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Salutes Covid Warriors

Pulmon (May - Aug 2020) is dedicated to the memories of those who lost their life by the clutches of Covid 19

Contents

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Pulmon

The Journal of Respiratory Sciences

General Information

Pulmon

The Journal of Respiratory Sciences

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Pulmon

The Journal of Respiratory Sciences

COVID-19 – New lessons every day

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COVID-19 (Coronavirus disease 2019) is a disease that needs no introduction at this point. It is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a strain of coronavirus and was first reported in Wuhan, China, in late December 2019 and thereafter it started spreading globally, becoming the most threatening pandemic humans ever faced. [1].

It is a zoonosis and is believed to have spread from bats to human in the wet markets of Wuhan. Transmission is primarily human to human via contact and droplets of upper airway secretions. Recently WHO has acknowledged the probability of airborne spread and evidence in this regard is growing. Transmission by fomites, sexual contact, oro fecal route and blood transfusion and vertical transmission has not been fully ruled out.[2]

The ever changing statistics[1]

The epidemiological parameters are daily changing and vary widely geographically. As of early August 2020, the total number of confirmed COVID-19 cases globally is over 19 million affecting virtually all continents except Antarctica. Even though asymptomatic transmission has not been documented, presymptomatic carriers and transmission have been documented. Most recent data suggest the incubation period is around 5 days and the average period of infectivity is 12 days. The case fatality rate is apparently 2-3%, but the actual rate may be lower because of not testing asymptomatics and thereby under diagnosing the condition.

Even though 1-2% of COVID patients are children, severe disease and mortality is luckily much less among children. However, infants under 12 months seem to be affected more seriously. The incubation period is likely to be 2 days lesser than in adults.

Pathophysiology: "Happy" or not? [3]

Observations have suggested that patients with COVID pneumonia do not behave uniformly in the pathology of lung lesions as well as clinical presentation. There seem to be two different phenotypes – some patients who are breathing normally even with hypoxemia (the so-called happy hypoxia), with good response to nitric oxide, have hypocapnia and good response to prone ventilation. The other group is severely dyspneic, poor response to nitric oxide and prone position and have normocapnia or hypercapnia.

This may be explained by the concept of L-type and H-type COVID-19 pneumonia. L type is characterized by low elastance, low ventilation-to-perfusion (VA/Q) ratio, low lung weight and low lung recruitability (most of the lung tissue is aerated, so the recruitability is low). While, the H type is characterized by high elastance, high right-to-left shunt, high lung weight and high lung recruitability.

The evolution of the disease and the so-called P-SLI[4]

Unfortunately, one cannot be relieved by having L type COVID, as the disease can evolve to H type any time! It has been found that L type accounts for 70-80% of all patients – while most of them remain stable for a few days and improve, but a minority can progress to H type. This transition from L to H type need not be always due to progression of COVID pneumonia. Two other factors may contribute to it – both ventilator-induced lung injury (VILI) and patient self-inflicted lung injury (P- SLI). P- SLI is rather a novel concept, which postulates that laboured spontaneous breathing associated with increased tidal volume results in increased negative intrathoracic pressure which can be detrimental. But this is not yet universally accepted, and it would be too premature to advise prophylactic intubation and mechanical ventilation to avoid PSLI since VALI is a proven culprit. Also, it is yet to be proven whether PSLI is a cause or effect or merely an epiphenomenon.

Clinical manifestations: are there some clues?

Apart from the usual influenza-like presentation with fever, myalgia, fatigue throat pain, dry cough, etc., some specific symptoms may arouse suspicion of COVID 19. 40 to 50 percent COVID-19 sufferers have reported having difficulty in appreciating smell and taste including anosmia, hyposmia, ageusia, and dysgeusia[5]. This is thought to be more of a neurological etiology rather than a conductive cause. Some of the patients may have diarrhoea, and this seems to be

more commonly reported among Indian patients than found globally. 1-2 % of patients seem to develop conjunctivitis which may be a heralding sign of impending respiratory involvement. There are case reports in which conjunctivitis was the only manifestation of the disease.

Radiology - neither diagnostic nor specific[6,7]

Chest radiographs may be normal in early or mild disease. Though up to 70% have a normal X-ray chest at the time of admission, almost 80 percent develop some abnormality in the due course. CT may be needed in symptomatic patients to detect early lung lesions. Most of the findings occur around 10-12 days of onset of symptoms .Even though the CT findings are not specific or diagnostic for COVID pneumonia, it may aid in diagnosis as well as management. The consensus statement of Radiological Society of North America, American College of Radiology and Society of Thoracic Radiology has classified CT findings in COVID 19 into 4 categories:

Typical appearance – characterized by bilateral, peripheral, multifocal ground-glass opacities +/- consolidation. Bronchovascular thickening in the areas of GGO also seen. Crazy pavement pattern or organizing pneumonia pattern with reverse halo sign also may be there.

Indeterminate appearance – characterized by absence of typical CT findings and the presence of multifocal, diffuse perihilar or unilateral ground glass opacities +/- consolidation lacking a specific distribution

Atypical appearance characterized by absence of typical or indeterminate features and the presence of isolated lobar or segmental consolidation without GGO, centrilobular nodules, tree in bud appearance, cavitation, interlobar septal thickening, mediastinal lymphadenopathy etc. Pleural effusion is uncommon (3%)

Negative for pneumonia – no GGO, no consolidation.

CT findings may progress over days through 4 stages –

1. Early-stage of 0-4 days with normal CT/ GGO only
2. Progressive stage, 5-8 days, with increased GGO and crazy pavement pattern
3. Peak stage, 9-13 days, with consolidation and
4. Absorption stage, after 14 days, with an improvement, may show fibrosis.

CT may help to diagnose pulmonary thromboembolism (PTE) which is found to common in the segmental and subsegmental arteries of the consolidated segments. Higher CT scores along with higher values of d-dimer and C- reactive protein values indicate a high risk for PTE.

CO-RADS & COVID-RADS

CO-RADS (COVID-19 Reporting and Data System) is a proposed CT scoring system for COVID 19 to ensure uniformity in CT reporting. This system assigns a score of 0 to 6, depending on the CT findings. 0- CT uninterpretable, 6 is confirmatory. COVID-RADS is a similar one developed by American radiologists.

Ultrasound

Lung ultrasound may help pick up lesions due to COVID 19. Focal to diffuse predominantly posterobasal multiple B- lines representing thickened subpleural interlobar septa, subpleural consolidations typically associated with preservation of flow, dynamic air bronchograms, etc. are some of the signs. Light beam sign, the vanishing border between normal and consolidated lung is also described.

PET CT The experience is limited, However, FDG uptake has been noticed o be high in the GGO areas.

Risk factors and complications[8]

Even though more than 90 percent of COVID 19 patients are cured without any significant morbidity, patients with risk factors like smoking, elder age, diabetes, underlying malignancy, immunosuppression, obesity, cardiovascular diseases, etc. develop complications like ARDS, pulmonary embolism, cerebrovascular accidents, acute cardiac injury, myocarditis, secondary bacterial pneumonia, sepsis, dissceminated intravascular coagulation (DIC) and multi organ failure (MOF). The cytokine storm is thought to be responsible for most of these. Hypoxemia, lymphopenia, elevated CRP, rising titres of D –dimer may suggest the impending doom.

Diagnosis- Making Sense of the Jigsaw puzzle

There are principally two types of tests available for COVID-19: viral tests and antibody tests. The viral tests are direct and detect the virus and hence reflect current infection. The antibody tests are indirect tests, as they detect established seroconversion to a previous infection or early seroconversion to ongoing infection.[9]

Direct Tests

These tests detect viral RNA using nucleic acid amplification tests (NAAT), such as reverse transcription (RT)-PCR. Technical considerations including specimen collection (variable collection methods), which samples to collect (upper or lower respiratory tract biospecimens, or other samples), time of collection in relation to the course of the disease, and the availability of different laboratory test methods and kits and transportation of samples affect the results but is generally accepted as a single discriminatory test.[9]The viral load in throat swabs is greatest at the time of onset of symptoms and decreases monotonically thereafter. Specimens should be collected as soon as possible once a decision has been made to pursue SARS-CoV-2 testing, regardless of the time of symptom onset as it has been shown that viral shedding may begin 2–3 days before the appearance of the first symptoms, facilitating pre-symptomatic or asymptomatic transmission.[10]

A nasopharyngeal swab is preferred but when not possible, acceptable alternatives are: an oropharyngeal specimen, a nasal mid-turbinate specimen (using a flocked tapered swab), an anterior nares (nasal swab) specimen (using a flocked or spun polyester swab) or a nasopharyngeal wash/aspirate or nasal aspirate specimen. Lower respiratory tract specimens are also recommended if available, though not recommended to be obtained for diagnosis. The virus can also be detected in other specimens, such as blood and stools but are less reliable.

TrueNat is a chip-based, battery-operated RT-PCR kit. Initially, it could only identify the E-gene in the SARS-CoV-2 virus, but the newer generation can detect the RdRp enzyme also found in the virus RNA. ICMR has approved it as a comprehensive assay for screening and confirmation of COVID-19 cases. The assay comprises of two steps first of which detect E gene. All negatives are to be considered as true negatives. If positive second phase RdRp gene confirmatory assay is performed. All samples that test positive by this assay are considered as true positive.[12]

Detection of isolated viral antigens

Antigen detection tests directly detect viral particles in biological samples like nasopharyngeal secretions. Several rapid antigen tests have been proposed and are in pipeline however, false-negative rate is high owing to either a low or variable viral load and the variability in sampling. Variability is sampling can augment negativity due to low viral loads and hence increasing the false-negative rate.[13]

Standard Q COVID-19 Ag kit and has been developed by SD Biosensor has been validated by ICMR to have high specificity with moderate sensitivity and are recommended for use as detection test (point of care diagnostic assay) in the containment zones as well as hospitals in combination with the gold standard RT-PCR test.[14]

Detection of Antibody

The data suggest that seroconversion occurs after exposure to SARS-CoV-2 as in other acute viral infections, with IgG concentration beginning to rise as IgM concentrations reach a plateau. IgM and IgA rise are relatively slow, relative to other respiratory viruses. In the early phase of illness (within 7 days since the onset) the NAAT test only has a sensitivity of around 70%, with the antibody assay having an even lower positive rate around 40%.

After 8 days of onset of symptoms sensitivity of antibody testing increases and reach over 90% across day 12 after onset. In the later phase (day 15-39 after onset), the sensitivities for total antibody, IgM and IgG were 100.0%,94.3% and 79.8%, respectively. In contrast, RNA was detectable in nearly 50 %. Hence the timing of testing is of prime importance.[15]

Most point of care rapid test are LIFA (lateral flow immunoassay) which uses gold nanoparticle antibody conjugate. Generally, rapid assays have a low diagnostic performance compared with ELISA assays.[16] A recent meta-analysis found inadequate evidence to advise the use of serology testing especially in point of care testing. Hence the idea of immunity passports and rapid diagnosis using serological testing is enticing extreme caution is required to be not overzealous and it is advisable to stick to National guidelines. ICMR has advised using IgG based ELISA and CLIA assays only for the conduct of serosurveys.[14]

At present, NATT based tests are the most reliable of tests available for SARS-CoV-2. There is an urgent need for the development of serological assays with high sensitivity for screening and adequate specificity and confirmation that seropositivity equates to immunity.

Treatment -There is no failure, except in no longer trying

"Time is of the essence" is apt to describe current scenario not just in need to bring out newer drugs but also to make sure they are effective and safe. A strong foundation of scientific thinking should be the way forward even as we try to expedite therapeutic options.

No definitive cure for COVID 19 exist as of now however there has been huge optimism in the medical community with recent results of RECOVERY trial showing Dexamethasone to reduce mortality by one third in ventilated patients and one fifth in patients requiring oxygen support. The dose used was dexamethasone 6mg IV/oral and in pregnant or breastfeeding women, prednisolone 40 mg administered by mouth (or intravenous hydrocortisone 80 mg twice daily) was used instead .[17,1819]

Though initially touted as a potential game-changer much of interest in Hydroxychloroquine has waned especially with Solidarity trial which is a multinational Phase III-IV clinical trial organized by the World Health Organization involving multiple countries. With the availability of interim results WHO has stopped two arms – Hydroxychloroquine and Ritonavir/lopinavir for lack of benefit while the other two arms - Remdesivir and Lopinavir/Ritonavir with interferon beta are ongoing.[20] Remdesivir has shown improve recovery time but has no effect on mortality however this could be of extreme relevance when hospitals are flooded with patients and every available bed is a potential lifesaver. Interestingly a short 5-days course was found to have similar efficacy to a long 10-days course with a lesser side effect.[21,22]

Favipiravir is approved for restricted emergency use in COVID 19 by Drugs Controller General of India (DCG)I, however scientific data available remain scarce and inconclusive. The drug is approved for use only in India, China and Russia whilst not approved in all other countries.[23]Available data suggest that the drug may reduce recovery time in mild to moderate cases and further studies are essential.[24]

Data suggest that the IL-6 pathway may play an important role in the overactive inflammatory response in the lungs of COVID-19 patients and hence provided the background for use of Tocilizumab, a humanized interleukin-6 (IL-6) receptor antagonist. Multiple small studies have shown benefit in severely ill patients.

Hypercoagulable state seen in COVID 19 appears to adversely impact prognosis, however, there are no high-quality studies to support interventions that go beyond standard indications, and antithrombotic therapies carry risks of increased bleeding. All patients with COVID-19 admitted to the intensive care unit (ICU) or wards require thromboprophylaxis. Usually, medical patients can be treated with prophylactic-dose low mo-

lecular weight (LMW) heparin (or unfractionated heparin if LMW heparin is unavailable) for thromboprophylaxis. Perioperative VTE prophylaxis is important for patients with COVID-19 who are hospitalized for a surgical procedure as well as in obstetric patients with COVID-19 who are in the hospital prior to or following delivery. LMW heparin is adequate if delivery is not expected within 24 hours and after delivery; unfractionated heparin is used if faster discontinuation is needed.[25]

Other potential therapeutics includes Ivermectin, Nitazoxanide, Convalescent plasma therapy.

Vaccines – Where we are?

Though no vaccine is currently approved, there are at least 16 vaccines in clinical trials and a plethora of other candidates in pre-clinical stages.[26] Vaccination may be used to reduce disease severity, control transmission by viral shedding and/or prevent future infections.

The average vaccine, taken from the preclinical phase, requires a development timeline of 10.71 years and has a market entry probability of 6% and this, in fact, represents a major hurdle.[27] Much of vaccine development strategies have been based on advanced in SARS coronavirus which like SARS-CoV-2 bind to similar ACE2 receptors found in the human lung and exhibit genomes of approximately 30 kb (approximately 89% nucleotide similarly to SARS-like coronaviruses found in Chinese bats)

Eosinophilic infiltration or increased infectivity post-vaccination represent the most important hurdles in the early development of SARS coronavirus vaccines and is seen with both whole virus vaccines and complete spike protein vaccine[28]. Though significant hurdles exist and much of the route untraveled, vaccines offer currently one of the best probable solutions.

