicutes phylum, due to higher burden of Lactobacillus genus, in severe COPD patients which could be though attributed to the fact that the lung tissue samples studied were mainly parenchymal. Later, Zakharikina and researchers, enriched our knowledge about the core microbiota identified in lower respiratory tract of COPD patients, publishing that Moraxella, Curvibacter and Corynobacterium are some of the genera shown to characterize COPD state. Importantly, they correlated the presence of P. aeruginosa with a significant decrease in microbiome diversity identified in COPD patients BAL samples.

It is worth noting that microbiota of COPD patients clustered not according to disease stage but according to the use of inhaled corticosteroids and other bronchodilators, a fact attributed to their interference with immune response to lung microbiota.

b. Asthma

It is well-known that asthma is another major pulmonary disease characterized by chronic inflammation, either precipitated by external stimuli (allergens) or microbial communities causing acute infection thus triggering an asthma exacerbation. Early studies based on traditional culture-techniques or serologic testing implied chronic colonization or acute infection by Mycoplasma pneumoniae and Chlamyphila pneumoniae is associated with asthma beginning in adulthood. Later, C. pneumoniae were later proved by PCR techniques to be highly present in induced sputum, BAL and endobronchial biopsies of asthmatics compared to healthy controls. Bisgaard and co-workers in 2010 showed that during exacerbations lower respiratory tract of asthmatic young children is inhabited by Moraxella catarrhalis, Haemophilus influenzae, or Streptococcus pneumoniae. This is in accordance with previous study showing that early colonization of neonates hypopharynx by these species is associated with asthma beginning by the age of 5 and is a putative predictive marker for early-life wheeze, asthma and atopy. Later studies based on sequence analysis by PCR revealed that nasal colonization by Moraxella catarrhalis and Streptococcus pneumoniae correlates highly with severe asthma exacerbations caused by rhinovirus.

Generally, adult asthmatics sputum samples showed greater variety in terms of bacteria than healthy controls, as well as a rich profusion of Proteobacteria. Specifically, adults suffering from severe asthma had sputum samples rich in Actinobacteria and Klebsiella species, whereas those showing signs of moderate asthma had sputum samples abundant in Proteobacteria phylum. Interestingly, Proteobacteria were also highly abundant in induced sputum samples of patients with mild asthma, who were not under treatment with corticosteroids, thus suggesting that this...
microbiota alteration may be an inherent characteristic of asthma and not a result of immunomodulatory therapy.

c. Interstitial Lung Disease (ILD)

An alteration of lung microbiota is anticipated to be also present in the case of patients with ILD since a dysregulation in immune response and excessive inflammatory response is proven in lower respiratory tract of such patients. Surprisingly, only recently Garzoni and collaborators using culture-independent techniques showed that lung microbiota of patients diagnosed with idiopathic interstitial pneumonia, non-idiopathic interstitial pneumonia and sarcoidosis is comparable with that of healthy individuals. Earlier researchers reported the presence of Haemophilus influenza in BAL samples coming from patients with different interstitial lung diseases and a putative antagonistic relation between Pneumocystis jirovecii and bacteria inhabiting their lower respiratory tract.

As far as idiopathic pulmonary fibrosis (IPF) is particularly concerned, it was shown that specific genera, including Staphylococcus and Streptococcus, were more abundant in progressive disease rather than stable IPF state. In more details, IPF patients were reported to have double the bacterial burden of healthy controls and specifically Haemophilus, Streptococcus, Neisseria, and Veillonella species. Furthermore this bacterial abundance was shown to be predictive of progressive lung dysfunction and death, shedding light to the pathogenesis of the disease.

d. Lung Cancer

Keeping in mind the proven association between the risk for several types of cancer and the presence of specific bacteria in human body niches (such as Helicobacter pylori and stomach cancer) researchers are currently studying a putative disturbance of lung microbiota in patients diagnosed with lung cancer and the impact of changes in lower respiratory tract microbiome in carcinogenesis. Supporting this association, the meta-analysis of Brenner and co-workers revealed a pooled relative risk of 1.76 for patients with Mycobacterium tuberculosis infection to show later lung cancer. A restricted study concerning females, with no smoking history who were diagnosed with lung cancer, showed a significant microbiome disturbance in sputum, which surprisingly was not recognized in oral washes samples. Their sputum samples showed higher abundance in Streptococcus, Granulicatella and Abiotrophia genera when compared to healthy controls. Given that these bacteria are well known to cause infections of nervous system, higher and lower respiratory tract and chronic vascular inflammation, and that a major pathway in carcinogenesis involves inflammation, these results may suggest a new role of microbiota in lung cancer pathogenesis.

A first study involving diagnostic endoscopic sampling of single pulmonary nodule has been published, showing taxonomic differences between the microbiomes of benign and malignant lesions sampled. Specifically, Staphylococcus aureus, Pseudomonas and Haemophilus species were more abundant in malignant cases, while no differences between peri-lesional and peripheral (coming from other lung segments) samples was reported. To support these findings, Yu and co-workers in a recent publication showed that there is significant difference in microbial diversity between malignant and non-malignant lung tissue samples from cancer patients. Non-malignant tissues in paired samples showed higher microbial abundance. Specifically, non-malignant tissues from patients with advanced disease (stages IIIB, IV) were highly abundant in Thermus genera, whereas those from patients with metastatic disease were highly inhabited by Legionella. These results raised the issue of whether microbiota plays a role in tumorigenesis or the disease influences the microbiota of neighboring lung areas.

e. Cystic Fibrosis

Cystic fibrosis (CF) is an autosomal recessive disorder characterized by mutations of the gene coding for cystic fibrosis transmembrane regulator (CFTR) protein, which plays an important role in ion transport (chloride, thiocyanate, bicarbonate) across epithelial surfaces. Disruption of the lungs mucus layering leads to recurrent respiratory infections, chronic inflammation, progressive airway obstruction, damage of lung parenchyma and eventually death.

Since early times, culture-based techniques have been used to identify the microbiota inhabiting the lower respiratory tract thus establishing the main views of CF microbiology and treatment options for CF respiratory infections. Apart from Pseudomonas aeruginosa, Haemophilus influenzae and Burkholderia cepacia being major pathogens, methicillin-resistant Staphylococcus aureus (MRSA) has been isolated from CF patients and associated with higher risk of death.

During the last decade studies based on culture-
independent techniques proved the polymicrobial aspect of CF lower respiratory tract microbiota. *A. fumigatus, Stenotrophomonas maltophilia*, *M. avium-intracellulare* complex (MAC), *M. abscessus* complex (MABSC), Achromobacter spp., and Streptococcus milleri/anginosus group have been added to the list of microbes inhabiting CF patients lung. Rogers and co-workers proved that CF lung microbiota is characterized both by high complexity and large quantities of bacterial species. Recently, anaerobic bacteria were shown to be part of both “healthy” and “infected” CF lungs microbiota. Interestingly, higher airways inflammation and lower lung function is associated with reduced anaerobic load, a finding opposing the fact that anaerobes were shown to produce mediators of virulence, causing inflammation and acting synergistically with other putative pathogens.

In terms of disease progression it is well established that microbiota diversity reduces from early years to older ages. Coburn and co-workers showed that lower bacterial diversity correlates with deteriorating lung function, both forming a plateau at the age of 28. This decreasing diversity, which was recently shown to be great among individuals, has been primarily attributed to the use of antibiotics. Interestingly, during disease exacerbations an alteration of the bacterial burden and diversity has never been proved, thus raising exacerbations to events of “intrapulmonary spread of infections” and opening up new horizons to CF treatment.

**IMPLICATIONS**

It is now common knowledge that gut microbiota, which has been the target of intense study during the past decades, was shown to interact in a mutual way with host immune system. Importantly gut microbial communities were reported to be involved in pivotal signaling, thus boosting maturation of host immune cells and therefore protecting from infections. According to the “hygiene hypothesis” in early life years harmless pathogens such as, hemlinths, saprophytic mycobacteria, bifidobacteria and lactobacilli, induce abberant production of T regulatory cells, suppressing function of T effector cells, thus indirectly playing a protective role against inflammatory bowel disease and establishing immune tolerance.

In the same context, it has been shown that whereas farm and pet exposure during early infancy reduces the risk of atopy and asthma in coming years, the colonization of lower respiratory system of neonates by *S. pneumoniae* and/or *H. influenzae* and/or *M. catarrhalis* can lead to childhood asthma. Further studies on healthy lung microbiota could give answers on what defines healthy microbial contact and shed light on the topic of healthy lung immune response and how this is disturbed in case of disease.

Furthermore, taking into consideration that in the case of gut microbiota three distinct types, so-called “enterotypes”, have been suggested as three main clusters of all human gut microbiomes, it would be of researchers interest to find out if this applies for lung microbiome too. Putative “pulmotypes” could help researchers classify these highly dimensional microbial communities into easily manipulated groups. Importantly, if human lung microbiome could be classified in distinct groups, individualized therapies, as well as diagnostic tools, could be designed for diseases already correlated with disturbed microbiota.

Recently lower respiratory microbiome has been extensively studied in a plethora of diseased lung states, including COPD, asthma, CF, ILD and lung cancer. These studies have shed light to the pathogenesis of the diseases, revealing unknown host to microbe and microbe to microbe interactions thus loading numerous quarrels to the quiver of treatments for clinicians to use. Nevertheless, it is the thorough knowledge of healthy lower respiratory tract microbiota that would allow further understanding on lungs physiology and reveal putative pathogens still hiding under the mask of healthy lung microbiota variation.

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Neuromuscular diseases and respiratory system

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Key words:
- Neuromuscular diseases
- Lung disorders
- Pulmonary functional tests

SUMMARY
The balance (gas exchange) between the atmosphere and the human body depends not only on the lungs but also in large part on the function of the “ventilator pump”, which consists of the respiratory control center which is located in the brain, the body rib cage, the diaphragm the intercostal, accessory and abdominal muscles. A wide variety of neuromuscular disorders (NMDs) can result in dysfunction of the ventilatory pump that can lead to respiratory failure, pneumonia and even death. Breathing disorders are recognized as the leading cause of mortality in neuromuscular disease and appropriate interventions can prevent complications and prolong life in individuals with neuromuscular disease affecting the respiratory system.1


INTRODUCTION
Neuromuscular disorders (NMDs) are disorders that affect the central nervous system such as the motor-nerve cells in the spinal cord, the peripheral nerves, the nerve-muscle (neuromuscular) junction and the muscles. This diverse group of disorders vary markedly in etiology, prognosis, rate of progression, therapy and of course, pattern of respiratory involvement.2

The ventilation impairment in MNDs depends on the specific disorder, such as disorders that affect one level (e.g. isolated diaphragm paralysis) or different levels (e.g. multiple sclerosis) and the severity of impairment may be minimal and totally resolve with time and proper treatment (e.g. Guillain–Barré syndrome) or is characterized by relentless progression to eventual respiratory death (e.g. amyotrophic lateral sclerosis). Moreover, some NMDs concomitantly affect several structures (e.g. swallowing dysfunction in poliomyelitis, interstitial lung disease in polymyositis).

NMDs can be classified into diseases that involve the upper motoneuron, lower motoneuron or muscles themselves (Table 1).

The response of the respiratory system to moderate or severe NMDs is relatively stereotyped. The most common features are a reduced forced vital capacity (FVC), reduced respiratory muscle strength and malfunction of the neurons that control breathing. The breathing pattern is usually abnormal
and in early phases of NMDs, patients are either free of respiratory symptoms or have exertional dyspnea, not explained by obvious obstructive or restrictive lung disease. Physical examination may be unremarkable because generalized muscle weakness does not correlate with the degree of respiratory muscle involvement.3

Patients with respiratory muscle weakness have a low tidal volume (TV) and a high respiratory rate that persists in response to hypoxic or hypercapnic challenges. This rapid shallow breathing is not due to abnormalities in gas exchange (i.e. hypoxemia or hypercapnia) but is more likely to be due to severe muscle weakness and/or impaired afferent and efferent connections of the motoneurons by the underlying neuromuscular disease (Table 3).

### UPPER MOTONEURON LESIONS/ PYRAMIDAL INSUFFICIENCY

**Stroke**

Hemispheric ischemic strokes reduce chest wall and diaphragm movement on the side contralateral to the cerebral insult. Bilateral hemispheric strokes are usually associated with Cheyne-Stokes respiration, which is

| TABLE 1. Neuromuscular Diseases and motoneuron- muscles lesion. |
|-------------------|-----------------|
| **Upper Motoneuron** Lesions (pyramidal insufficiency) | Stroke | Spinal Cord Injury | Parkinson’s Disease | Multiple Sclerosis (MS) |
| **Lower Motoneuron** Lesions | Poliomyelitis | Amyotrophic Lateral Sclerosis (ALS) |
| Disorders of Peripheral Nerves | Diaphragm Paralysis | Guillain–Barré syndrome (GBS) | Critical Illness Polyneuropathy (CIP) |
| Disorders of the Neuromuscular Junction | Myasthenis Gravis | Eaton–Lambert Syndrome | Botulism |
| Inherited Myopathies | Duchenne’s Muscular Dystrophy (DMD) | Myotonic Dystrophy | Facioscapulohumeral Dystrophy (FSH) | Limb- Girdle Dystrophy | Acid Malate Deficiency | Mitochondrial Myopathy |
| Acquired Myopathies | Inflammatory Myopathies | Systemic Lupus Erythematosus (SLE) | Steroid Myopathy |

| TABLE 2. NMDs and clinical assessment |
|-----------------|-----------------|
| **Assessment** | **Clinical History** | **Physical Exam** | **Pfts** | **Sleep Testing** | **Thoracic Imaging** |
| Bulbar Weakness | Aspiration | Change in appearance | Difficulty with testing due to poor mouth seal | In any patient with nocturnal symptoms or progressive decline in FVC | Low lung volumes |
| | Drooling | Drooling | | | Pneumonia |
| | Voice change | | | | Pulmonary embolism |
| | Post prandial cough | | | | |
| Diaphragm/ inspiratory muscles | Orthopnea | Sleepy | Restrictive pattern | Decrease Sat <90% for >5 min | Hypoventilation |
| | Dyspnea on bending | Increased RR | ↓TLC, ↑RV | | and/or obstructive sleep apnea |
| | Sleepiness | Shallow breathing | ↓PIP | | |
| | Morning headaches | Orthopnea | ↓SNIP | | |
| | Decreased stamina speaking | Accessory muscle use | FVC (supine): drop >20%-30% | | |
| Expiratory muscles | Recurrent infections | Decrease in cough volume | MIP ≤30ccH2O | | |
| | Cough | | DLCO: normal or ↓ | | |
| | | | Sniff test :abnormal | | |

progressive hyperventilation alternating with hypoventilation and ending in apnea. This breathing pattern may result from increased responsiveness to carbon dioxide as a result of interruption of normal cortical inhibition.