BCG vaccine is being investigated for effectiveness to prevent and/or reduce the severity of SARS-CoV-2 infection however remains controversial and is currently not recommended by WHO.[29,30]

A recombinant vaccine called AZD1222 by the University of Oxford and AstraZeneca[31], Moderna's mRNA-1273, a nucleotide-based vaccine and a protein subunit approach- spike antigen by Sanofi and Glaxo SmithKline are some of the vaccines in the pipeline.

Multiple vaccines have recently shown effectiveness in eliciting an immune response with acceptable safety profile. The world eagerly awaits results of Phase 3 trials which already is underway for Oxford/AstraZeneca vaccine in Brazil and South Africa; Sinopharm in the United Arab Emirates; Sinovac Biotech- inactivated vaccine called CoronaVac. The Murdoch Children's Research Institute in Australia is conducting Phase 3 trial with The Bacillus Calmette-Guerin vaccine.

Other Vaccines that show promise are the ones developed by Moderna, Bharat Biotech designed a vaccine called Covaxin. Trump administration has awarded a $1.9 billion contract for 100 million doses to be delivered by December and the option to acquire 500 million more doses for the Vaccine developed by BioNTech in collaborations with Pfizer and the Chinese drug maker Fosun Pharma.

At present, there seems no way out, as disease ravages even places which have done excellently, and aroused hope of a future model for others. The chances are, the majority of the population might be infected before vaccines or drugs become available. Vaccines along with repurposed drugs appear promising. Further drugs that can improve recovery times can help free up beds during a pandemic which can reduce mortality due to the disease. We must prepare ourselves for a life with COVID at least in foreseeable future till either a vaccine or cure or effective therapy is available especially in the context of recent trials showing an absence of protective antibody in the majority. It is upon us, medical professional to be steadfast and be the beacons of resolve and fight that the community desperately needs.

The current issue of PULMON contains two articles about COVID 19. Fathahudeen et al describe one of the earliest experiences of treating COVID 19 successfully in Kerala. Safe performance of spirometry during COVID 19 era is a major concern and is well addressed by Jai Mohan Unnithan et al. Both these articles shall increase our insight into this disease.

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Biomarkers in Sepsis

Raghavendra Goudar[1], Pooja Murthy[1], Ajith Kumar A.K[2]

Introduction

Sepsis is defined as life-threatening organ dysfunction caused by dysregulated host response to an infection[1]. Global burden of sepsis is estimated to be about 31.5 million leading to around 5.3 million deaths per annum[2]. A five year data from a tertiary care Indian ICU found 6.2% incidence of severe sepsis with the ICU mortality, hospital mortality and 28-day mortality being 56%, 63.6% and 62.8% respectively[3].Sepsis when not identified or treated appropriately carries a very high mortality.

Sepsis: the continuing diagnostic and treatment dilemma:

Despite widespread awareness and constantly evolving definitions of sepsis,it still poses a significant diagnostic and treatment dilemma. As mentioned above, diagnosis of infection is one of the key components of sepsis definition.The conventional clinical signs and traditional markers like ESR and CRP have not been shown to be sensitive or specific enough to make a definitive and early diagnosis of infection or sepsis. The 2016 Surviving sepsis campaign guideline has come up with the qSOFA scoring system for early diagnosis of sepsis in non ICU settings though it turns out to be an in hospital mortality predictor rather than an accurate diagnostic tool [1,4].

Many studies have shown an increase in mortality when sepsis is not treated on time. One landmark retrospective cohort study has shown that every one hour delay in administration of antibiotics after the onset of septic shock is associated with a significantly increased risk of death upto 6 hours [5]. These findings have been supported by various similar studies which have led to significant physician anxiety resulting in antibiotic misuse and abuse in the form of antibiotic prescriptions even for non-infectious conditions and non-bacterial infections. This trend in turn has promoted natural selection of resistant bacteria contributing not only to increased global antibiotic resistance but also to increased incidence of potentially lethal infections like clostridioides difficile colitis.

Thus,there is an urgent need for an early diagnostic tool with high sensitivity and specificity which can be used to detect sepsis and helps in making appropriate clinical decisions. Sepsis biomarkers based on molecular diagnostics have proven to be of immense help in the early diagnosis, monitoring of the severity and response to the

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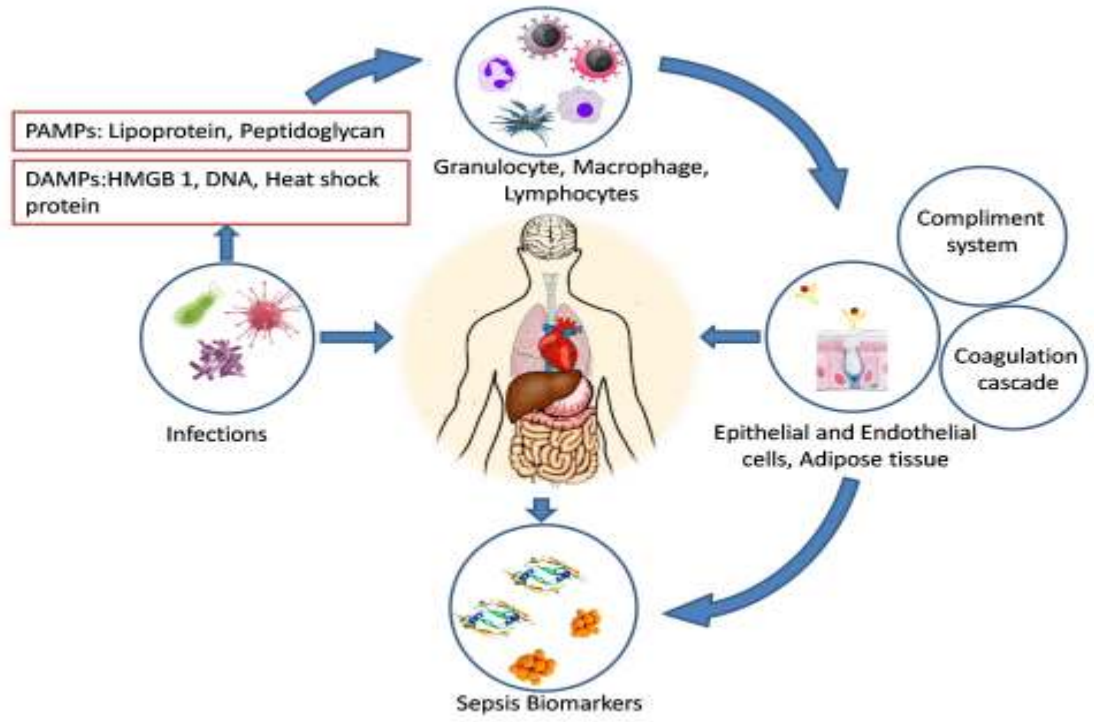

Fig.1 Pathophysiology of Sepsis

PAMPs : Pathogen Associated Molecular PatternsDAMPs: Danger Associated Molecular Patterns

treatment [6]. The currently available clinical studies have shown promising results with biomarker guided antibiotic stewardship programmes.

Biomarkers in sepsis

Sepsis has traditionally been considered as a result of uncontrolled inflammatory response, a "cytokine storm" that results in organ dysfunction[1].

Any infection triggers release of PAMPs (Pathogen Associated Molecular Patterns) and DAMPs (Danger Associated Molecular Patterns) which are sensed by the receptors on the cell surface[7,8]. This leads to activation/release of host factors like complement system,endothelial stress response molecules,acute phase reactants,cytokines/ chemokines and cell surface markers resulting in multi organ dysfunction as shown in figures 1 & 2.Biomarkers are "molecules that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" [9].Currently, there are more than 170 potential biomarkers for sepsis that have been identified and proposed for clinical use[10].

Role of sepsis biomarker and novel sepsis biomarker (Table 1 illustrates the same)

Table : 1

Role of Sepsis Biomarker:
To establishearly diagnosis that helps in the initial management of sepsis. Identify patients who might benefit from specific therapies. Monitoring the response to the therapy. To predict and prognosticate disease outcomes. Screening patients at risk of sepsis.
What makes Novel Sepsis Biomarker ?
Ideal biochemical markers are characterized by the following features: Should be highly specific and sensitive for sepsis: Should allow the differentiation between infectious and non-infectious conditions. Should be done at point of care and rapidly diagnose before the appearance of clinical signs of sepsis. They could be easily and widely applied clinically. Should be cost effective. Should provide opportunities for intervention and help in improving outcomes.

Table 2

Cytokine Biomarkers	TNF alpha, IL-6,IL- 8, IL-4, IL-10
Cell Surface Biomarkers	CD 64,CD 48, sTREM, suPAR, C5aR
Miscellaneous Biomarkers	C-Reactive Protein,Procalcitonin,Lactate, Mid-Regional Pro Adrenomedullin, Angiopoietin

Table 3

Early Response Biomarkers	1.Cytokines and Chemokines 2. Lipopolysaccharide-Binding Protein (LBP)
Late Response Biomarkers	1. High-Mobility Group box 1 protein 2. Macrophage Migration Inhibitory Factor

Table 4

Diagnostic Biomarkers	CRP, TNF alpha, IL- 6, IL-8,IL-11,IL-18
Prognostic Biomarkers	suPAR, Troponin T
Diagnostic as well as Prognostic	Pro ADM, sTREM -1,Presepsin,BNP,
Biomarkers	MicroRNA(miRNA)

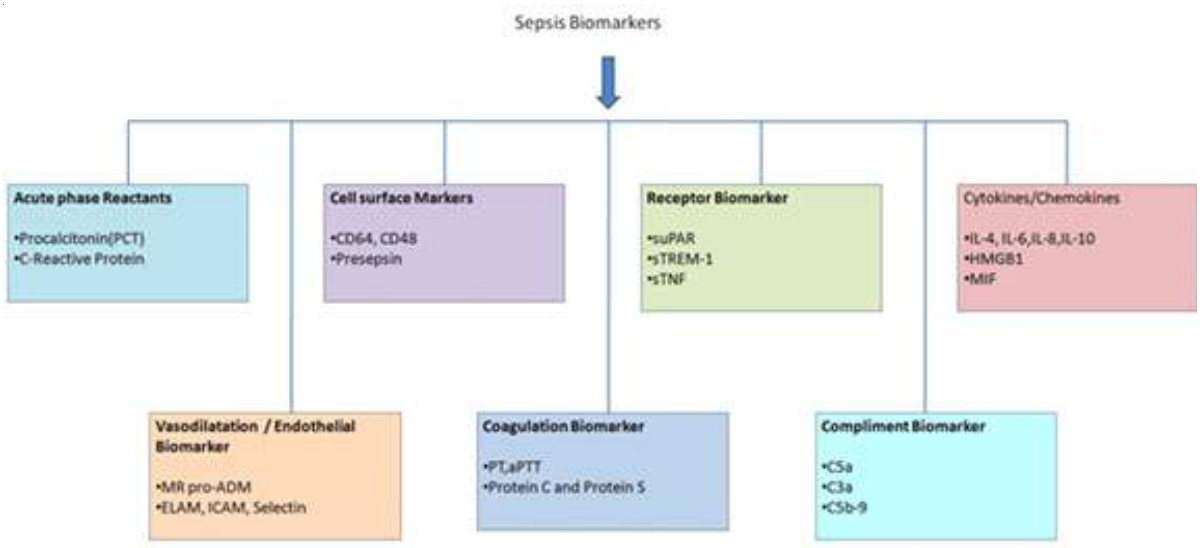

Figure .2 Classifications of Biomarkers of Sepsis

suPAR : soluble urokinase-type Plasminogen Activator Receptor; sTREM-1: soluble Triggering Receptor Expressed on Myeloid cell 1; sTNF: soluble Tumour Necrosis Factor; IL: Interleukin; HMGB1: High-Mobility Group protein Box 1; MR-proADM :Mid-Regional proADrenoMedullin; ELAM: Endothelial cell Leukocyte Adhesion Molecule; ICAM: Intercellular Adhesion Molecule, PT: Prothrombin Time; aPTT: activated Partial Thromboplastin Time

Biomarkers are classified on the basis of varying parameters [11,12].

I. Cytokine biomarkers, Cell surface biomarkers and Miscellaneous biomarkers [Table 2].

II. Early response biomarkers and late response biomarkers [table 3].

III. Diagnostic biomarkers and Prognostic biomarkers [table 4].

C - reactive protein

C-reactive protein (CRP) is an acute phase reactant produced in the liver as a response to inflammatory process or stressful insults like trauma which involves tissue damage [13,14]. CRP has extensively been studied as a biomarker for sepsis,but has not been an ideal biomarker due to low specificity. However, some data suggest the use of CRP in combination with other biomarkers such as procalcitonin (PCT) which could increase its specificity [14,15]. Thus we suggest use of CRP only in combination with other biomarkers in sepsis.

Procalcitonin

Procalcitonin is generally not detected in normal conditions but increases in response to bacterial infections[16]. It is a precursor protein of calcitonin. In case of viral infections, its production is blocked by interferon gamma [8,17]. Surviving sepsis 2016 guidelines mention the use of PCT in sepsis only for shortening the duration or discontinuation of antibiotic therapy, that too as a weak recommendation[1].

Kinetics of Procalcitonin

The kinetics of procalcitonin is unique in a way

that the levels rise rapidly to an infectious insult and the peak levels are reached in 25-30 hours.

Pocalcitonin levels also decline rapidly as the inflammatory insult resolves [18]. This property is advantageous not only in the diagnosis of sepsis but also in prognostication by measurement of serial values. The above property has been utilised to de escalate or stop antibiotics in sepsis management.

Is Procalcitonin specific for bacterial infections?

Procalcitonin is mainly studied in bacterial infections and is more specific for the same. Interferon gamma released in viral infections block the production of Procalcitonin as mentioned previously. A 2019 systematic review concluded that Procalcitonin should not be used as a stand alone tool to differentiate between candidemia & bacteremia [19] .

Procalcitonin can be erroneous in few conditions. False positive results are seen in non specific intense inflammatory states like major surgery, significant trauma, circulatory shock, severe burns, and pancreatitis[14] . False negatives are observed, in localized or encapsulated infections which do not have systemic spread like mediastinitis, empyema, or abscesses [15]. A retrospective observational study which applied Sepsis-3 definition for diagnosis, calculated the optimal cutoff values for diagnosing sepsis,pre septic shock and septic shock as 0.41 ng/dL (sensitivity: 74.8% and specificity: 63.8%; AUC: 0745), 4.7 ng/dL (sensitivity: 66.1% and specificity: 79.0%; AUC: 0.784), and 2.48 ng/dL (sensitivity: 72.8%,specificity: 72.8%, AUC: 0.781), respectively [20].

Evidence for the use of procalcitonin as a biomarker:

Various studies and meta analyses have extensively evaluated the usefulness of procalcitonin as a biomarker in sepsis. The Procalcitonin to Reduce Antibiotic Treatments in Acutely ill patients (PRORATA) trial is a large multicentre study in France which included 630 patients with suspected bacterial infections. The procalcitonin group used procalcitonin based strategies to guide physicians in initiation, de escalation or cessation of antibiotic therapy[21].Patients in the procalcitonin group had significantly more antibiotic free days (14.3 days vs 11.6 days) and an overall 23% relative reduction in days of antibiotic exposure (mean 10.3 vs 13.3 days).The study showed non inferiority for 28-day and 60-day mortality, with no difference in other clinical outcomes.