In descending order of frequency, the most fatal complications of ischemic stroke are bacterial pneumonia, pulmonary embolism, myocardial infarction, and sepsis without pneumonia (often in the setting of a urinary tract infection).4

Stroke-associated pneumonia (SAP) has been implicated in the morbidity and mortality after acute ischemic stroke. SAP is described as early when it happens in the first 72 h of admission to the hospital. Another classification in use divides SAP into acute (when pneumonia develops within a month of stroke) and chronic (when it occurs later than a month).5

SAP is thought to be secondary to aspiration. Aspiration and its related risk factors such as impaired level of consciousness and dysphagia have been found to be important risk factors for SAP.6 Many stroke patients have impaired swallowing mechanisms leading to aspiration of oral content during sleep, which theoretically may be related to abnormal dopamine transmission.5,7

Spinal Cord Injury

Spinal cord injury is most commonly due to traumatic injury caused by motor vehicle accidents, falls, sports accidents and gunshot wounds. Cervical spinal cord injury (SCI) severely compromises respiratory function due to paralysis and impairment of the respiratory muscles. Respiratory complications such as atelectasis, pneumonia and respiratory failure are common,8,9 and a primary cause of both short and long-term morbidity and mortality.10

After acute SCI, a number of pathophysiologic changes can affect the respiratory system and predispose to respiratory complications, such as 1) cough inefficiency due to expiratory muscle weakness, 2) ciliary dysfunction, 3) mucous hypersecretion possibly due to impairment of the peripheral autonomous nervous system, 4) glottis dysfunction or gastric hypomotility increasing the risk of aspiration and 5) loss of consciousness at the time of the injury, increasing the risk of aspiration.11,12

The degree of respiratory impairment depends on the level and extent of the spinal cord injury.13

Initially the patients are threatened by acute loss of motor function of the respiratory muscles and the development of neurogenic pulmonary edema, whereas changes in lung function, pulmonary infections and airway problems are the main reasons for life threatening respiratory failure. The FEV1 and PEF are much lower in smokers as well.14

Early surgical stabilization, careful weaning from mechanical ventilation, meticulous search and treatment of infections, aggressive posturing and mobilization and support of impaired mechanical functions by physiotherapy, muscle training, noninvasive respiratory support and in some cases electrophrenic pacing increase survival and independency of quadriplegic patients.15

Various types of respiratory muscle training (RMT) (i.e. respiratory muscle strength and endurance) have been described in the literature, in order to improve respiratory function for people with cervical SCI. RMT is effective for increasing respiratory muscle strength and perhaps also lung volumes for people with cervical SCI.16,17

Parkinson’s Disease

Respiratory abnormalities are common in Parkinson’s disease, with pneumonia being the most common cause of death. Parkinson’s disease results in disordered coordination and activation of upper airway and chest wall muscles that may result in functional glottis obstruction and/or failed coordination of repetitive respiratory tasks. The substantial problem with Parkinson’s disease is glottis muscle dysfunction. An abnormal flow-volume loop contour showing regular or irregular flow oscillations commonly occurs. Physiological evidence of upper-airway...
obstruction may be present. In addition to the presence of flow oscillations, a rounding off of the peak of the midexpiratory flow-volume curve and a lowered peak expiratory flow rate have been observed in Parkinson’s patients. All these abnormalities are favorably treated with antiparkinson medications. 18

Levodopa is considered the gold standard therapy for Parkinson’s disease (PD). Some studies suggest that levodopa therapy improved FVC and PEF, whereas no changes are observed in FEV(1) and FEV(1)/FVC. These findings may provide some indirect evidence regarding the efficacy of levodopa in restrictive parameters of pulmonary function.19,20

Multiple Sclerosis

MS is a demyelinating disorder of the CNS, characterized clinically by “relapsing remitting” pattern, in which there are clearly defined symptomatic attacks lasting 24 hours or more, followed by complete or almost complete improvement. Because MS can cause focal lesions anywhere in the central nervous system, different patterns of respiratory impairment can occur. Involvement of the respiratory center in the medulla can cause failure of automatic breathing (Ondine’s curse), apneustic or neurogenic pulmonary edema. The three most common respiratory manifestations of MS are respiratory muscle weakness, bulbar dysfunction and abnormalities on respiratory control.

When respiratory motor pathways are involved, respiratory muscle impairment and subsequent acute or chronic ventilatory failure may ensue. As in other neuromuscular diseases, respiratory muscle weakness, abnormal control of breathing, or increased respiratory system elasstance with increased work of breathing may be involved in the respiratory disturbances in MS.21

In patients with clinically stable, moderately severe multiple sclerosis, the respiratory muscle function is abnormal.22 Diaphragmatic paralysis resulting in respiratory insufficiency has also been reported. Even with severe disability and impaired respiratory muscle strength, patient with MS seldom complain of dyspnea. This paucity of respiratory complaints may be due to restricted motor activities and greater expiratory than inspiratory muscle dysfunction. Clinical signs that may be helpful is predicting respiratory muscle impairment are weak cough and inability to clear secretion, limited to count on a single exhalation and upper extremity involvement, Advanced MS is frequently complicated by aspiration, atelectasis and pneumonia. Acute respiratory failure rarely occurs in this disease, but it can occur in case of severe demyelination of the central spinal cord.23

Trends for improvement following respiratory muscle training are observed for expiratory muscle strength, vital capacity and residual volume.2 Breathing exercises with positive expiratory pressure are often recommended to patients with advanced neurological deficits and may be beneficial in patients with mild to moderate stages of MS.24,25

LOWER MOTONEURON LESIONS

Poliomyelitis

Paralytic poliomyelitis, an acute disease caused by poliovirus, has been part of human history for thousands of years. In the mid-twentieth century, after the introduction of an effective vaccination program, the number of new cases dramatically dropped. Respiratory muscle paralysis is the most devastating of the clinical manifestations of poliomyelitis infection and is preceded by a period of fever and mild illness. Respiratory motor nuclei may be directly involved, resulting in diaphragmatic or other respiratory muscle dysfunction. Moreover, the central respiratory centers can be directly affected, resulting in irregular respirations. In contrast to Guillain-Barré syndrome, sensation is intact. Many patients require aggressive ventilator and hemodynamic support during the acute phases of their illness.26

Some patients with a history of poliomyelitis report late-onset neuromuscular symptoms and a decline in functional capacity.27 These late symptoms are referred to as post-poliomyelitis syndrome (PPS). The syndrome is characterized by new and increased muscle weakness, fatigue, muscle pain, joint pain, muscle cramps, cold intolerance, and lung problems.28,29