Chanu Rheeet al has summarized the use of Procalcitonin in the following scenarios, in their review: In critically ill patients with sepsis, the cutoff values to discontinue antibiotics is a procalcitonin level of <0.5 micro g/L or $\geq$ 80% decrease in peak level after initiation of treatment [18].

SAPS trial [22]showed that procalcitonin levels can be used in non-critically ill patients as well with suspected or proven respiratory infections. The cutoff value of <0.25 micro g/L to withhold antibiotics is used in non critical and low-risk patients.

A 2019 editorial in CHEST has concluded that though many reports support the PCT-guided antibiotic de-escalation and discontinuation in critically ill patients, there is only weak evidence

to prove mortality benefits at this stage. The authors also concluded that in view of global abuse of antibiotics & the ongoing threat of emergence of multidrug resistant organisms, Procalcitonin is an important potential biomarker for mediating effective antibiotic stewardship strategies.[23] Hence the use of Procalcitonin as a biomarker at this stage is largely to de-escalate or stop antibiotic therapy.

Soluble Triggering Receptor Expressed on Myeloid cells 1(sTREM-1)

Soluble triggering receptor expressed on myeloid cell 1 (sTREM-1) is a soluble form of TREM-1. It is a glycopeptide receptor expressed on the surface of myeloid cells like PMNs (poly morpho nuclear neutrophils), mature monocytes and macrophages. TREM-1 expression is increased in bacterial or fungal infections [24]. The levels of TREM 1show a rapid decrease and thus serial levels can be used in prognosis. However, the usefulness of this molecule as a suitable biomarker still needs to be validated.

CD-64

CD64 has been evaluated as a biomarker for sepsis. Few characteristics have been advantageous in clinical application. CD64 levels are rapidly upregulated in a few hours of activation and once the activation stimulus disappears, CD64 expression returns to its basal level in few days [25]

Neutrophil CD64 (nCD64) expression rapidly increases as a physiological response to microbial stimulus or infectious insult reaching upto 10-fold higher levels in 4–6 hours [26].Dimoula *et al*. found that nCD64 identified sepsis with a sensitivity of 89% (81%–94%) and a specificity of 87% (83%–90%)[27] . Thus CD64 is a promising biomarker in sepsis.

Soluble-urokinase-type-Plasminogen-Activator-Receptor (suPAR)

The soluble urokinase-type Plasminogen Activator Receptor (suPAR) is a cellular binding site for urokinase. suPAR can be detected in urine, blood and cerebrospinal fluid [28]. suPAR levels are elevated in severe sepsis and serious bacterial infections and is a marker of poor outcome and mortality.

A recent study by Ni et al [29] showed that high suPAR levels especially in bacterial infections are associated with an elevated risk of mortality, with a risk ratio of 3.37 (95% CI 2.60 to 4.38), and an AUC of 0.77 for the prediction of mortality. It showed a sensitivity and specificity of 70% and 72%.

Mid-Regional pro ADrenoMedullin(MR-proADM)

Adrenomedullin (ADM) is a peptide based hormone which is synthesised in various tissues like bone, adrenal cortex, kidney, lung, blood vessels and heart. The mid region of pro ADrenoMedullin(MR-proADM) is the more stable form of ADM and stays in circulation for a longer duration. High levels of MR-proADM are seen in septic patients in response to bacterial infections. The advantage of MR-proADM is that they are related to the severity of the disease, and accurately predict the risk of organ failure and mortality.

The TRIAGE[30]& PEDCRIP[31] studies showed that MR-proADM is a good biomarker for

differentiating between septic patients and non-septic patients with SIRS. MR-proADM has also shown to be a good mortality predictor at 28 days.

Presepsin

Presepsin in a promising immunologic biomarker which has been studied in early diagnosis of bacterial sepsis in ICU as well as emergency department settings.It is believed to be a regulatory molecule of adaptive immunity and is a stimulator of monocyte phagocytosis[32].It can be measured within few minutes at the bed side and can also prognosticate the infections.Presepsin measurements could be influenced by age, renal dysfunction, burns, gut translocation of bacteria, bacteremia &haemophagocytosis.

Angiopoietin

Angiopoietins (Ang)1 and 2 are endothelial-derived vascular growth factors that play opposing roles during sepsis. Studies using angiopoietins have shown that a ratio of Ang-2/Ang-1 can risk stratify patients with critical illness and sepsis[33]. Angiopoetins help in diagnosis and prognosis of sepsis and show a great promise in predicting sepsis outcomes.

Lactate

Lactates, mainly metabolised in the liver and produced as a result of anaerobic metabolism has been a traditional biomarker in circulatory shock and sepsis. Tissue hypoxia is an important contributor to hyperlactatemia which is an independent predictor of mortality. However, lactate clearance is not clearly identified as a variable that can be used to determine the therapeutic endpoint for patients with sepsis. Currently, monitoring serum lactate levels over a time could help to ensure that treatment was effective and may provide valuable information regarding response to therapy[34].

Future trends:

None of the currently available biomarkers are capable enough to diagnose early sepsis with high sensitivity and specificity. Thus the search for a unique biomarker or a magic bullet to precisely diagnose sepsis has been unsuccessful so far. The focus has now been shifted to combination of various biomarkers, and development of various predictability scores to have a faster and accurate diagnosis of sepsis.

Bioscore is a recently studied scoring system which includes combination of neutrophil CD64, PCT and sTREM-1[35]. The *Infection Probability Score (IPS)* is another scoring system which includes six different variables such as temperature, heart rate, respiratory rate, white blood cell count, C-reactive protein, and Sequential Organ Failure Assessment. IPS is used to assess probability of infection in suspected sepsis and is simple & has been clinically validated [36]. Various combinations have been tried with similar results; these include combination of PCT, proADM and TNF-alpha. Studies on combination of Alpha 2-macroglobulin and PCT are also in the pipeline[37].

Conclusion:

Sepsis continues to be a major killer of mankind. Early diagnosis of sepsis is of utmost importance for appropriate management decisions including early antibiotics and source control.

The diagnostic dilemma compounds the treatment dilemma which results in under treatment aswell as over treatment in the form antibiotic misuse or abuse. Early accurate diagnosis or sepsis may also influence the type of monitoring of the patient,shifting from a non-ICU setting to an ICU setting or a high dependency care.

Biomarkers in sepsis herald a new ray of hope in the early diagnosis & treatment.Procalcitonin has been evaluated by numerous studies & meta analyses which showed some evidence for its role in helping the decision making regarding shortening or discontinuation of antibiotic therapy.Considering the unrelenting growth of multidrug-resistant bacteria along with scanty antibiotics in the pipeline, biomarker utilisation in stewardship strategies may prevent antibioic misuse to many extent. However, as of yet, there is no single, ideal standard biomarker or a biomarker combination that are universally reliable for definitive early diagnosis. The ongoing current trend appears to be the utilisation of biomarker combinations with addition of sepsis scoring systems like Infection Probability Score (IPS) & APACHE II which is expected to increase the sensitivity as well as specificity of early sepsis diagnosis.

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Editor in Chief Pulmon

Special article

Progressive Fibrosing ILDs (PF-ILDs)- A namesake terminology or a treatment essentiality?

Sajitha M

Abstract

Interstitial lung diseases comprises of a heterogeneous group of parenchymal disorders. Majority of ILDs is comprised of IPF and major studies have been centred around IPF because of its progressive nature as well as ease of determining progression and significant end points. However, a proportion of ILDs classified as NSIP, CTD ILD, HSP, occupation related ILDs, and sarcoidosis may also exhibit a progressive fibrosing phenotype. A group of un-classified ILDs also have a Progressive fibrotic nature. All of them have a similar disease progression and mortality similar to IPF and have been designated as Progressive fibrosing ILDs. There is a huge unmet need in terms of evidence-based management as well as addressing patient-related problems of Progressive deteriorating lung function and quality of life.Ongoing trials on PFILDs reveal possibilities of a common treatment pathway and monitoring progress despite the varying etiology and primary inciting factors.

Key Words& Abbreviations

ILD – Interstitial Lung Disease ;PF-ILD – Progressive Fibrosing Interstitial Lung Disease; IPF – Idiopathic Pulmonary Fibrosis; NSIP - Non-Specific Interstitial Pneumonia; HP/HSP – Hypersensitivity Pneumonitis; CTD ILD - Connective Tissue Disease-related Interstitial Lung Disease; RA ILD - Rheumatoid Arthritis Interstitial Lung Disease; SSc ILD - Systemic Sclerosis Interstitial Lung Disease; ERS - European Respiratory Society; ATS - American Thoracic Society; FVC – Forced Vital Capacity; HRCT – High Resolution Computed Tomography; EULAR - European League Against Rheumatism; EUSTAR - European Scleroderma Trials and Research group; SLS-Scleroderma lung study

Introduction

Interstitial lung disease (ILD) includes a large group of parenchymal lung disorders, of varying etiology which overlap in their clinical presentations,clinical progression, and patterns of lung injury.[1]The commonest of ILDS, IPF is, is of unknown cause and is a progressive fibrosing ILD characterised by a decline in lung function and early mortality[2].But a proportion of patients with certain other typesof ILD are also at risk of developing a Progressive fibrosing phenotype like that of IPF.These include idiopathic nonspecific interstitial pneumonia (NSIP)[3], unclassifiable idiopathic inter-

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stitial pneumonia[4], autoimmune ILDs[5], chronic sarcoidosis[6], chronic hypersensitivity pneumonitis (HP)[7]and exposure-related diseases such as asbestosis and silicosis[8].The proportion of patients with non-IPF ILDs who develop a progressive fibrosing phenotype is not known but has been estimated to be up to 40%[9, 10].The term Progressive fibrosing ILD(PF-ILD) best describes this subset which resembles IPF in clinical and radiological pattern and prognosis.

Pathogenesis of Fibrosing ILDs

The process can be either an initial alveolar epithelial injury followed by fibro-proliferative process or an inflammatory process leading to chronic fibrosis as seen in CT-ILD[11]. Regardless of initial trigger, a proportion of ILDs have a self-sustaining mechanism of fibro proliferation which leads to progression by cellular injury and unregulated repair[12].The fibroblasts migrate from different sites sources of injury transform to myofibroblasts and secrete extracellular matrix.This, together with reduced matrix degradation leads to increased tissue stiffness and loss of alveolar tissue.[13,14]. Macrophages and lymphocytes are recruited to the site and propagate the fibroblast activation, by profibrotic mediators and the process offibrosis continues.The increased lung tissue stiffness further activates and stimulates fibroblasts to drive a self-sustaining process of fibrosis[15]. Hence the volume of lung is reduced and gas exchange impaired, resulting in worsening breathlessness and capacity for exertion, and ultimately in respiratory failure.

Need for an Umbrella Terminology

PF-ILDs and IPF draw many parallels clinically and prognostically[17,80],as the progressive fibrosis causes deterioration in lung function, quality of life and worsening of respiratory symptoms [18].

Patients having non-IPF progressive fibrosing ILDs have a resistance to immune-modulatory therapy and early mortality, i.e. around 4–5 years after an initial diagnosis of ILD.[19]This is similar to patients with IPF prior to the availability of therapies that slow disease progression.These similarities in pathological,radiological and clinical progression suggest the potential for a common treatment pathway. Hence for clinical research and, potentially, for treatment, IPF can be "lumped" with other forms of fibrosing ILD that have similar biological and clinical behaviours. [20,17]This can aid management , categorisation of ILDS and also identify pathogenetic pathways that may help in prevention .

Epidemiology and Defining progression in patients with fibrosing ILDs

PF- ILDs include a proportion of connective tissue disease-related ILDs (CTD-ILDs) such as those related to rheumatoid arthritis (RA-ILD)[5], systemic sclerosis (SSc-ILD), and polymyositis/ dermatomyositis; chronic hypersensitivity pneumonitis (HP)[7]; ILD related to chronic sarcoidosis[6]; idiopathic non-specific interstitial pneumonia (iNSIP)[3] and unclassifiable ILD[4]. IPAF or interstitial pneumonia with autoimmune features is an entity constructed by European Respiratory Society (ERS)/American Thoracic Society (ATS) Task Force to describe unclassifiable ILDs characterized by fibrosis,to standardize research. However the classification

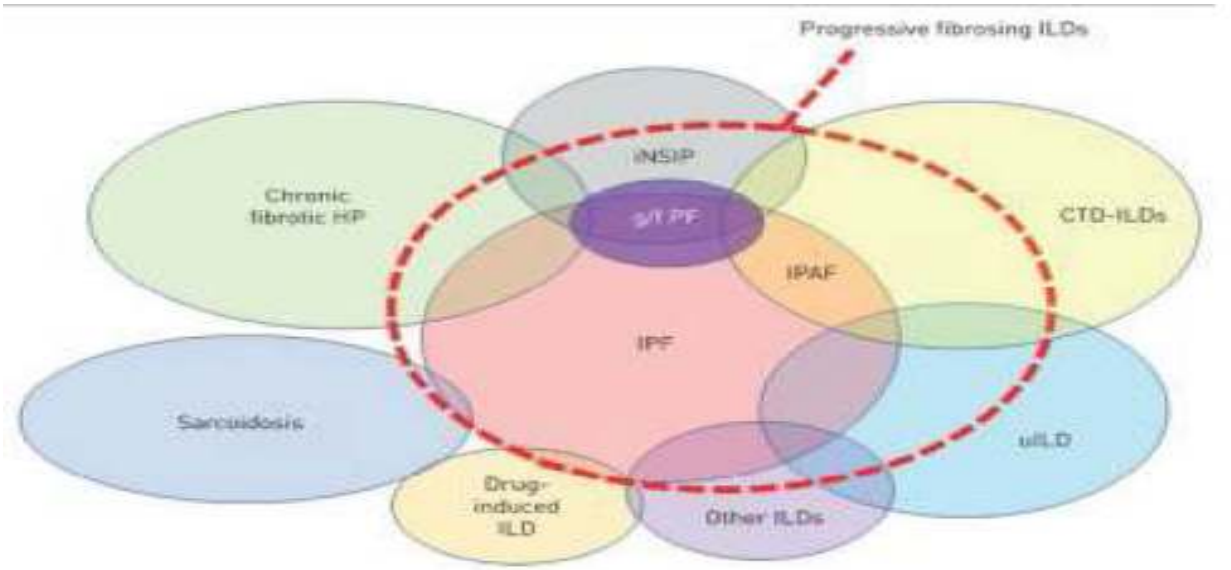

Fig-1 103 Classification of ILDs (Courtesy-European Respiratory Review 2019 28: 190109)

is yet to be validated in clinical practice.[21,22](Fig. 1)

There is a definite decline in lung function, worsening of symptoms and deterioration in health-related quality of life. Most clinical trials and observational studies in patients with ILDs have defined progression in terms of decline in FVC, the change from baseline in mL or as a percentage of the predicted value, or as a categorical change (typically ≥ 10% predicted) and diffusion capacity of the lungs for carbon monoxide (DLCO).

IPF

In patients with IPF, a decline in FVC is a well-established predictor of mortality[2]. In a retrospective study of data fromTOMORROW, INPULSIS, CAPACITY and ASCEND trials, in 1132 patients with IPF who received placebo , patients with 10–15% absolute decline of predicted FVC had two-times greater risk of mortality compared to those with an absolute FVC decline of <5% predicted[10]. In addition to lung function parameters, several other end-points have been used to assess disease progression in clinical trials in IPF.

iNSIP

Idiopathic NSIP accounts for about one-quarter of all IIPs[23]. The disease is more common in women, never-smokers and in the sixth decade of life[3]. iNSIP can be cellular or fibrotic type of which cellular sub-type is responsive to treatment with corticosteroids and immunosuppressive agents and has a good prognosis[3],while fibrotic type[Fibrosis in HRCT/biopsy and lack of lymphocytosis in BAL] fare worse. Early treatment have good response[24].Histopathologically in lung specimens neovascularisation in fibrotic areas is more common in iNSIP than UIP. iNSIP has a five year survival of 82% while IPF has medium survival of less than five years.[26] However the survival of patients with NSIP is poorer than patients with iNSIP associated with CTDs. A histopathological finding of UIP overlap or a clinical diagnosis of chronic HP overlap has been associated with increased risk of death[25].

Autoimmune Related ILDs

Autoimmune diseases like SSc,RA-ILD and less commonly polymyositis/dermatomyositis, Sjogren's syndrome may present with ILDs and a proportion of them are the progressive type. In SSc, diffuse cutaneous disease or anti-topoisomerase I antibodies positivity has more propensity for ILD and the risk of development and progression is greatest in the initial years after diagnosis[27]. In the European League against Rheumatism (EULAR) Scleroderma Trials and Research group (EUSTAR) cohort, among 695 patients one-third had a DLCO less than 50% within the first three years after diagnosis. A seminal study inferred that a greater extent of fibrosis on HRCT (more than 30% of fibrosis or 10-30% of fibrosis + FVC less than 70%) predicted a three-fold risk of mortality.[28]

The risk of developing clinically significant ILD in RA is estimated to be about 10%. The *MUC5B* promoter variant rs35705950, a genetic risk factor for IPF, was identified as a risk factor for usual interstitial pneumonia (UIP) pattern ofRA-ILD[29].In a study at single tertiary care centre among 167 patients with RA-ILD the proportion of patients with FVC <50% predicted,RA-ILD rose from 14% at diagnosis to the 22% after five years[5]. In an analysis of 107 patients with ILD associated with polymyositis/dermatomyositis, 16% had a decline in FVC of ≥ 10% predicted and/or a decline in DLCO of ≥ 15% predicted over a median follow-up of 34months, despite treatment[7].The exact factors which preclude to progression needs to be further evaluated.

Unclassifiable IIP

15-20% of IIPs may remain unclassifiable according to the present classifications due to the non-specific or conflicting clinical and radiological findings, mixed patters of lung injury by histopathological findings, or non-performance of lung biopsy. But patients with unclassifiable IIPs may have one or more of the defined pathological entities (UIP or NSIP) which characterize the progressive fibrosing phenotype. Though the prognosis for unclassifiable IIPs are better than that of IPF, the mortality rate is still high (31% at 5years). In fact, survival of this group is intermediate between IPF and other non-IPF ILDs. Low DLCO the presence of a UIP-like fibrotic pattern on HRCT, high fibrosis score predict poor prognosis and mortality in unclassifiable ILD[20].

Chronic Hypersensitivity Pneumonitis

A proportion of acute hypersensitivity patients go on to develop chronic HP, and some of these patients develop a progressive fibrosing phenotype[30], which is associated with a worse health related quality of life than IPF and poor survival(≥ 5years)[31,32,33]. In a retrospective study of chronic HP, amongst 147 patients with fibrosis on HRCT and surgical lung biopsy had a median survival of 4.9 years as to 16.9 years in those without fibrosis. The other factors predictive of mortality were an inability to identify the inciting antigen, older age, lower FVC% predicted and a history of smoking[31]. As in IPF, the single nucleotide polymorphism MUC5B rs35705950 and short telomere length have been associated with reduced survival in patients with chronic HP[34]. On HRCT, a "possible UIP"

pattern of fibrosis[80]and greater extent of fibrosis is also predictive of mortality[31,35].

Acute exacerbations of HP which may be potentially fatal have been reported as that of IPF

Sarcoidosis

Sarcoidosis tends to progress more gradually than other fibrotic ILDs. But Stage IV sarcoidosis which comprises 20% of pulmonary sarcoidosis patients is characterized by progressive fibrosis and is associated with significant morbidity and mortality[36]. Here pulmonary fibrosis is peribronchovascular and affects mostly the upper lobes (posterior segments). Relapse or remission of the disease can result in either increase or decrease of both FVC and DLCO[37].

Drug Induced Lung Disease

Drug induced ILD may progress into a fatal progressive fibrotic phenotype despite the withdrawal of the offending agent[38,39].

ILDs related to other occupational exposures

Common diseases of this group are asbestosis, which is caused by asbestos fibres and silicosis, which is related to free crystalline silicon dioxide or silica[40,41]. Exposure to farming, livestock, metal dust, coal, sand and dust arising from the preparation of dental prosthetics have also been linked to the development of ILDs[42]and are diagnosed principally from exposure history and imaging. Simple silicosis is characterized by bilateral nodules especially in upper lobes which are actually hyalinised collagen[42]. Progressive massive fibrosis sets in when these nodules converge and is seen as larger opacities radiologically which is called the advanced complicated silicosis.Avoidance of exposure is the only means of reducing the burden of disease and there is no known treatment.

Acute Exacerbations

Acute exacerbations are defined as episodes of rapid respiratory worsening accompanied by evidence of new ground-glass opacities on HRCT and occurs in 5–10% of patients per year. These may be triggered by an insult such as infection or aspiration or may be idiopathic and are associated with very high morbidity and mortality, with a median post-event survival of only 3 to 4months. Acute exacerbations do occur in patients with other fibrosing ILDs like IPF and are often fatal. In a study from tertiary centres, it was found that 14 patients (17%) with RA-ILD had an idiopathic acute exacerbation over a median follow-up of 33months, and 13 of those 14 patients died within 1.5months of the event[43]. Another study of 51 patients with RA-ILD found that 22% had an acute exacerbation over 8years. UIP pattern on HRCT or histology predisposed to acute exacerbation in patients with SSc-ILD[44]. In a study of 100 patients with chronic HP among the patients who had an exacerbation over 2 year follow-up period, 78% died within one month. Patients with a UIP pattern on histology were more likely to experience acute exacerbations than other patterns [45]

Predictors/markers of disease progression, mortality and scoring systems

Several factors that predict mortality have been identified, but a careful interpretation is required because of variations in methodology and retrospective nature of studies. A low FVC

predicted is a predictor of mortality in patients with progressive fibrosing ILDs, as established by studies spanning IPF[46], RA-ILD[5,47], SSc-ILD[28], chronic HP[35,48] and fibrotic iNSIP[31]. The same is true of DLCO[33,37,38,39], and decline in FVC >10% predicted over 24 months [47, 33, 48, 39].However smaller declines in FVC have also been shown to be associated with a poorer prognosis, if persistent and cumulative, at least in patients with IPF[49]. The smaller annual decline of FVC predicted becomes substantial in patients with earlier age of onset of disease.

Radiological markers

Composite scoring systems based on the extent of fibrosis in HRCT have been developed specifically for the prediction of progression of SSc-ILD[50] and for taking decision to treat. HRCT scoring as limited lung involvement(<20% on HRCT) and extensive involvement (>20% on HRCT) devised since diffuse lung involvement has worse prognosis and risk of death. If indeterminate lung extent, FVC<70% determines decision to treat. Usage of radiological markers i.e.extent of fibrosis as predictors of disease progression and mortality has also been demonstrated in IPF, RA-ILD[51], chronic HP[35], pulmonary sarcoidosis[52] and unclassifiable ILD. Honeycombing and traction bronchiectasis specifically have been associated with worse prognosis[53,54]. But subjectivity and large inter-observer variability in reading HRCT scans hampers clinical utility and necessitates validated, low-cost, automated scoring systems[55] in patients with fibrosis.

Composite scoring systems like the GAP (gender, age, physiology) model,to predict mortality in IPF based on gender, age, FVC % predicted and DLco % predicted[56], is also useful in patients with RA-ILD[57], SSc-ILD[58], unclassifiable ILD[59] and a mixed cohort[60].

Recently, a cluster analysis in a cohort of 770 patients with diverse ILDs (IPF,CTD-ILDs, chronic HP, IPAF) identified four phenotypic groups based on factors such as age, race, smoking, and radiological features that differed in FVC decline and survival[61]. Whilst interesting from an academic perspective, the use of such groupings in clinical practice remains to be established.

Unfortunately, none of the scoring systems provides an accurate prediction of the way that ILD will progress in an individual patient,which challenges therapeutic decision-making and patient counselling.

Blood Markers

Biomarkers studied in disease progression are Krebs von den Lungen-6 protein (KL-6) in IPF and CTD-ILDs,[62,63] surfactant protein-D (SP-D)[64]Matrix metalloproteinase which is involved in re-modelling of extracellular matrix in IPF[65].Protein fragments generated by the breakdown of the extracellular matrix have also been investigated as predictors of disease progression[66]. Among these KL-6 used in practice in some IPF centres.

Genetic Markers

MUC5B, a gene encoding a component of mucus secretionshas been studied for predicting survival. In patients with IPF, rs35705950, a single nucleotide polymorphism (SNP) in MUC5B has been associated with

improved survival[67]. While rs5743890, SNP of a Toll Interacting Protein(TOLLIP), has been associated with worse survival[68]. But in patients with IPAF, MUC5B rs35705950 was associated with worse survival and no association was noted between TOLLIP mutation and survival [69]. In chronic HP, neither of these alleles was associated with survival[70]. In RA patients MUC5B rs35705950 is associated with the development of ILD [71].

Recently mutations in genes related to telomere maintenance, like telomerase reverse transcriptase (TERT) and telomerase RNA component (TERC), have been observed in patients with IPF with short telomeres being associated with reduced survival. Short telomeres are also associated with worse survival in patients with IPAF[42] and chronic HP. Association between survival and variants in telomere-related genes are yet to be validated in patients with RA-ILD[71] and SSc-ILD. Atleast in some cases, genetics may serve as a stronger predictor of progression as in studies with familial pulmonary fibrosis than radiological markers.

Assessing progression in clinical practice

At present, the progressive fibrotic phenotype can be designated only by observed disease progression, despite the treatment considered to be appropriatefor the LDs.

Progression of fibrosing ILD is reflected as a decline in lung function, worsening of symptoms and deterioration in health-related quality of life and less frequently by chest imaging. (Table 1). There is no consensus as to how disease progression should be defined in patients with ILDs however it is suggested that patients satisfying any of the following criteria within a 24-month period have experienced disease progression: a relative decline of ≥10% in forced vital capacity (FVC); a relative decline of ≥ 15% in diffusing capacity of the lung for carbon monoxide (DLCO)[72]; or worsening symptoms or a worsening radiological appearance accompanied by a ≥ 5– 10% decline of FVC predicted.[20,74,75].

Comparable criteria was used in INBUILD and UILD trials.

Unknowns & Unmet Factors

Correlation of objective measurements with actual scenario

Though the predictors of disease progression have been identified, the course of disease for an individual patient remains impossible to predict.The previous decline in FVC is not a good predictor of future decline in FVC. In an analysis from two databases of FVC measurement , of IPF patients, among 50 patients with a decline in FVC of ≥ 10% predicted during first year, 84% had stable FVC over second year ; while of 135 patients who had stable FVC during the first year, 19% had a decline in FVC of ≥ 10% predicted during the second year[75]. In ASCEND and CAPACITY trials pooled analysis, only a weak negative correlation between changes in FVC in two consecutive 6-month intervals was noted[76].In contrast, if persistent and cumulative, smaller declines in FVC have also been shown to be associated with a worse prognosis at least in patients with IPF[49]. The smaller annual decline of FVC predicted becomes substantial in

Table 1[81]

Proposed criteria that may be used in clinical practice to assess disease progression in fibrotic interstitial lung diseases

Lung function	·Rate of decline in FVC (mL·year^{-1})· Absolute or relative changes in FVC (mL or % predicted)· Absolute or relative changes in DLCO % predicted
Exercise capacity	·Absolute change in 6-min walk test distance
	· Change in oxygen saturation nadir during 6-min walk test
Symptoms and patient -reported outcomes	Change in symptoms regularly assessed by Questionnaires on shortness of breath, cough, and/or quality of life·
	Change in everyday life exercise capacity
Acute worsening	Acute exacerbation of fibrosis (idiopathic or triggered)
	Non-elective hospitalisation for a respiratory cause·
HRCT	·Change in quantitative fibrosis scores on HRCT#
Need for supportive care	Initiation of ambulatory oxygen therapy at exercise ·
	Initiation of supplemental oxygen therapy at rest, or change in flow of oxygen
Serum biomarkers	·None validated

patients with earlier age of onset of disease. These ambiguities need to be clarified with large RCTs with uniform treatment protocols.

Aside from IPF, ILDs with progressive fibrosing phenotype may be clinically stable, especially if the disease is mild. On the contrast, a phenotype progressive in terms of quality of life can exist, even in the absence of pulmonary fibrosis. So many contradictions exist. Deterioration in health-related quality of life is highly meaningful for the patients but lacks objectivity in practice. Non-elective hospitalisation[77], acute worsening of shortness of breath and initiation of ambulatory or long-term supplemental oxygen are directly relevant to the patients, predict long-term mortality in IPF [78], and may be used as landmarks to assess progression and the effects of treatment interventions throughout the course of the disease.These can be evaluated for non-IPF progressing PFILDs also[81]. The use of long-term oxygen therapy in ILD is likely to indicate that lung function impairment has progressed and predicts an increase in the risk of mortality[77,79].

There is a huge challenge in defining treatment

response and defining endpoints as disease progression does not necessarily equal treatment failure,many of which are being validated in ongoing trials.

Treatment woes

There are no advances in the pharmacological treatment of PF-ILD patients, other than IPF and no RCTs apart from those in SSc ILD[81]. The novel antifibrotics, Nintedanib and Pirfenidone, are recommended conditionally for IPF only[2].

A study[9] conducted across pulmonologists,rheumatologists and internal medicine experts found that, in non-IPF ILDs time from symptom onset to death was 61–80 months. Drug treatment was given to 50–75% of patients with non-IPF progressive fibrosing ILDs[82]. Reasons for patients not being treated included physicians considering patients to have mild or slowly progressing disease, or not believing available treatments are effective or well tolerated. Corticosteroids were the preferred first-line treatment for all types of non-IPF ILD. This is consistent with recent data from the EXCITING-ILD registry in Germany, which showed that prednisone was used by two-thirds of patients with ILD. This may lead to short-term improvements in lung function, but no randomized clinical trials have evaluated their efficacy in patients with progressive fibrosing lung disease. There was considerable heterogeneity in preferences for second and third-line treatments.These patients also experience significant delays in the diagnosis of ILD, and the detection of progressive fibrosis. Between 25% and 50% of patients with progressive fibrosing ILDs do not receive drug therapy and this further leads to faster progression. There is an unmet need for effective and well tolerated treatments for progressive fibrosing ILD.