In patients with poliomyelitis, respiratory failure requiring ventilatory support may occur during the acute illness. Some patients continue to require long-term nocturnal ventilatory support; others are weaned but subsequently require support because of a late deterioration in ventilatory function.30

Amyotrophic Lateral Sclerosis (ALS)

ALS is a progressive neurodegenerative disease of the motor neurons in the cerebral cortex and spinal cord, which results in weakness and atrophy of voluntary skeletal muscles.31
The result is a combination of upper and power motoneuron dysfunction, manifested by spasticity and hyperreflexia muscle wasting, weakness and fasciculations. The usual clinical presentation is progressive weakness of the distal extremities, although severe respiratory muscle weakness, particularly intercostal muscle and diaphragm weakness presents with respiratory insufficiency as the initial manifestation. Progression of respiratory impairment as well as reduction in FVC and MMV are much faster in ALS than other chronic neuromuscular disorders. In contrast to patients with other neurological disorders, slightly elevated transpulmonary pressure at FRC and RV is usually increased and continues to rise as the disease progresses with maintenance of normal TLC. These changes are thought to be due to earlier involvement of the abdominal musculature, with preservation of intercostals and diaphragm function.

The shape of the flow-volume curve may also pinpoint the subgroup of ALS patients with greater weakness of the expiratory muscles. In patient with severe expiratory muscle weakness, the flow-volume curve near RV shows a sharp drop in flow such the maximum expiratory curve has a concave appearance. Additionally to respiratory dysfunction, sleep—disordered breathing is common and noninvasive nocturnal ventilation can be highly effective. The use of respiratory muscle testing has been used to help determine the prognosis and help clinicians decide when to initiate ventilatory assistance. Recently, the sniff nasal inspiratory pressure (SNIP) was used to predict survival in ALS. A sniff is a short voluntary inspiratory maneuver, which has been shown to correlate with invasive non volitional test of diaphragm strength.33

Difficulty in swallowing food or even saliva predisposes ALS patient to markedly increased risk of pulmonary aspiration. The advanced age at diagnosis and airway mucus accumulation represent factors of poor prognosis for ALS patients treated with NIV while airway intubation may be required because of bulbar dysfunction further impairing cough and the inability to clear secretions.33,34,37

Currently, guidelines from the American Academy of Neurology recommend treatment with noninvasive mechanical ventilation once the FVC is below 50 per cent of predicted. Also, ventilation with bi-level positive airway pressure has been shown to increase both survival and quality of life in patient with ALS, while those with orthopnea seemed to derive the most benefit.35,36 However, concerns remain that progressive disability may negate these benefits.38

**DISORDERS OF PERIPHERAL NERVES**

**Diaphragm Paralysis**

Unilateral or bilateral diaphragm paralysis following phrenic nerve injury can result from cardiac surgery, trauma, mediastinal tumors, infections of the pleural space and forceful manipulation of the neck. Diaphragm paralysis may also be seen with a variety of motoneuron diseases, myelopathies, neuromopathies and myopathies, as well COPD and other diseases that cause lung hyperinflation.

Unilateral diaphragm paralysis is more common than the bilateral one. The list of potential causes is similar. The most frequent causes of unilateral paralysis include phrenic nerve injury related to cardiac or thoracic surgery, herpes zoster, cervical spinal disease, or invasive or compressive tumors.39 In unilateral diaphragm paralysis, patients may be asymptomatic at rest but have dyspnea with exertion.40 Orthopnea may be present but is not as common or severe as in bilateral paralysis.

The diagnosis is often suggested by an elevated hemidiaphragm on chest x-ray and confirmed with a fluoroscopic sniff test. In this test, an upward or “paradoxical” movement of the paralyzed hemidiaphragm is seen during a vigorous sniff maneuver performed by the patient.41 Bilateral diaphragm paralysis is characterized by severe restrictive ventilatory impairment, with impaired pulmonary function and significant drops of VC in the supine position of up to 50%.42 TLC is also markedly decreased, as well as FRC and static pulmonary compliance. In most patients with nontraumatic bilateral diaphragm paralysis, the most important clinical feature is orthopnea out of proportion to the severity of the underlying cardiopulmonary disease. Sleep-disordered breathing with hypoventilation and hypoxemia is common as well and can be treated with NIMV.43,44

Interestingly, bilateral diaphragmatic paralysis can be difficult to diagnose. For one thing, there is no normal hemidiaphragm to use for comparison with an abnormal one. Therefore, chest radiography and fluoroscopic sniff testing can yield false negative results. Two-dimensional echocardiography of the movement of the diaphragm dome shares the same limitations as fluoroscopy.45 Phrenic nerve conduction studies can be useful in diagnosing a neuropathic cause of diaphragmatic paralysis but can have technical limitations.46 Diaphragm electromyography measurements may be useful, but technical issues such as “cross-talk” from adjacent muscles, electrode placement,
or variable muscle-to-electrode distances due to subcutaneous fat limit its utility. The “gold standard” diagnostic test is measuring transdiaphragmatic pressure using a thin balloon-tipped polyethylene catheter placed in the esophagus and stomach and showing a lack of ability to generate a transdiaphragmatic pressure. 47

Electromyography of the diaphragm is technically difficult and its results can therefore be hard to interpret or rely upon. It can potentially help differentiate between a myopathy and neuropathy, if one of these is strongly believed to be the cause of diaphragmatic dysfunction. Finally, dynamic MRI of the diaphragm has been recently described in the literature as a tool allowing more detailed study of diaphragmatic dysfunction.48,49

Guillain–Barré syndrome (GBS)

GBS precipitates respiratory failure more often than any other peripheral neuropathy. It is an acute idiopathic polyneuritis and usually presents as paresthesia and ascending paralysis of the lower extremities with absent deep tendon reflexes in a symmetrical distribution. The distribution of muscle weakness between respiratory and nonrespiratory muscles is not uniform in GBS and peripheral muscle strength does not correlate with the presence or absence of respiratory muscle weakness. However, ventilator failure collates with diaphragmatic weakness.

GBS affects the respiratory system by causing (1) weakness of the upper airway muscles, (2) weakness of the inspiratory and expiratory muscles, and (3) secondary complications such as pneumonia or pulmonary embolism.30 Approximately 25% to 50% of patients develop respiratory insufficiency severe enough to necessitate intubation and mechanical ventilation.51,52 Despite medical treatment, GBS often remains a severe disease; 3-10% of patients die and 20% are still unable to walk after 6 months.