Despite the fact that pulmonary involvement causes significant morbidity and mortality,not many drugs are licensed for the treatment of ILDs related to autoimmune diseases[81]. Treatment guidelines issued by the European League Against Rheumatism (EULAR) recommend tailored therapy with cyclophosphamide (CYC) for SSc-ILD, in particular for patients with progressive ILD[81,82] . This recommendation was made based on the results of two randomised controlled trials[82,83]FAST and SLS studies demonstrated a significant improvement in FVC with cyclophosphamide and MMF, with MMF having lesser adverse effects and less treatment withdrawals.Biological therapies such as rituximab[84] have shown positive effects on lung function in some studies in patients with autoimmune ILDs, but are not approved for the treatment of ILD. In RA-ILD the treatment options become complicated since commonly prescribed drugs of proven articular benefit like methotrexate, leflunomide and anti-TNF-álfa agents are implicated in *ex novo* occurrence and acceleration of existing ILDs[85].There is considerable ambiguity since not all patients with ILD require immunomodulatory drug treatment. In some patients, it is appropriate not to initiate therapy, but to keep watch for disease progression. Non-pharmacological approaches like pulmonary rehabilitation,supplemental oxygen and supportive care, along with management of extrapulmonary manifestations of disease and comorbidities is considered corner stone of current management. However, patients with progressive disease warrant consideration of anti fibrotic drug therapy, either alone, or in addition to immunomodulators[81].

In iNSIP, immune-modulating drugs such as prednisone, azathioprine, cyclophosphamide, and mycophenolate have been used empirically; however, their doses are based on clinical response as assessed by clinicians rather than evidence. Antifibrotics may be considered in these patients.

There is no established treatment algorithm for HP and a poor evidence base to inform therapeutic decision-making. Management of HP typically involves removal of the antigen (if identified) and the use of corticosteroids or other immune-suppressants, despite very limited evidence to support their efficacy[30]. As such, much remains unknown regarding the treatment of patients with chronic HP, including the risks and benefits of immunosuppression and antifibrotic therapies.

Treatment Options

Choice of Treatment

The first step in selecting an appropriate treatment for a patient with ILD is a correct diagnosis. Multidisciplinary team discussion to integrate clinical, radiological, and laboratory data is the gold standard for making a differential diagnosis of ILD.

All patients with IPF, should be offered treatment with nintedanib or pirfenidone to slow the progression of their disease. Both nintedanib and pirfenidone are approved by regulatory agencies worldwide for treatment of IPF and recommended in the IPF guidelines[2,86]. The IPF patients treated with antifibrotics may have lower rates of acute respiratory decompensations i.e. acute exacerbation of IPF (nintedanib) and reduced frequency of respiratory related hospital stays (pirfenidone)[87,88]. The molecular mechanism of pirfenidone has not been fully elucidated, but is thought to involve antifibrotic, anti-inflammatory and antioxidative effects to reduce collagen synthesis, deposition and suppression of TGF- â[89].Nintedanib is an oral intracellular tyrosine kinase inhibitor with antifibrotic and anti-inflammatory activity in various animal models of lung fibrosis, thus providing a strong rationale for its clinical efficacy in patients with IPF and, therefore, patients with other progressive-fibrosing ILDs [90].

Hence the use of drugs with antifibrotic action, pirfenidone[91] and nintedanib,as treatments for other forms of fibrosing ILDs seems rational[90]. Nintedanib, after showing efficacy in the treatment of Scleroderma ILD [SENSCIS trial[91] (which showed a 44% relative reduction in the rate of decline in FVC over time) is being aimed to prove efficacy in patients suffering from progressive lung fibrosis of other etiology in INBUILD trial[99]. Patients were carefully phenotyped as progressive ILDs(PF-ILD), as assessed by documented FVC decline and CT abnormalities consistent with progressive fibrosis; >10% extent of fibrosis, UIP-like fibrotic pattern over 24 months. In this trial, Nintedanib proved effective in reducing the rate of progression, as assessed by the annual FVC decline, by 57%. The authors report an absolute difference of 107ml in the FVC decline at week 52 compared to placebo, benefits were comparable to the IPF trials[100].

The efficacy and safety of pirfenidone in subjects with SSc-ILD[SLS3] (in combination with MMF versus MMF alone), pirfenidone in

unclassifiable progressive fibrosing ILD[98], rheumatoid arthritis-associated ILD [TRAIL 1][102], fibrotic sarcoidosis and fibrotic HP are currently being investigated in clinical trials and show promising initial results. Studies with Pirfenidone in patients fulfilling pre-defined criteria for all PF-ILDs on anti-inflammatory therapy(Relief Stud) was undertaken and was terminated early due to slow recruitment and is about to report final results.[20,73]

If a benefit of antifibrotic therapy is demonstrated in PF-ILDs by ongoing trials, major changes can be expected in how PF-ILDs are diagnosed and managed. The management would require an establishment of a diagnosis of progressive fibrosis and assessment of the disease behaviour with the first line therapy. Defining point for anti-fibrotic therapy could beprogressive fibrosis of whatever etiology, especially when fibrosis progresses despite conventional management of corticosteroids and/ or immune-suppressive therapy.

A combination of immunomodulatory therapy with antifibrotic drugs [92,94,95] may be beneficial as suggested by the study of nintedanib in patients with systemic sclerosis[92].Likewise the antifibrotic therapy could be added to the systemic therapy in other cases of PF-ILD too. However, the value of a precise diagnosis of IPF cannot be under estimated since immunosuppressive therapy is deleterious in IPF.

Another point of consideration is, the disease progression on therapy has to be standardized and clinical end points need to be defined. Serial pulmonary function tests and genetic/blood bio-markers may have to be used in measuring disease activity especially to differentiate progression to fibrosis from inflammation. Stringent patient monitoring should include longitudinal assessments of FVC, DLCO, symptoms, exercise capacity and HRCT scans, in addition to a careful assessment of medication tolerance. In most cases, stability of these factors may be considered a successful outcome.

Autologous stem cell transplantation (ASCT)[105]

ASCT has been recently approved by the American Society for Blood and Marrow Transplantation for refractory Ssc. Studies have proved that the benefit is more for patients with more extensive ILD and it lasted for at least 5 years. The presence of groundglass opacities as well as absence of honeycombing on the baseline CT are predictors of better results with ASCT.

Overall care of patients with fibrosing ILDs

All patients with fibrosing ILDs should receive supportive care. Measures to alleviate symptoms and preserve the quality of life, should be initiated early and tailored to the needs of the patient[96]. Pulmonary rehabilitation or exercise training can improve exercise capacity and quality of life in patients with ILDs, particularly if started when the patient's physiology is less impaired[97]. The use of supplementary oxygen in patients with ILD and hypoxia can help to alleviate dyspnoea and improve quality of life[99]. Vaccines should be recommended to reduce the risk of respiratory infections. Prophylactic antibiotics may also be considered to reduce the risk of infections and associated hospitalisations. For patients with HP, a thorough investigation for the inciting antigen should be performed and,

Table 2 - Immunomodulatory treatment options for PF-ILDs[102]

Type of ILD	Treatment Options
SSc-ILD	1st line: MMF or cyclophosphamide, 2nd line: rituximab
RA-ILD	Rituximab, MMF or cyclophosphamide
ILD associated with polymyositis/ dermatomyositis	High-dose corticosteroids Rituximab or cyclophosphamide
MCTD-ILD	According to dominant histopathology
IPAF	According to dominant histopathology
Hypersensitivity Pneumonitis	Corticosteroids ± cytotoxic agent
Pulmonary Sarcoidosis	Corticosteroids, methotrexate, azathioprine,infliximab, leflunomide, hydroxychloroquine, thalidomide
Unclassifiable ILD	Treat according to working diagnosis but undertake regular reas sessment and refine diagnosis according to treatment responseand/or the emergence of new symptoms, clinical signs or radiological features

if the antigen can be identified, the patient advised of the importance of antigen avoidance. Management of extra-pulmonary manifestations of disease and identification and treatment of comorbidities can improve patient outcomes.[101]

Conclusions

Progressive fibrosing ILDs show commonalities in underlying pathogenetic mechanisms, suggesting that drugs, that slow the progression of IPF with its specific antifibrotic actions in lung,may also have utility in slowing the progression of other fibrosing ILDs. Ongoing trials have provided valuable insights into the potential use of immunomodulatory and antifibrotic agents in the management of these ILDs with a progressive phenotype. Further studies and meta-analyses are required for the precise defining points for progressive ILDs, monitoring of treatment, disease progression and treatment end-points. The concept of precision medicine may hold the key in definitive management of individual patients and may have improved outcomes.

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Pulmonary Function Testing Protocol during COVID - 19 pandemic

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INTRODUCTION:

Pulmonary function testing comprises of tests which are essential for accurate diagnosis and management of respiratory diseases. These tests are of paramount importance to clinicians and researchers for setting up goals and assessing outcomes.

Estimation of peak flow, spirometry, FeNO and DLCO are essentially blowing procedures and can be associated with serious cross infections among the health personnel and others in the vicinity if not done carefully following infection prevention control practices[1].

After the outbreak of Corona virus infections all over the world, restrictions were imposed over these tests as they could cause devastating spread of the disease. COVID-19 has created a stumble-block in the performance of these near mandatory maneuvers. With restrictions and non performance of them, there has been a fall in the standard of care in these groups of patients. With this background we, a team of Pulmonologists, decided to come up with a protocol which will minimize cross-infections and ensure the safety of our patients, medics and paramedics[2].

Aim:

To ensure safety of patients, paramedical staff, medical personnel and others who are involved in the care of patients who require spirometry and diffusion studies involving blowing of air.

Responsibility:

Consultants are responsible to frame the protocols in accordance with national and international guidelines

Consultants are also responsible for training of PFT technicians in infection prevention control measures and protocols.

All PFT technicians are responsible to get trained and follow these protocols and infection prevention control measures.

Consultants should check and ensure compliance and implementation of these protocols.

Protocol (Strategies considered):

PFT laboratory:

The room in which the patient/ subject undergoes the testing should have adequate ventilation and should be fitted with negative suction to blow the air out. Exhaust equipment has to be fitted at the windows to blow out the air and aerosol generated inside to the exterior. Choosing the room should be such that there is no or minimal contact of the air to any humans [9,10].

The floors and surfaces need to be mopped, sponged and air dried with 1% Sodium hy-

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pochlorite solution everyday in the morning. The same has to be repeated before and after

every time the procedure is repeated[3].

The surfaces of the tables, door knobs and every possible contaminable area have to be cleaned with soap solution and or 1% Sodium Hypochlorite solution as per the Washington University protocol of April, 2020[4].

Aerosol Generating Procedure sign should be placed on the door

PFT eqipment:

The equipment surfaces, the calibration syringes need cleaning with a sanitizer containing 70% Isopropyl alcohol[5].

For performance of Spirometry , FeNo and DLCO, bacterial and viral filters (BVF) of high quality approved by CDC should be used[5, 6, 7].It is notable that use of quality mouth pieces and filters are mandatory in laboratories[21,22].

Due consideration should be given to use of equipment. Unnecessary handling of the equipment by the patient/subject should be avoided. Detachable components should be as far as possible disposable. Example will be the use of mouth pieces, BVF's and turbines. Non disposable components should undergo sterilization as per sterilization protocol viz. 70% Isopropyl alcohol, twenty minutes of submerging in gluteraldehyde or gas sterilization (ethylene oxide) [7].

PFT technitian:

The staff performing the procedures should be provided with personal protective equipment (PPE). Adequate training in donning and doffing should be provided to them. In no circumstances they should undertake any procedure without PPE[14, 15].

Thorough hand sanitization protocol should be followed before, after and in between procedures. Adequate training needs to be imparted to paramedics as per the guidelines[8].

Patient:

It is mandatory that the patient should be putting on a mask when he / she comes to the lab and is given disposable hand gloves

Consent of the subject prior to undergoing spirometry, FeNo or DLCO has to be obtained for both the procedure itself and to undergo a COVID test by RT-PCR or else whatever approved methodology in suspects, whenever prescribed as per regulations. In such situations , it is advisable to wait for the results of COVID tests preferably RT-PCR (23). If the test results are positive then extra precautions need to be taken for everyone's safety. We recommend postponing of the test in subjects with positivity. It will be further advisable to treat everyone as a potential patient in order to avoid security lapses[16, 17, 18, 19].

Pre procedure:

Disinfection of the room is undertaken by cleaning staff supervised by the technician[20].

The technician goes through a check list prepared for performance of spirometry.

The Spirometry, FeNo /DLCO technician dons PPE.

The calibration of the machine is undertaken followed by sterilization of the equipment with sanitizer containing 70% Isopropyl alcohol[20].

Consent of the subject prior to undergoing spirometry, FeNO or DLCO has to be obtained for the procedure.

Procedure:

Ideally, PFT should be performed in an airborne infection isolation room (AIIR) whenever feasible, if AIIR not available a room from which air does not circulate to other areas and minimizes the exposure risk for health care workers to be used.

In all cases, leave the room vacant with the door closed for 30 minutes after the procedure and the patient has vacated the room.

The subject is invited in. It is mandatory that the subject should be putting on a mask and is given disposable hand gloves. He/she is made to sit comfortably.

No bystander is allowed inside in usual routine tests. If unusual situation prevails and an attendant has to be allowed, then he/she has to be adequately protected with PPE.

The technician explains the procedure in detail to the subject as per ATS/ERS guidelines[10].

Hand sanitization is performed on the gloved hands of the technician followed by that of the subject.

The subject removes his own mask and the procedure is performed as per ATS/ERS protocol[10].

If a post bronchodilator test is contemplated, 400mcg of Salbutamol or 160mcg of ipratropium bromide by a MDI and spacer with valve is used. The direct use of MDI or DPI is not to be permitted bronchodilator reversibility testing but can be used as a regular therapeutic in privacy and special settings. Strict surveillance is needed for any device that needs blowing into open air. Medications through nebulizer are strictly prohibited for its potential for transmission of corona virus[11, 12, 19].

Bronchodilator reversibility is performed the same way as the pre-bronchodilator test.

Post procedure:

After completion of the test, the subject undergoes removal of the hand gloves and hand sanitization. The gloves are disposed into the bin designated for this purpose. He/ She is escorted out to the waiting area where social distancing has to be maintained.

The respiratory technician sanitizes the equipment, the seat of the subject and potential areas of possible contamination or body contact.

Sufficient interval has to be provided for sanitization of the room and equipment. Hand-washing is performed for 20 seconds and a change over to fresh gloves is made between two patients. Restriction on the number of tests done will depend on the resources of the centre including trained manpower. We recommend a different technician to take over after two tests.

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Original article

Can coffee plantation in Wayanad be a source for increased fungal infections in chronic obstructive pulmonary disease? A prospective study

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Abstract:

Background: Increased rate of hospitalization among COPD patients due to exacerbation is seen during coffee ripening season in Wayanad.

Objective: to find out the increased prevalence of AECOPD due to fungal infections in Wayanad districts, to establish its link to coffee ripening season and to study the risk factors and outcome among these patients.

Materials and methods:The study was a descriptive comparative study done over 2 phases of 4 months duration each among hospitalized AECOPD patients in a tertiary care hospital in Wayanad district of Kerala State. Phase 1 was the crop period, which extended from October 2018 to January 2019. Phase 2 was the non-crop period, which extended from March to June 2019.

Results:There is an increased prevalence of AECOPD due to fungal infections in Wayanad during coffee ripening season. Diabetes mellitus, frequent use of broad-spectrum antibiotics and proximity of residence to coffee plantations were found to have associations with fungal infections. Patients with such fungal infections had a significantly longer duration of hospital stay and increased risk of mortality.

Conclusions: Fungal infection is a common complication among COPD patients during the coffee plucking season extending from November to January particularly among those residing near these coffee plantations. Diabetics are at an increased risk of acquiring these infections.

Keywords :Coffee plantations, Aspergillus fumigatus, Invasive aspergillosis, Sabouraud's agar.

Introduction:

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death worldwide. It accounts for 5% of the annual deaths globally[1]. In the INSEARCH study conducted by Dr SK Jindal and his team, the burden of COPD was estimated as 3.49% in persons above the age of 35 years[2]. However, its burden in the state of Kerala, estimated at 10%; was much higher than the national average. 6.03% of the total deaths in Kerala and 4.36% of total DALYs were accounted for by COPD[3-5]. While being a chronic illness, a large proportion of these patients experience worsening of symptoms warranting a change in treatment.

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Such exacerbations pose a serious health problem and significantly affect the mortality and morbidity of these patients[6]. Bacteria and viruses are the most implicated causative agents in acute exacerbations of COPD (AECOPD). The role of fungi in these exacerbations has not been elucidated even though COPD has been recognised as an important risk factor for acquiring aspergillus infections[7]. The presence of underlying diseases like diabetes mellitus, frequent use of broad-spectrum antibiotics and corticosteroids are the other contributing factors that have been documented[8,9].

The state of Kerala has a tropical climate with an average rainfall of 300cm. This climate usually offers a hostile environment for many microorganisms like fungi[10]. Wayanad is a hill district in Kerala with predominant coffee and tea plantations. These coffee plants and berries contain white flakes, which are abundant in fungi. During the crop season,these spores are dispersed into the environment[11], resulting in increased quantities of these fungal spores in the environment leading to fungal infections in susceptible individuals like COPD patients. This study tries to explore the possibility of increased fungal spores in the environment during coffee ripening season as a possible cause of AECOPD in this district.

Objectives:

1) To study the prevalence of fungal infections in COPD patients during coffee crop season and to compare it with that of non-crop season.
2) To study the risk factors involved in acquiring fungal infections among COPD patients.
3) To study the effect of fungal infections on the length of hospital stay and mortality in COPD patients.

Materials and methods:

Study design:

The study was a descriptive comparative study done over 2 phases of 4 months duration each. Phase 1 was the crop period, which extended from October 2018 to January 2019.

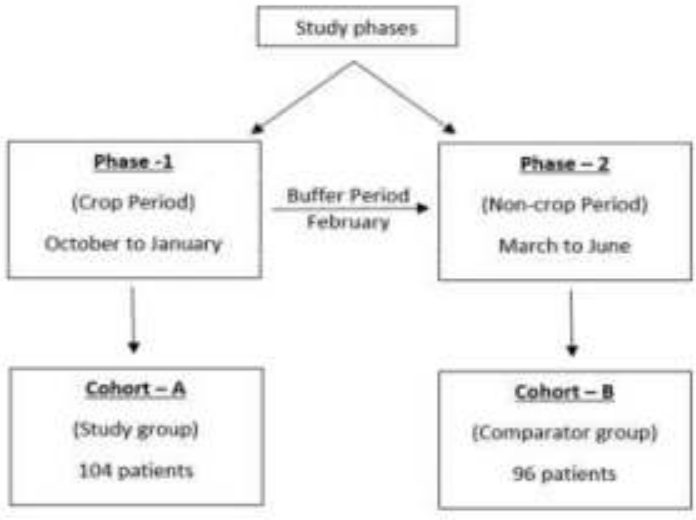

Fig-1: Flow chart showing study design

Phase 2 was the non-crop period, which extended from March to June 2019 (Fig-1). Approval from the institutional ethics committee was obtained for this study.

Study sample:

Inclusion criteria:

Only known COPD patients as per hospital records and on treatment for COPD for more than one year were considered for the study. These patients were included when they were hospitalized due to an exacerbation of COPD in a tertiary teaching hospital in Wayanad district during the study phases.

Exclusion criteria:

Patients less than 40 years, patients with active tuberculosis, asthmatics, immunosuppressed individuals and patients with other chronic respiratory illnesses were excluded from the study.

Study protocol:

All patients admitted in the pulmonary medicine ward and ICU of a tertiary teaching hospital during the study period were evaluated clinically and investigated with blood investigations, chest X-ray, spirometry, sputum gram stain and culture, fungal smear and culture. All these patients received standard treatment as per GOLD guidelines. Systemic corticosteroids were started when indicated for a period not exceeding 7 days. Broad-spectrum antibiotics predominantly comprising beta-lactams like third and fourth generation cephalosporins, penicillins or carbapenems were started when deemed necessary and if the clinical response was inadequate. If they were still not responding to treatment, CT thorax and bronchoscopy, to obtain bronchial washings and biopsy, were done in selected patients. Patients, in whom fungal growth was detected,were started on Itraconazole or Voriconazole in standard adult dose. Clinical course and outcome of the patients were recorded.

Data collection:

The demographic variables including age, sex, residence and proximity to coffee plantations were collected. Data regarding smoking status, FEV_1%, presence of comorbid illnesses like diabetes and hypertension, use of inhaled or oral corticosteroids and frequent treatment with broad-spectrum antibiotics in the past were also recorded. The length of hospital stay, need for ICU admission and outcome of the disease were followed up and recorded.

Microbiological analysis:

On admission, the sputum samples were collected from patients before initiation of antibiotic therapy. Samples were collected in sterile containers under aseptic precautions and immediately transported to the microbiology laboratory. Gram stain, KOH smear, bacterial and fungal cultures were done on the samples. Fungal species were identified by initially incubating the sample on Sabouraud's dextrose agar. This was done at two temperatures of 25^0C and 37^0C for 7 days and specimens were observed for the growth of fungi each day. This was followed by performing a lactophenol cotton blue mount and slide culture.

Bronchoscopy was done in selected patients when they were not responding to standard treatment or when sputum sample could not be obtained. Bronchial washings were col-

lected, and biopsy was done when bronchial inflammation was seen. These samples were sent for microbiological analysis and histopathological examination.

Samples were also collected from the various coffee plantations in Wayanad. 33 such samples from 5 different plantations were sent to the microbiology laboratory. These samples were processed with KOH mount and inoculated in Sabouraud's dextrose agar. Mycelial elements were examined on the KOH mount and the species were identified after observing the colonial growth on fungal culture.

Statistical analysis:

Data were analysed using SPSS version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). Categorical variables are presented as absolute numbers and relative frequencies, while continuous variables are presented as the mean, standard deviation (SD) in parametric data, or median with interquartile range (IQR) in non-parametric data. Categorical variables were compared using paired chi square analysis and a p value of <0.05 is taken as significant.

Results:

Patients were grouped into two cohorts. Cohort A was the study group and included the patients from the crop seasons (Phase-1). Cohort B was the comparator group and included patients from the non-crop season (Phase-2). Cohort A comprised of 104 patients and cohort B, 96 patients. All patients belonged to group C or group D COPD. A significant proportion of these patients were on inhaled corticosteroids either since they had an eosinophil count of >300 cells or since their symptom control was inadequate on an inhaler containing a combination of inhaled beta-2 agonists and muscarinic antagonists. 126 patients (63%) were more than 65 years old. The remaining 74 patients (37%) were less than 65 years old (Table 1). Majority of the patients (90.5%) were male and the rest female. The study population also predominantly consisted of people residing or working near coffee plantations (79.5%). Systemic hypertension (37.5%) was the most common comorbidity associated with the study population and 18% of the people were diabetic. Most of the patients received antibiotics (82.5%) and corticosteroids (82%) for management of AECOPD. A significantly higher proportion of patients who presented during the crop season (89.42%) resided or worked near coffee plantations (Table 2). This may be attributed to the huge influx of workers seen in these coffee plantations, during this season,to pluck the coffee berries.

29 (14.5%; 95%CI – 9.93%, 20.16%) of COPD patients had fungus isolated. Even though a higher proportion of fungal infections was seen in the crop season (16.35%) compared to the non-crop season (12.5%), the association was not significant (p-0.440) (Table 2). Aspergillus fumigatus was isolated in all the 29 patients and 2 of the patients in the crop period were co-infected with Mucor spp. Aspergillus fumigatus was also the most common species isolated from the samples obtained from the coffee plantations (83.34%) (Table – 4). An incidental correlation was found between the bacteria grown in both the seasons. The majority (67.31%) of the patients in the crop season had not grown any bacteria in their sputum cultures. On the con-

trary, 84.12% of the patients in the non-crop season had grown bacteria when their sputum was cultured of which 63.29% were gram-negative bacteria. The investigators intend to analyse this peculiar finding during the coming season.

On evaluating the factors associated with fungal infections, we found that diabetes mellitus was the most significant risk factor (p-0.048). 9 (31.03%) out 29 patients who had fungal infections were diabetic and only 27 (15.79%) out of 171 patients who did not grow fungi were diabetic (table-3). The frequent use of broad-spectrum antibiotics was also significantly associated with fungal infections (p=0.031) (table-3). 96.5% of the patients with fungal infections were subjected to repeated course of broad-spectrum antibiotic therapy. A higher proportion of patients with fungal infections were also on inhaled or oral steroids (89.66%). This association was however not significant (p=0.24). Most of the patients who had fungal infections were older than 65 years (72.41%), residing or

Table 1 - General Characteristics of the study population (n=200)

Study variable	**Group**	**Number**	**Percentage**
Age	<65	74	37.00%
	65 or above	126	63.00%
Sex	Male	181	90.50%
	Female	19	9.50%
Residence or work near a coffee plantation	No	41	20.50%
	Yes	159	79.50%
Hypertension	Absent	125	62.50%
	Present	75	37.50%
Diabetes	Absent	164	82.00%
	Present	36	18.00%
Growth on sputum culture	No growth	96	48.00%
	Gram-positive	25	12.50%
	Gram-negative	79	39.50%
Antibiotic given for exacerbation management	No	35	17.50%
	Yes	165	82.50%
Steroid given for exacerbation management	No	36	18.00%
	Yes	164	82.00%

Table 2 - Differences in study population and outcomes during the crop and non-crop seasons

Variable	Group	Fungus not isolated n=171		Fungus isolated n=29		p value
		Number	Percent	Number	Percent	
Age	<65	66	38.60%	8	27.59%	0.25
	≥65	105	61.40%	21	72.41%	
Sex	Male	156	91.23%	25	86.21%	0.39
	Female	15	8.77%	4	13.79%	
Residence or work near coffee plantation	No	35	21.05%	5	17.24%	0.64
	Yes	135	78.95%	24	82.76%	
Hypertension	Absent	109	63.74%	16	55.17%	0.37
	Present	62	36.26%	13	44.83%	
Diabetes	Absent	144	84.21%	20	68.97%	0.048
	Present	27	15.79%	9	31.03%	
Growth on sputum culture	No growth	81	47.37%	15	51.63%	0.61
	Gram positive	23	13.45%	2	6.90%	
	Gram negative	67	39.18%	12	41.38%	
Antibiotic given for exacerbation management	No	34	19.88%	1	3.45%	0.031
	Yes	137	80.12%	28	96.55%	
Steroid given for exacerbation management	No	33	19.30%	3	10.34%	0.24
	Yes	133	80.70%	26	89.66%	
Duration of hospital stay	<5 days	73	42.69%	5	17.24%	<0.001
	5-10 days	70	40.94%	12	41.38%	
	>10 days	28	16.37%	12	41.37%	
Outcome	Alive	165	96.49%	26	89.66%	0.10
	Death	6	3.51%	3	10.34%	

working near coffee plantations (82.76%) and normotensive (55.17%). However, these associations were also not significant. Almost half of the patients (48.28%) with fungal infection also grew bacteria on their sputum cultures with a majority of them being gram-negative bacteria (41.38%).

On evaluating the morbidity and mortality of the patients, we found that those with fungal infection required a longer duration of hospital stay (Fig-3). 24 (82.8%) out of the 29 patients with fungal infection required hospitalisation for more than 5 days. 12 (41.4%) of these patients needed hospitalisation for more than 10 days (table-2). This association was found to be significant with p=0.003. 10.34% of the patients who had fungal infection succumbed to their illness (Table-2) whereas mortality was 3.51% among patients who had no fungal infection (p=0.101).

Discussion:

COPD patients frequently experience worsening of symptoms warranting a change in

Table 3 - Factors associated with isolation of fungus in patients with COPD

Variable	Group	Fungus not isolated n=171		Fungus isolated n=29		p value
		Number	Percent	Number	Percent	
Age	<65	66	38.60%	8	27.59%	0.25
	≥65	105	61.40%	21	72.41%	
Sex	Male	156	91.23%	25	86.21%	0.39
	Female	15	8.77%	4	13.79%	
Residence or work near coffee plantation	No	35	21.05%	5	17.24%	0.64
	Yes	135	78.95%	24	82.76%	
Hypertension	Absent	109	63.74%	16	55.17%	0.37
	Present	62	36.26%	13	44.83%	
Diabetes	Absent	144	84.21%	20	68.97%	0.048
	Present	27	15.79%	9	31.03%	
Growth on sputum culture	No growth	81	47.37%	15	51.63%	0.61
	Gram positive	23	13.45%	2	6.90%	
	Gram negative	67	39.18%	12	41.38%	
Antibiotic given for exacerbation management	No	34	19.88%	1	3.45%	0.031
	Yes	137	80.12%	28	96.55%	
Steroid given for exacerbation management	No	33	19.30%	3	10.34%	0.24
	Yes	133	80.70%	26	89.66%	
Duration of hospital stay	<5 days	73	42.69%	5	17.24%	<0.001
	5-10 days	70	40.94%	12	41.38%	
	>10 days	28	16.37%	12	41.37%	
Outcome	Alive	165	96.49%	26	89.66%	0.10
	Death	6	3.51%	3	10.34%	