With rapidly progressive disease, the intensive care unit (ICU) is considered the best setting for monitoring.53 Absolute indications for intubation include impaired consciousness, respiratory or cardiac arrest, shock, arrhythmias, blood-gas alterations, or bulbar dysfunction with confirmed aspiration. Noninvasive ventilation is generally not an option for these individuals because they are at a high risk for aspiration because of bulbar muscle involvement.34

Critical Illness Polyneuropathy (CIP)

Critical illness polyneuropathy (CIP) and myopathy (CIM) are major complications of severe critical illness and its management. CIP/CIM prolongs weaning from mechanical ventilation and physical rehabilitation since both limb and respiratory muscles can be affected.55 The major risk factors for critical illness polyneuromyopathy include sepsis and systemic inflammatory response syndrome (SIRS),56 treatment with steroids57 or neuromuscular blocking agents,58 and hyperglycemia.59 Other factors that are thought to contribute to this disorder include total parenteral nutrition, aminoglycoside use, catecholamines, hyperosmolality, female gender, longer duration of multiorgan failure, greater illness severity, and renal failure.60

The patients typically exhibit varying degree of musculoskeletal weakness, which range from mild weakness to near total paralysis with diminished deep tendon reflexes. Unfortunately, physical examination is unreliable as the sole means of diagnosis and electromyography with nerve conduction studies (EMG/NCS) are required to confirm the diagnosis.

Patient who develop CIP tend to require a longer period of mechanical ventilation and longer hospital stays compared with those without CIP. Additionally, the weakness associated with CIP results in an extended rehabilitation period, and there is evidence of persistent neuropathy on EMG/NCS as long as 5 years after discharge from the intensive care unit. Because no specific therapy for CIP exists, treatment is purely supportive and includes aggressive rehabilitation, nutrition support and treatment of any medical complication.

Compared with demyelinating diseases such as Guillain–Barré syndrome, critical illness polyneuromyopathy has normal nerve conduction velocity; compared with rhabdomyolysis, critical illness polyneuromyopathy will have a normal creatine phosphokinase, lack of muscle tenderness, and minimal electromyographic changes; and compared with cachectic myopathy, critical illness polyneuromyopathy is not usually associated with severe malnutrition.51

DISORDER OF THE NEUROMUSCULAR JUNCTION

Myasthenia Gravis

Myasthenia gravis is the most common disease affecting neuromuscular transmission and nowadays the term ‘myasthenia gravis’ includes heterogeneous autoimmune diseases, with a postsynaptic defect of neuromuscular transmission as the common feature.52,53 It causes weakness of several muscle groups including
the respiratory muscles, which are particularly susceptible to fatigue during the severe, potentially life-threatening, exacerbations, requiring intubation and mechanical ventilation, known as myasthenic crisis.\textsuperscript{64-66}

Acute respiratory failure and recurrent pneumonias due to aspiration from bulbar involvement and impaired cough are the most common complications of myasthenic crisis. BiPAP can be tried first in selected patients with acute respiratory failure especially due to myasthenic crisis and possibly other causes, while awaiting improvement from other specific therapies. However, it should be stressed, that there is a need for closer cardiac monitoring, as patients with myasthenic crisis are prone for cardiac arrhythmias. Also, repeated clinical assessment and arterial blood gas measurements for the first 6–8 hours are generally required to judge the efficacy of NIV and the need for invasive ventilation.\textsuperscript{67}

**Eaton – Lambert Syndrome**

LEMS is a myasthenic syndrome associated with small cell lung cancer (3% of cases) that can affect the respiratory muscles in a fashion similar to that of myasthenia gravis.\textsuperscript{68} In patients with LEMS, SCLC may also be occult and should be sought for up to 5 years after the diagnosis of LEMS. Although respiratory involvement is often a late finding, true respiratory failure can be a manifestation of LEMS,\textsuperscript{69} and this disorder should be considered in individuals with unexplained neuromuscular weakness.

LEMS shares a similar pathophysiologic mechanism with myasthenia gravis, but the clinical presentation is different and is characterized by:

1. an increase in the compound muscle action potential with repetitive nerve stimulation, a feature not seen in myasthenia,
2. more frequent presence of proximal leg weakness, which is worse in the morning,
3. greater autonomic dysfunction, and
4. frequent association with malignancy.\textsuperscript{70} Respiratory muscle weakness is often detected on pulmonary function tests but respiratory failure is infrequent.\textsuperscript{71}

**Botulism**

Botulism is a neuroparalytic syndrome that is caused by a toxin produced by the gram-positive bacterium *Clostridium botulinum* (A-F toxins).\textsuperscript{72} The botulinum toxin is one of the most potent toxins known. The clinical syndrome is one of progressive paralysis with early cranial nerve involvement causing blurred vision, dysphagia, dysarthria, and facial weakness. Descending muscle weakness is the usual course and involvement of the upper airway, diaphragm, and intercostal muscles often leads to the requirement for intubation and mechanical ventilation.\textsuperscript{73}

Spirometry usually reveals a restrictive ventilatory defect and recovery of muscle strength from the injury requires regrowth of new synapses, a process than can take 6 months, often requiring prolonged mechanical ventilation. Early diagnosis and management rely on history and physical examination. Treatment requires intensive care that includes mechanical ventilation, when necessary, and administration of antitoxin. Delay in treatment may allow progression of paralysis, protracted hospitalization and deaths of long-term mechanical ventilation and intensive care unit care. The clinicians must take this disease into consideration of a possible outbreak.\textsuperscript{74,75}

**INHERITED MYOPATHIES**

**Duchenne muscular dystrophies**

DMD is a progressive myopathic disorders caused by mutations of the dystrophin gene on chromosome Xp21.\textsuperscript{76}

Symptoms usually present in the early childhood.\textsuperscript{77} Gait disturbances and delayed motor development are common manifestation with proximal weakness resulting in an exaggerated lumbar lordosis. Kyphoscoliosis commonly develops as a result of severe muscle weakness and further contributes to a restrictive ventilatory deficit. Pulmonary symptoms are often minimal early on, despite significant weakness of the respiratory muscles. Maximum inspiratory pressure is reduced at all lung volumes in patients with DMD and declines time. FVC increased with growth during the first decade and may mask early respiratory muscle dysfunction before it plateaus and progressively decreases about 5 to 6 percent after 12 years of age.

Despite the severe and progressive muscle weakness, hypercapnia is uncommon in patients with DMD in the absence of pulmonary infections. This absence of hypercapnia is believed to be due to relative preservation of diaphragm function until very late in the illness. Once hypercapnia occurs, however, the course is rapidly progressive and the mean survival is approximately 10 months. Management of a patient with DMD is mainly supportive.

Maintenance of cough and adequate airway clearance is extremely important in attempting to prevent atelectasis and pneumonia in this patient population.
Respiratory tract infections are a serious complication in DMD patients and must be treated aggressively with physiotherapy, postural drainage, assisted cough techniques and appropriate antibiotics. All patients, regardless of cough status should receive vaccination against pneumococcal pneumonia and influenza. In some patients, assisted ventilation is required once respiratory insufficiency or symptoms of sleep-related breathing disorders are present.78,79

Myotonic Dystrophy (MD)

MD is the most common form of hereditary muscular dystrophy in adults. Symptoms such as muscle weakness are common and can be severe, despite mild limb muscle weakness. Myotonia of the respiratory muscles contributes to an increased work of breathing by increasing inspiratory impedance. Studies have suggested that the presence of a chaotic breathing pattern may explain the higher prevalence of chronic hypercapnia in patient with myotonic dystrophy than in patient with other forms of muscular dystrophy.78

Patient with myotonic dystrophy are particularly susceptible to development to respiratory failure with general anesthesia and sedatives. Postoperative respiratory monitoring is essential if surgery or the use of these agents is required. Pharyngeal and laryngeal dysfunction increases the risk of aspiration and sleep-related breathing disturbances are common and may include both central and obstructive forms of sleep apnea.80

In patients with MD, chronic pCO2 retention was associated with the presence of impaired central control of breathing81 and nocturnal positive-pressure ventilation should be tried when hypercapnia and hypoxemia are present.