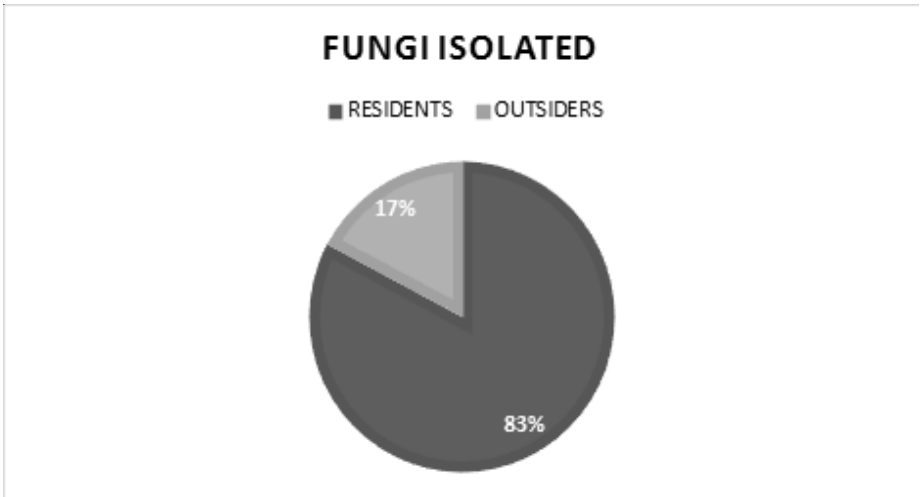

Fig-2: Diagram showing fungal infections among residents near coffee plantation

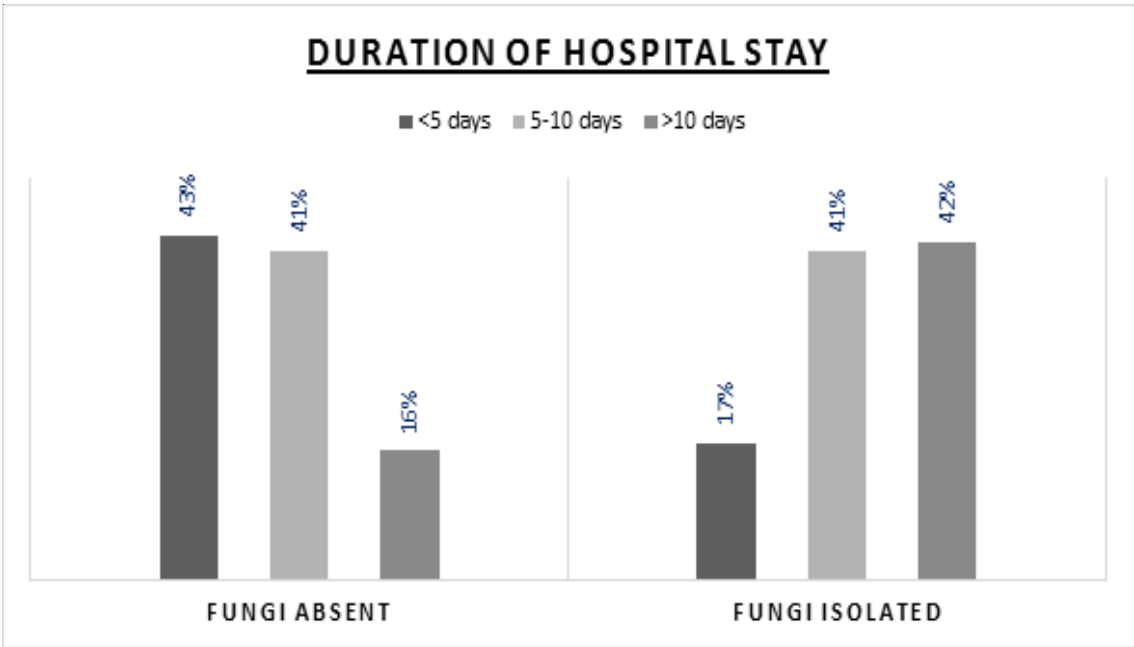

Fig-3: Length of hospital stay among AECOPD due to fungal infection

Fig-4(A &B): Branches and berries of the coffee plant during ripening season showing white flakes on it (black arrows) which harbour large quantities of fungal elements.

treatment. Such exacerbations pose a serious health problem and significantly affect the mortality and morbidity of these patients[6]. Bacteria and viruses are the most implicated causative agents in AECOPD. The role of fungal infections in AECOPD is poorly understood. The commonest fungal genus implicated is Aspergillus of which aspergillus fumigatus is the most common organism isolated from patients with AECOPD[12]. Aspergillus is responsible for a wide spectrum of clinical diseases ranging from saprophytic colonisation to rapidly invasive and life-threatening disseminated disease. The common risk factors associated with aspergillosis include neutropenia, corticosteroid therapy, organ transplantation, haematological malig-

Table-4: Fungi isolated from environmental samples collected from coffee plantations

FUNGI ISOLATED	NUMBER OF ISOLATIONS (N =30)	PERCENTAGE
Aspergillus fumigatus	25	83.34
Aspergillus niger	2	6.67
Rhizopus spp.	1	3.33
Mucor spp.	1	3.33
Fusarium spp.	1	3.33
Aspergillus fumigatus +Rhizopus	2	6.67
Aspergillus fumigatus +Mucor spp.	2	6.67
Aspergillus fumigatus +Fusarium spp.	4	13.33

nancies, chemotherapy, HIV infection and chronic respiratory diseases [13-19].

The progression of Aspergillus infection occurs following its colonisation in the upper respiratory tract. This process requires the penetration of the fungal spores through the epithelial layer of the lower airways[20]. Since the spores of aspergillus are of small size (2-3 mm), they can reach the lung parenchyma, through the airways, with relative ease [21]. In COPD patients, the activity of the cilia in the bronchial epithelium, airway defence mechanism and phagocytic host defence is often impaired. This leads to conidial binding to the epithelial layer[22]. Prolonged use of corticosteroid therapy, frequent hospitalisation, broad-spectrum antibiotic treatment, invasive procedures and comorbid illnesses such as diabetes mellitus, alcoholism and malnutrition are also contributory factors[23].

Aspergillus spp. is ubiquitous and is commonly found in both the outdoor and indoor environment. Besides immunosuppressed individuals, COPD patients are at an increased risk of acquiring these infections. Pulmonary disease is most commonly caused by aspergillus fumigatus which also happens to be the most common fungi isolated from the white flakes in coffee berries[24]. In the hill district of Wayanad, there is abundant coffee and tea plantations. Unlike tea plantations where harvesting happens all around the year, coffee harvesting is confined to a specific season. During the crop season, due to the plucking of these berries, these spores are dispersed into the environment increasing the concentration of these spores in the atmosphere. Thus, at-risk patients like COPD can easily acquire these infections especially those residing near coffee plantations.

Our study is the first study done in this part of the country to prospectively determine the seasonal prevalence of aspergillus infection along with the associated risk factors and outcome in COPD patients. There is an increased prevalence of fungal infections in cohort A when compared to cohort B, but it did not show any

statistical significance. This is further supported by our finding that the majority of the patients (83%) with fungal infections were either working or residing near coffee plantations. Studies were done to estimate the prevalence of aspergillus spp. in COPD patients like the one done by Huerta et al also reported similar figures[25].Diabetes was found to be clinically and statistically significant risk factor in acquiring fungal infections. Almost half of the patients with fungal infections had coexistent bacterial growth in their sputum cultures. Thus, it is prudent to start antibiotics even in this subset of patients. However, fungal infections were commonly seen among patients who were subjected to repeated courses of broad-spectrum antibiotics. This can be attributed to the fact that the infection in most of them was not resolving and a trial of broader spectrum antibiotics were given until the fungal culture reports were available. However, in a few patients where the index of suspicion was high, antifungal therapy was initiated early based in KOH smear reports which are usually available within a day. The increased length of hospital stay is statistically correlated with fungal infections. Mortality is found to be higher among patients having fungal infections though not statistically significant.

Pulmonary fungal infections tend to have a varied presentation. A high degree of suspicion is needed to diagnose these cases since the clinical and radiological presentation is frequently nonspecific. In most cases, a diagnosis is only considered once pneumonia is not responding to conventional antibiotics. Unfortunately, in COPD patients with poor lung function, any delay in initiation of treatment may prove to be fatal. Thus, if we can establish a seasonal correlation, our clinical judgement may improve and help in promptly starting antifungal drugs.

Limitations of the study are that we only included COPD admitted with an acute exacerbation. Hence, only group C and D patients were accounted for. The relevance of the study in group A and B patients remains doubtful.

Conclusion:

Fungal infections were diagnosed among 16.3% of the COPD patients admitted during the crop season and 12.5% of the COPD patients admitted during the non-crop season. Diabetes and broad-spectrum antibiotic usage were found to have a significant association with these infections. Since the seasonal prevalence is higher during the coffee plucking seasons extending from November to January, a fungal aetiology should be suspected in any atypically presenting pneumonia in COPD patients during these months. Patients with such fungal infections had a significantly longer duration of hospital stay and increased risk of mortality.

Conflicts of interest:

Authors have no conflicts to declare. This study is not funded by any agency.

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Correlation of DLCO versus DLCO/VA with functional parameters in interstitial lung disease

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Abstract

Background:

Diffusion capacity of the lungs is measured in two ways, DLCO and DLCO/VA. DLCO/VA is more useful in conditions where the parenchymal size is reduced especially after surgery. Since ILD is a diffuse and heterogeneous disease, there is a controversy as to whether DLCO or DLCO/VA is a better indicator of functional parameters. As there are only a few studies on DLCO and DLCO/VA in ILD, this study was done in an attempt to look at which parameter would be a better indicator of functional parameters in ILD.

Aim and Objective

Correlation of DLCO and DLCO/VA with functional parameters like 6 Minute Walk Distance, Borg Dyspnoea Scale, baseline oxygen saturation, percentage desaturation during 6MWD.

Materials and methods

This was a prospective observational study conducted in the Department of Pulmonary Medicine, Amrita Institute of Medical Science, Kochi; between the months of August 2017 to February 2018. Patients with ILD referred for functional evaluation to the Department of Pulmonary Medicine were assessed with DLCO, DLCO/VA, 6MWD, baseline saturation, percentage desaturation was noted and correlation was analysed using IBMSPSS version 20.0 statistical software.

Observations

The mean DLCO was 0.50±0.22 ml/min/mmHg and mean DLCO/VA was 0.86±0.28 ml/min/mmHg/L. DLCO showed a mild positive correlation(statistically significant) with 6MWD (r = 0. 255, p value =0.015) and baseline saturation (r =0.326, p=0.002), while it showed a mild negative correlation(statistically significant) with Borg dyspnoea scale(r = - 0.384, p = <0.001) and percentage desaturation (r = -0.452,

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p<0.001) during 6 MWT.

DLCO/VA showed a mild positive correlation with 6MWD (r=0.202, p=0.056) (not statistically significant) and baseline saturation (r=0.247,p=0.019) (statistically significant).A mild negative correlation(statistically significant) was shown with Borg Scale (r= -0.227,p 0.008) and Percentage desaturation (r= -0.408, p < 0.001) during 6MWT.

Conclusion

DLCO and DLCO/VA showed a mild but statistically significant correlation with the functional parameters in patients with interstitial lung disease. Hence DLCO and DLCO/ VA both can be used in predicting the functional capacity in patients with interstitial lung disease.

Keywords

Diffusing capacity, Transfer factor, Interstitial lung disease

Introduction

Carbon monoxide (CO) acts as an excellent molecule to measure diffusing capacity of the lungs as the concentration of CO normally in the blood is minimal and its haemoglobin binding is 230 times stronger than Oxygen. The carbon monoxide transfer factor (DLCO is also known as TLCO) measurement is one of the most complex tests performed in the PFT laboratory. The transfer factor estimates the transfer of carbon monoxide from the alveolar gas to the haemoglobin in the red blood cell. It represents the integrity of the alveolar-capillary membrane. The commonly used method to measure the TLCO is the single breath method. Primarily in research, the steady-state and rebreathing methods of determining DLCO are employed.[1]

Measurement of diffusing capacity is useful in evaluation of dyspnoea or hypoxemia, evaluation of emphysema, evaluation for the presence of interstitial lung disease, monitoring of known interstitial lung disease, differential diagnosis of lung volume restriction, detection of pulmonary vascular disease, pulmonary disability/impairment evaluation[2].The rate of gas transfer in the lung depends on the surface area available for transfer; thickness of the alveolar-capillary membrane; solubility and molecular weight of the gas concerned: CO2 has a similar molecular weight to O2 but diffuses about 20 times more rapidly because of its high solubility.[3]

DLCO decreases with a reduction in alveolar volume either because of inadequate inspiration to TLC with normal lungs (e.g. neuromuscular disease, kyphoscoliosis), Loss of lung units (e.g. pneumonectomy, atelectasis, localised lung destruction), Poor mixing of inspired gas (e.g. significant airflow obstruction) or in those patients whose total lung capacities have been reduced as a result of a disease of the interstitium. Therefore, to determine the true loss of diffusing capacity, the transfer coefficient KL (Krogh index) can be derived as the CO gas that is taken up per time relative to the ventilated volume, VA (DLCO/VA). The application of KCO is useful if there is a linear relation between DLCO and VA, which is not true in cases of diseases where the lung volumes are less than 50% of the TLC and also if DLCO is directly proportional to VA which is not the case in diseased states. KCO is inversely propor-

tional to VA and therefore, KCO changes as the alveolar volume changes.

Reduction in alveolar volume by disease is the largest potential source of error in interpreting DLCO. Correction for the effect of altered alveolar volume has been attempted by reporting the ratio of DLCO/VA.However, this attempt to normalize measurements by alveolar volume leads to errors because DLCO/VA does not remain constant as alveolar volume changes. [1]

In disease states, the DLCO and DLCO/VA values are usually not concordant and the dilemma is to decide which value represents the diffusion abnormality more accurately. Especially in interstitial lung diseases, will DLCO alone or the DLCO/VA would be more accurate is a question of investigation as in ILD, diffuse alveolar-capillary damage leads to decrease in DLCO and the loss of aerated alveoli leads to decreased VA. Mostly ILD is a heterogeneous disease where there is a diversion of blood flow to the less affected region from the more affected region leading to decreased KCO (DLCO/VA)[4]. 6MWT is already an established reliable, valid, and responsive measure of disease status in IPF[5] and other ILDs. Therefore, we conducted a Prospective study of patients with interstitial lung disease to determine if DLCO or DLCO/VA closely correlates with the 6minute walk distance, baseline saturation, exercise desaturation and the level of dyspnoea.

Methods

This Prospective observational study was conducted in the Pulmonary Medicine Department of Amrita Institute of Medical Sciences and Research Centre, a multi-speciality teaching and tertiary care hospital from August 2017 to February 2018. Patients with clinical/ radiological diagnosis of ILD who came for functional evaluation to our pulmonary function test lab were included in this study. Patients who were acutely ill were excluded.

We analysed the pulmonary function test (PFT) records of all patients that included both diffusing capacity and 6 – min walk test with oximetry. All the baseline characteristics such as age, sex, height, weight were collected, lung function measurements including DLCO, DLCO/VA, 6MWD, baseline oxygen saturation, percentage desaturation, Borg Dyspnoea Scale were also taken.

All tests were done at our PFT laboratory by certified technologists using commercial equipment. Diffusing capacity by the single breathe method was done by Bodybox, Geratherm Respiratory GmbH, using Carbon monoxide, methane and incorporating a breath-hold time. We assessed the level of dyspnoea in patients using the Borg Dyspnoea Scale.Diffusing capacity measurements met quality guidelines as established by the American Thoracic Society. Oximetry during the 6 min walk test was recorded with a pulse oximeter (Shiller OxywaveTM pulse oximeter machine, Schiller healthcare India private limited, Pondicherry).The study was approved by the institutional review Board of Amrita institute of medical sciences, Kochi.

Statistical analysis

The sample size was calculated based on the study done by David A. Kaminsky et al[6].

Statistically analysis was performed using IBMSPSS version 20.0 software. Categorized variables are expressed as frequency and percentage; continuous variables are expressed using mean and standard deviation. To test the statistical significance correlation of variables, the Pearson correlation method was used. A P-value of less than 0.05 was considered statistically significant.