Facioscapulohumeral Dystrophy (FSH)

FSH is an autosomal dominant dystrophy that primarily affects muscles of the face and proximal portion of the upper extremities. Respiratory muscle weakness is relatively unknown in FSH, but it is not uncommon.82 In patient with FSH, the FVC is significantly reduced, although facial weakness complicates spirometric assessment.

Limb- Girdle Dystrophy

Limb-Girdle Dystrophy is a heterogeneous group of autosomal dominant recessive disorders. The disease usually presents in the 2nd or 3rd decade of life and several case reports have documented the development of chronic hypercapnia in patients with limb-girdle dystrophy who have severe diaphragm weakness or bilateral diaphragm paralysis as the basis for hypercapnia. However, not all patients develop hypercapnia, most have moderate respiratory muscle weakness with normal gas exchange.83

Mitochondrial Myopathy

Mitochondrial Myopathy represents a heterogeneous group of disorders that affect mitochondrial function and may present as complex multisystem disorders with brain and striated skeletal muscle being the predominant organs affected: (a) Kearns – Sayre syndrome (b) myoclonic epilepsy, “ragged red fibers” and mitochondrial myopathy (c) encephalopathy, lactic acidosis and stroke-like episode.84

The clinical manifestations may be broad and include myalgia and exercise intolerance, proximal muscle weakness and external ophthalmoplegia. All three disorders are characterized by hypoventilation and depressed responses to hypoxia and hypercapnia and in some cases, unexplained respiratory failure.85

ACQUIRED MYOPATHIES

Acquired Myopathies include inflammatory polymyopathies (polymyositis and dermatomyositis), systemic lupus erythematosus, endocrine myopathies (hyper- hypothyroidism), hyperadrenocorticism, electrolyte disturbances, rhabdomyolysis and the use of high dose exogenous corticosteroids.

Pulmonary complications are the major cause of morbidity and mortality in polymyositis and dermatomyositis. These include interstitial pneumonitis, pulmonary vasculitis, and recurrent aspiration from oropharyngeal dysfunction and rarely hypoventilatory failure from respiratory muscle weakness.

Symptoms related to respiratory muscle weakness usually are not the presenting complaints. However, respiratory muscle weakness can occur in 5% to 10% of the patients with dermatomyositis and polymyositis85 and may be found in as many as 75% of individuals if respiratory muscle function is carefully evaluated.86 Interstitial lung disease may occur in up to 70% of patients with dermatomyositis or polymyositis.87 Individuals diagnosed with dermatomyositis or polymyositis should be evaluated for the presence of restrictive pulmonary disease, which may be due to respiratory muscle weakness as well as underlying interstitial lung disease.

In patients with systemic lupus erythematosus, dia-
phragm dysfunction and respiratory muscle weakness with small lung volumes occur without apparent involvement of the peripheral skeletal muscle. This syndrome has been called “the shrinking lung syndrome.” Decreased lung volumes do not appear to be due to parenchymal lung disease or phrenic neuropathy but, rather to a myopathic process affecting diaphragm strength. It is estimated that approximately 25 percent of SLE patients have diaphragm weakness, even in the absence of a generalized myopathy.

Finally, although steroid-induced myopathy was first described almost 30 years ago, the development of severe respiratory muscle weakness and prolonged respiratory failure following the use of high dose steroids, usually in asthmatic patients in ICU, has received renewed interest. Some patients require months of mechanical ventilation before eventual recovery. The serum CPK and EMG data show nonspecific changes. Overall, it is difficult to incriminate specific neuromuscular blocking agents or steroids as the only factors responsible for myopathic changes because an underlying severe illness, under nutrition, multiple medication and disuse atrophy are usually concurrent.

NONINVASIVE VENTILATION IN NEUROMUSCULAR PATIENTS

NIV can be used to deliver bilevel (Bipap) or continuous positive airway pressure (CiPAP). CiPAP can help to overcome upper airway resistance and keep alveoli open but this mode provides little support to the respiratory

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<th>TABLE 4. Indications and relative contraindications for the use of NIV in patients with NMDs.</th>
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<td><strong>Indications</strong></td>
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<td>Myasthenic exacerbation/crisis</td>
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<td>Neuromuscular failure with persistent weakness after extubation</td>
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<td>Motor neuron disease (ALS etc)</td>
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**Indication for Invasive Ventilation in Patient with NMDs**

**Acute Respiratory failure**
- Severe dyspnea
- Excessive accessory muscle use
- Copious Secretions
- Instable hemodynamic state
- Severe hypoxemia refractory to supplemental oxygen
- Acute ABGs disturbances (increased pCO₂ with pH >2.25)

**Chronic respiratory failure**
- Nocturnal Hypoventilation
- Increased work of breathing

*Cor pulmonale or pulmonary hypertension*

**Nocturnal desaturation (Sat <88%) despite supplemental O₂ therapy**

**Indications for Tracheostomy in patients with NMDs**
- Constant and fixed large airway obstruction (prolonged translaryngeal intubation)
- Intermittent and dynamic large airway obstruction (eg multisystem atrophy)
- Secretion management in patients choking on excessive oropharyngeal secretions in bulbar motor neuron disease or severe neuromuscular disease
- Weaning from prolonged invasive mechanical ventilation
- Management of patient with NIV who either cannot be ventilated effectively or are spending most of the day on ventilation

COPD: chronic obstructive disease; CHF: congestive heart failure; ALS: amyotrophic lateral sclerosis
muscles and it is not ideal in neuromuscular patients. For this reason, the use of BiPAP is the best strategy because Inspiratory positive airway pressure (IPAP), expiratory positive pressure (EPAP) and flow of oxygen, should be tailored to the individual situation.91

Additionally, the patients with NMDs are likely to require ventilatory support in the long term but the issue about whether to perform a tracheostomy is a big problem, as once performed it may be difficult to decannulate the patient and provide support with NIV. The ability of clinicians to predict which patients will require prolonged ventilatory support is limited but the ability to clear secretions is the key if the patients can be decannulated.

Finally, ventilation via tracheostomy is an alternative for NMDs patients but the decision on tracheostomy depends more on professional and patient views and wishes than on strictly medical criteria, however decannulation usually is difficult if cough flows are <160 lt/min, even with the addition of cough insufflations-exsufflation device91 (Table 4).

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A hereditary methemoglobinemia case: Estimation of tissue oxygenation

Case Report

Summary

We present the case of an adult patient presenting with clinical features suggestive of asthmatic bronchitis due to respiratory tract infection and a low pulse oximetry reading, who was finally diagnosed suffering from hereditary methemoglobinemia. Presence of a wide discrepancy in haemoglobin oxygen saturation, as assessed by pulse oximetry (SpO₂) and arterial blood gas analysis (SaO₂), along with normal arterial blood partial pressure of oxygen (PaO₂) are findings highly suggestive of elevated methemoglobin concentration. Measurements of SpO₂ and SaO₂ are, due to technical reasons, unreliable in cases of, either hereditary or due to exposure to an oxidizing agent, Methemoglobinemia and alternative methods estimating tissue oxygenation have to be used.


Case Report

We report the case of a 48-year-old male, with no previous medical history, who presented with wheezing and symptoms suggestive of respiratory infection at the outpatient Pulmonary Clinic. His physical examination revealed mild expiratory wheezing and a low pulse oximetry reading (SpO₂) of 86%. His chest X-Ray was normal and spirometry findings were within normal limits (FVC: 86%, FEV₁: 96%, FEV₁/FVC: 91%). Bronchodilatation test was negative, though post FEV₁ was markedly increased by 300ml. Given that neither the radiologic nor the spirometry findings were compatible with the patient’s low SpO₂, he was referred for cardiology evaluation and Computed Tomography angiography, both of which were negative for cardiac disease or pulmonary embolism. Arterial Blood gas analysis (ABGs) were pO₂: 78mmHg, pCO₂: 36mmHg, pH: 7,41, SaO₂: 96%, HCO₃: 22,5mEq/L. Alveolar-arterial Gradient was elevated (27 mmHg). Since SaO₂ and pO₂ were normal, a macrolide antibiotic, as well as inhaled corticosteroid in a fixed combination with a long acting beta adrenoceptor agonist, were prescribed.

Four days later he came back for re-evaluation and significant clinical improvement was noted. However, his SpO₂ reading was still abnormal at
86%, leading to a new ABGs analysis on a different blood gas analyzer, with the following results: pO2: 76mmHg, pCO2: 38mmHg, pH: 7,423, SaO2: 96.7%, HbO2: 83.6%, COHb: 0.7%, HHb: 2.9%, MetHb: 13%, HCO3: 24.2mEq/L.

The high methemoglobin (MethHb) level of 13% was consistent with the patient’s low SpO2 reading and suggestive of methemoglobinemia. Upon closer inspection mild peripheral cyanosis of the fingernail beds was noted (Figure 1), which had been observed for years by the patient and his family members, with no apparent worsening recently. Two weeks later and after discontinuation of all medication, patient had fully recovered, while a new ABG sample confirmed the presence of MetHb at high levels. The patient was advised on avoidance of oxidative agents (e.g. local anesthetics, antibiotics and common household products, such as naphthalene) and was referred to an Haematology specialist for further evaluation and genetic testing to confirm the diagnosis of hereditary methemoglobinemia. Unfortunately such genetic testing is not available in any laboratory in Greece.

Given the fact that his MethHb was elevated at two different time points, there had been no prior oxidising agent exposure, comprehensive physical and laboratory testing were normal, and in view of the peripheral cyanosis present for several years according to the patient himself, final diagnosis of hereditary methemoglobinemia was reached.

DISCUSSION

The most interesting point regarding this case report is the wide discrepancy in haemoglobin oxygen saturation, as assessed by pulse oximetry and arterial blood gas analysis, which are the two most commonly used methods in clinical practice. Haemoglobin oxygen saturation measured by pulse oximetry (SpO2) was 84-86%, while measuring on a blood gas analyzer (SaO2) it was 96-97%. The SaO2-SpO2 difference is called the saturation gap, which is normally no more than 5%, whereas higher results should always raise high clinical suspicion of methemoglobinemia1-3 (in this case: Saturation Gap= SaO2-SpO2=96%-85%=11%).

Methemoglobin (MethHb) is produced by haemoglobin oxidation, during which the iron in the heme group (which is in the Fe2+-ferrous-state) loses an electron and changes to the Fe3+-ferric-state.1,2 Along with carboxyhemoglobin (COHb) and sulfhemoglobin (SHb), MethHb is one of the dyshemoglobins (dysHb), which are abnormal Hb derivatives incapable of reversibly binding with oxygen.5 Haemoglobin oxidation to MetHb has two important effects. The first one is that MetHb cannot bind with O2, and therefore does not contribute to O2 transfer to the tissues. Secondly, ferric iron in partially oxidised Hb (by means of allosteric modulation) increases its O2 affinity, hindering oxygen release to the tissues, and therefore causing a leftward shift of the oxygen-hemoglobin dissociation curve.1,2,4,5 When MethHb concentration increases, both these mechanisms are responsible for the resulting tissue hypoxia, and the upcoming wide clinical presentation, varying from asymptomatic peripheral cyanosis (when MethHb concentration is up to 15%) to death (when MethHb concentration exceeds 70%).1,2,4

Assessing tissue oxygenation in methemoglobinemia is challenging. MethHb absorbs light at both wavelengths used in pulse oximetry (660 and 940nm), at the same or even higher level than oxyhemoglobin (O2Hb) and deoxyhemoglobin (HHb), leading to inaccurate results.5-7 Animal studies since the ’90s have shown that pulse oximetry yields results that lead to either over- or under-estimation of tissue oxygenation (depending on whether SpO2 is over or under 70%). Moreover, pulse oximetry in methemoglobinemia rarely exceeds the value 85%.6,7

On the other hand, blood gas analysis is also unreliable as in most commonly used ABGs analyzers, SaO2 is not directly measured but is rather calculated, by means of the PaO2 measurement and the normal Hb-O2 dissociation curve.2 Although PaO2 is within normal limits in methemoglobinemia, the Hb-O2 dissociation curve is shifted to the left, resulting in false SaO2 calculation.

The only reliable non-invasive method to detect MethHb and estimate tissue oxygenation at the same time is by means of the new technology CO-oximeters.1,2,4 While the widely used pulse oximeters measure light absorbance in 2 wavelengths, CO-oximeters can reliably detect and measure not only HbO2 and HHb but also all dyshemoglobulins, by measuring light absorbance in at least 6 wavelengths.5,7 They are invaluable tools in every day
clinical practice, although their use is still not very popular. Regarding this case, since there was no CO-oximeter available, we calculate the oxyhemoglobin fraction (FO\(_2\)Hb), by using the data from the second ABGs analysis: 

\[
FO_2Hb = \frac{HbO_2}{(HbO_2+HHb+COHb+MethHb)} = 83.6\%
\]

Oxyhemoglobin fraction is a reliable tissue oxygenation index, which is practically equal to SpO\(_2\) and SaO\(_2\), in the absence of dysHbs\(^{1,2,5}\). In this case, FO\(_2\)Hb was approximately 84% of the total Hb, pulse oximetry (SpO\(_2\)) resulted in a slight overestimation (84-86%) and SaO\(_2\) in ABGs was utterly false (96%).

Any oxidising agent (e.g. drugs, smoke, inhaled toxic substances) can theoretically lead to MetHb formation and this practically happens constantly. Natural reducing systems maintain the levels of MetHb under 2%, with the most important system being NADH-Methemoglobin reductase (NADH-NR). NADH-NR is responsible for the endogenous reduction of MetHb, corresponding to 99% of the reducing activity\(^{1,2,4}\) by transfer of one electron from NADH to MetHb, thus reducing heme Fe\(^{3+}\) to Fe\(^{2+}\).\(^{1,2,4}\) NADH-MetHb reductase synthesis is encoded by the CYB5R3 gene in chromosome 22q13qter, whereas more than 40 mutations have been identified in cases of hereditary methemoglobinemia.

Initial misdiagnosis of asthma or Chronic Obstructive Pulmonary Disease (COPD), late diagnosis and lack of laboratory confirmation (due to lack of proper equipment) of the diagnosis are common features among the limited reported cases of hereditary methemoglobinemia diagnosed in adult life.\(^{10-14}\) In order to overcome these technical difficulties, simple diagnostic tests are proposed in order to reach diagnosis.\(^{2,5,13}\) The simplest method proposed is by assessing the arterial blood sample colour, which is dark brown (chocolate) when MetHb >20%, as is evident on our patient’s sample in figure 2.\(^{1,2,14}\) Additionally, while HHb (which is also dark coloured) turns bright red when exposed to O\(_2\) (either by enriching the blood sample with 100% O\(_2\) bubbles or by exposing a single blood drop to room air), MetHb maintains its dark brown colour.

Although the reported cases of hereditary methemoglobinemia in adults in the literature are limited,\(^{3,8,11-13}\) the clinical presentation and the diagnostic and tissue oxygenation evaluation methods discussed are also applicable in the far more common cases of acquired methemoglobinemia due to an oxidising agent exposure. It is noteworthy that, in a recent review, 11 cases of acquired methemoglobinemia due to lidocaine use during bronchoscopy were reported, while the reviewers suggest that methemoglobinemia is probably underdiagnosed in everyday practice\(^{16}\).

In conclusion, high clinical suspicion is the key prerequisite for methemoglobinemia diagnosis\(^{17}\) and should be high in every case of peripheral cyanosis with normal PaO\(_2\) unresponsive to oxygen therapy.

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Endobronchial hamartoma

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A 46-year-old man presented with a 3-months history of productive cough and shortness of breath. He was an ex-smoker and had a history of hypertension, dyslipidemia and chronic obstructive pulmonary disease. On auscultation, wheezing over the right pulmonary area was noted. A chest X-ray showed a nodular lesion at the right middle pulmonary field while computed tomography revealed a soft tissue polypoid endobronchial lesion, of 1.5cm in diameter, arising from the carina between the right upper lobar bronchus and the bronchus intermedius and carrying calcifications (Figure 1). Flexible bronchoscopy revealed a polypoid mass inside the distal end of the right main bronchus (Figure 2). The patient underwent right thoracotomy with bronchotomy and mass excision. Histological features were consistent with hamartoma.

Endobronchial hamartomas are the most common benign lesions in adults, with an incidence of 2.5-3.2/1000 in the general population. They are usually located in the lung parenchyma and are asymptomatic. The endobronchial location is very rare (1.4%) and may produce respiratory symptoms and radiological findings. Hamartomas are traditionally treated by surgical manipulations. Less invasive procedures involving rigid or flexible bronchoscopy are being recently applied with fine results.

**FIGURE 1.** Soft tissue lesion in the right main bronchus of 1.5cm in diameter, areas of fat density and central and superficial calcifications.

**FIGURE 2.** Polypoid mass emanating from the carina between the right upper lobar and the intermedius bronchus (RC1) with superficial calcifications.

**REFERENCES**

Tracheal diverticulum
Virtual and flexible bronchocopy views

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The authors declare no conflict of interests.

A 73-year-old Caucasian male with a history of chronic cough was admitted to our outpatient clinic. On virtual endoscopy, administered prior to his examination, a congenital tracheal diverticulum, almost 3-4 cm above the level of carina was demonstrated (Figure 1). A flexible bronchoscopy followed. The procedure verified the presence of a tracheal diverticulum almost 4 cm above the level of the carina, located at the right posterolateral tracheal wall, filled with secretions (Figure 2).

Tracheal diverticulum is rare, can be considered as a supernumerary, malformed branch of the trachea and the histological structure resembles that of trachea. The frequency in some autopsy series has been estimated to 1% and a male predominance has been reported. They are usually asymptomatic, but in some cases chronic cough, stridor, dyspnocia, hemoptysis or repeated episodes of infection may be present. Cases of recurrent laryngeal paralysis, causing dysphonia and ineffective ventilation or pneumomediastinum due to perforation of the diverticulum have also described. The differential diagnosis includes a laryngocele, a pharyngocele, a Zenker’s diverticulum, an apical hernia of the lungs and apical paraseptal bullae. Computed tomography examination, including thin sections or reconstructed images, is the proper imaging modality. Bronchoscopy can establish the diagnosis, but diverticula with a narrow opening or just a fibrous connection with the trachea can be missed. Conservative measures, such as antibiotics, mycolytics agents and physiotherapy are proposed and surgical intervention is rarely advocated.

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Cystic Bronchiectasis in a 38 year old female

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A 38 year old non-smoker female was admitted to our hospital 7 months ago because of severe worsening of her symptoms: cough, purulent sputum, dyspnea, wheezing, fever, fatigue and hemoptysis. The patient had symptoms since childhood and recurrent exacerbations all her life. At the age of 28, she was diagnosed with bronchiectasis and Pseudomonas aeruginosa was isolated in sputum. During the last ten years the patient did not refer to a chest physician for follow up, she was self-prescribing antibiotics during exacerbations of her symptoms (3 times/year).

Her clinical examination on admission to the clinic revealed: SaO2 88%, FiO2 21%, HR 120 beats/min, RR 20/min and wheezing. The patient received antipseudomonal IV antibiotics, ceftazidime and amikacin, nebulized bronchodilators, mucolytics, oxygen therapy, physiotherapy. Sputum culture and bronchial washing grew again Pseudomonas aeruginosa, indicating chronic infection. HRCT was performed showing severe bilateral cystic bronchiectasis lesions affecting all lobes, more excessive in middle lobe, lingula and lower lobes bilaterally. Spirometry showed a severe obstructive pattern. Patient’s symptoms improved and she was discharged from hospital.

Subsequently, she was under follow up in the outpatient clinic. A complete etiologic investigation of bronchiectasis was performed and resulted to post infective etiology (severe pertussis in infancy). Eradication treatment was administered with ciprofloxacin 750mg×2 for 14 days, followed by nebulized colistin 1MU twice a day for 3 months. She then started chronic suppressive treatment with Azithromycin (250 mg 3 times a week). After these therapeutic interventions, a significant improvement was observed: improvement of dyspnea and fatigue, reduced sputum volume, improvement of sputum purulence, without any new exacerbation during the last 7 months.

Collectively, eradication treatments with systematic and nebulized antibiotics is indicated in patients with chronic infection with Pseudomonas aeruginosa as well as long term antibiotics for anti-inflammatory effect, in order to improve symptoms and reduce exacerbations.

**FIGURE 1.** HRCT was performed showing severe bilateral cystic bronchiectasis lesions affecting all lobes, more excessive in middle lobe, lingula and lower lobes bilaterally.
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Acknowledgements

Acknowledge the persons who made a true contribution and who endorse the data and conclusions. Acknowledge any funding sources.

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5ο Εθνικό Σεμινάριο
1-2 Δεκεμβρίου 2017
Μακεδονία Πάλας Ητέλ
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