Results

90 consecutive patients with interstitial lung disease who were evaluated with DLCO were included in this study out of which 46(51.1%) were males and 44(48.9%) were females. The mean age was 55.9± 14.1years. The mean DLCO was 0.50±0.22 ml/min/mmHg and mean DLCO/VA was 0.86±0.28ml/min/mmHg/L. The mean 6 MWD was 416.6±80.46meters. Mean baseline oxygen saturation was 96.57±02.82%. Mean percentage desaturation was 5.43±5.08%.

Discussion

In this study, we tried to assess the correlation of DLCO and DLCO/VA with the functional parameters in ILD patients. DLCO measurement is a complex procedure that requires effort from the individual and in the late stages of the disease, the objective parameter DLCO will not be able to detect subtle improvement in functional status

The primary objective of this study was to find out the correlation of DLCO with 6 MWD. In our study on correlation of DLCO with 6 MWD and Borg Dyspnoea scale, the result showed minimal correlation between DLCO and 6MWD (r=0.255, p= 0.015)similar to a study conducted by Caminati et al[7]which showed a significant correlation of DLCO with 6MWD(r=0.42, p= 0.01) and also similar to the study conducted by Pimenta et al[8] (r=0.58, p= <0.001).The assessment of correlation of DLCO with Borg Dyspnoeascale showed a mild negative correlation (r= - 0.384, p<0.001) which was similar to study conducted by Nishiyama et al[9], which showed a significant negative correlation of DLCO with Borg dyspnoea scale ($r = -0.38$, $p = 0.01$)

This appears to be the first study using DLCO/VA as a functional variable for ILD patients. In our study, it showed that correlation of DLCO/VA with 6-minute walk distance had no significant correlation (r=0.202, p=0. 056) and a negative correlation was shown with Borg dyspnoea Scale (r= -0.227,p 0.008).

The second objective was to study the correlation of DLCO with baseline saturation and percentage desaturation. There was a small positive correlation of DLCO with baseline saturation which was statistically significant(r =0.326, p=0.002) and a mild negative correlation with percentage desaturation which was also statistically significant (r = -0.452, p<0.001) and similar to the study conducted by Pimenta et al[8]where DLCO showed a significant negative correlation with percentage desaturation(r = -0.67, p<0.001)and Hu et al which showed a negative correlation between percentage desaturation and DLCO (r= -0.44, p<0.001). [10]

In the present study, DLCO/VA showed a mild positive correlation with baseline saturation, which was statistically significant(r =0.247, p=0.019) a mild negative correlation with percentage desaturation which was also

Table 1: Variable – Descriptive Statistics

Variable	Minimum	Maximum	Mean	SD. Deviation
Age	10	80	55.9	14.1
DLCO	0.12	1.17	0.50	0.22
DLCO/VA	0.35	1.85	0.86	0.28
6 MWD	80	600	416.6	80.46
Baseline SPO2	84%	100%	96.57%	2.82%
Exetrtion	70%	100%	91.2%	7.2
% Desaturation	0%	20%	5.43%	5.08%

Table 2: Correlation with 6 minute walk distance

Variable	N	6MWD	P value
		Correlation Coeffaciant	
DLCO	90	0.255	0.015
DLCO/VA	90	0.202	0.056

DLCO was found to have a mild but statistically significant correlation with 6MWD (r=0. 255, p= 0.015.) whereas DLCO/VA was found to have a mild positive correlation with 6MWD (r=0.202), which was not statistically significant (p=0.056)

Table 3: Correlation with dyspnea Borg Scale

Variable	N	BORG Scale	P value
		Correlation Coeffaciant	
DLCO	90	-0.384	<0.001
DLCO/VA	90	-0.277	0.008

Both DLCO and DLCO/ VA were found to have mild correlation with Borg dyspnoea which was significant statistically (r= - 0.384, p<0.001andr= -0.227, p= 0.008 respectively).

Table 4: Correlation with baseline saturation

Variable	N	Baseline Saturation	P value
		Correlation Coeffaciant	
DLCO	90	0.326	0.002
DLCO/VA	90	0.247	0.019

Both DLCO and DLCO/ VA were found to have mild correlation with baseline saturation which was significant statistically(r =0.326, p=0.002andr=0.247,p=0.019respectively).

Table 5: Correlation with percentage desaturation.

Variable	N	Percentage desaturation	P value
		Correlation Coeffaciant	
DLCO	90	-0.452	<0.001
DLCO/VA	90	-0.408	<0.001

Both DLCO and DLCO/ VA were found to have mild correlation with percentage desaturation which was significant statistically(r= -0.452, p<0.001andr= -0.408, p <0.001respectively).

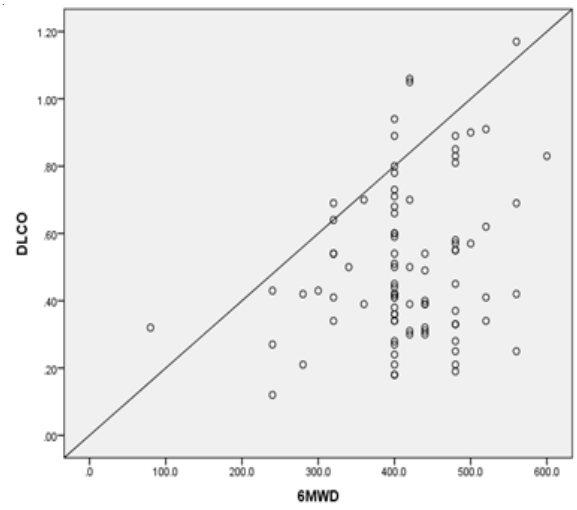

Figure 1: Correlation between 6 MWD and DLCO

statistically significant (r = -0.408, p <0.001). These results were similar to the results obtained in a study by Kaminsky et al[6](r = -0.40, p <0.0001)

Raquel Rosa et al also demonstrated an evident correlation between post 6MWT Borg dyspnoea index and DLCO (r=-0.25; p=0.04) and KCO (r=-0.32; p=0.01) similar to our study and demonstrated a stronger correlation of percentage desaturation with DLCO (r= -0.59, p <0.001) and KCO(r= -0.44,p <0.001).[11]

The results of this study show that just like DLCO, DLCO/VA can also be used to assess the diffusion capacity in patients with ILD. The advantage of DLCO or DLCO/VA over each other was not apparent as the study is done in a restricted set of patients. As this was a very small sample, it is difficult to draw conclusions.

The reason that the DLCO and DLCO/ VA had no advantage over each other maybe because the DLCO is a more global measure of diffusing capacity and DLCO/ VA takes into account, not only the intrinsic gas exchange ability of the lung (the DLCO/VA) but also the overall lungsize and distribution of ventilation (VA).[6]

The value of DLCO increases with VA whereas the value of KCO decreases as it is a ratio of DLCO and VA. So, the adjustment with the VA can be an important step in determining the diffusion capacity.

Strengths of the study were that it was a prospective study and the patients were diagnosed based on clinical details, examination findings in addition to PFT and radiology as cases of ILD. We also corrected the DLCO values for haemoglobin concentration of the patients.

Our results must be applied after understanding the limitations. First, it was a single centre study and the data may be only applicable to the population included, which may represent the regional distribution of interstitial lung diseases and prevalence of exercise desaturation. A different set of population may yield different results based on disease prevalence. ABG was not done to determine the A-a gradient and pulse oximetry was used to determine hypoxaemia and reading on pulse oximeter can be altered due to multiple causes especially in a patient with poor perfusion. ABG for all patients is not practically possible and exercise oximetry was used to measure the level of desaturation.

Summary

DLCO and DLCO/ VA both showed a mild but statistically significant correlation with the functional parameters in patients with interstitial lung disease. Hence DLCO and DLCO/ VA may be used in predicting the functional capacity in patients with interstitial lung disease.

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Orginal article

An analysis of genetic mutations in lung adenocarcinoma and their relationships to demographic patterns and histopathological features.

Theresa Scaria[1], Sreeja Raju[2], Rennis Davis[3], Anil Jose Thazhath[4]

Abstract

Background:

A better understanding of genomics of the carcinomas and its molecular pathways underlying oncogenes has led to the era of personalized medicine and targeted therapy.

Aim & objectives:

1. To assess genetic mutation analysis in lung adenocarcinoma.

2. To find out the relationship between EGFR,ALK and ROS1 mutations and demographic patterns and histopathological features.

Methods:

Following institutional ethics committee clearance, 112 patients diagnosed with lung adenocarcinoma over a period of 29 months (June 2017-October 2019) were identified. Of the 112 cases, 53 were selected in which mutation analysis has been done by using ARMS-Real Time PCR. Their demographic parameters and histopathological features were retrieved from the archives. Analysis was performed by using the SPSS23 software.

Results:

The male to female ratio in the study population is 1.6:1 with mean age of 63.56. Of the 53 cases analyzed. 20.7% were EGFR mutations, 5.6% were ALK mutations and 1.88% were ROS1 mutations respectively. The most common mutation detected in EGFR was deletions in exon19.EGFR, ALK and ROS1 mutations were common in the age group of 50-70. The predominant histopathological type was acinar pattern in EGFR mutations, solid pattern in ALK mutations and mixed pattern in ROS1 mutations.

Interpretation & conclusions:

In this study, the commonest mutation detected was EGFR mutations (deletions in exon 19) which can have therapeutic implications. EGFR mutations were more common in males, smokers, belonging to the age group 50-70. This contradictory result in our study may be due to less sample size included during the study period.

Keywords:

EGFR, ALK, ROS1

Introduction

Lung cancer can be broadly divided into small cell carcinoma and non-small cell lung carcinoma. (SCLC: NSCLC= 16:84).SCC has mainly neural crest origin and there is increased chance of recurrence and mortality. NSCLC originate in the lung epithelial cells.

NSCLC frequently over expresses receptors of the erbB family including EGFR encoded by erbB1(Her1).The EGFR is a 170 kDa receptor tyrosine kinase that dimerizes and phosphorylate several tyrosine residues after bonding of several specific ligands. These phosphorylated tyrosine serve as the binding site for several signal transducers that initiate multiple signaling pathways resulting in cell proliferation, migration and metastasis, evasion from apoptosis or angiogenesis, all of which are associated with cancer phenotypes.[1] T790M are the mutations in the EGFR gene that results in the substitution of threonine with methionine at amino acid position 790 and it is associated with increased resistance toward tyrosine kinase inhibitors.

ALK is a member of the insulin family of receptor tyrosine kinase and is typically expressed at low levels in regions of CNS.[2] ALK may be activated in cancer through multiple mechanisms including rearrangements as is the case in anaplastic large cell lymphoma and inflammatory myofibroblast tumor or through mutation and amplification as in neuroblastoma.[3] In NSCLC, ALK activation is typically caused by a chromosomal rearrangement that results in a fusion of 3'kinase domain of the ALK with various truncated portions of the (N terminal) echinoderm microtubule associated protein like 4(EMPL4) gene and its associated promoter.[4,5] EML4 is a cytoplasmic protein necessary for correct microtubule formation belonging to the family of echinoderm associated proteins.[6,7] The oncogenic activity of EML4-ALK comes from ligand independent dimerization and subsequent constitutive activation of the ALK kinase domain with subsequent effects on proliferation, migration and survival.[8] Mouse models where EML4-ALK is expressed specifically in lung epithelial cells develop hundreds of adenocarcinoma nodules in both lungs soon after birth which are eradicated following administration of ALK inhibitors.[9,10]

ROS1 is an orphan receptor tyrosine kinase of insulin receptor family. The gene was first discovered as homologue of a viral oncogene.[11] The ROS1 receptor has no known ligands [12] and there is only limited knowledge of its physiological function.[13] Increased expression of ROS1 fusion proteins as a consequence of chromosomal rearrangements is observed in several carcinoma including glioblastoma, cholangiocarcinoma and ovarian carcinoma. In oncogenic fusion transcripts ROS1 retain its kinase domain which is aberrantly expressed and constitutively active to trigger downstream sig-

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naling pathways, including mitogen activated protein kinase and phosphatidylinositol 4,5 bisphosphate 3 kinase/AKT/mammalian target of rapamycin pathway.[14]

This study has been done in the anticipation of promoting targeted drug therapy for EGFR, ALK and ROS1 mutations positive lung adenocarcinoma. Establishment of a relationship between these mutations and demographic and histopathological patterns would enable us to administer targeted therapies without doing expensive molecular studies.

Materials and methods

The study has been started after getting institutional ethical committee clearance. This is a descriptive study with a calculated sample size of 50 using formulae- $4PQ/d^2$, taking P as 70%[15], prevalence of EGFR mutations from previous study. One hundred twelve cases diagnosed with adenocarcinoma lung between the time interval of June 2017 to October 2019 was taken from our institution (Amala Institute of Medical Sciences, Thrissur, Kerala, India). From this population, 53 cases who satisfied the inclusion criteria (all NSCLC cases who have performed mutation analysis) were included in the present study.

Lung Tru-Cut biopsies were taken and histopathological studies were done in our institution. Mutation analysis was done using ARMS-Real time PCR, which was outsourced. In the outsourced specimen, DNA extracted from the sample is tested for the presence of indicated hotspot mutations Exon 19 deletions, Exon 20 insertion and substitution mutations G719X, S7681,T790M, L858R and L861Q in exons 18, 20 and 21 of the EGFR gene using ARMS real time PCR and the target exons are amplified with mutation specific primers. Information regarding various demographic patterns and other clinical parameters were retrieved from medical records archives. Demographic patterns included age, sex, various clinical parameters including smoking status, other medical comorbidities, presence of metastasis and history of carcinoma in first degree relatives.

The data collected was entered into Microsoft excel and analysis was done by SPSS software version 23.

Results

The male to female ratio in the study population is 1.6:1 with a mean age of 63. 566.Of the 53 cases analyzed, 20.7% were EGFR mutations, 5.6% were ALK mutations and 1.88% were ROS1 mutations respectively. The most common mutation detected in EGFR was deletions in exon19.EGFR mutations in our study showed a male preponderance (63.6%) while ALK and ROS1 mutations showed a female preponderance. EGFR mutations were more common amongst smokers while ALK and ROS1 mutations were more common amongst non- smokers. Distant metastasis was present in 36.3% of EGFR positive cases while no distant metastasis was noted in ALK and ROS1 positive cases. EGFR, ALK and ROS1 mutations were common in the age group of 50-70. The predominant histopathological type was acinar pattern in EGFR mutations, solid pattern in ALK mutations and mixed pattern in ROS1 mutations. Carcinoma in first degree relatives and distant metastasis

were absent in the majority of EGFR, ALK and ROS1 mutation positive cases.

Discussion

In the present study with sample size of 53, 20.7% of the cases were positive for EGFR mutations, the mean age was 66.54 and the mutations were more common among males and smokers. The most common mutation observed was deletions in exon 19 and the predominant histopathological pattern observed was of acinar type. In a similar study conducted by[16] Celina Vila et.al with the sample size of 200, 20.5% of the cases were positive for EGFR mutations, the mean age was 64.8 and the mutations were more common among women .Smoking history was present in 49% and the most common mutations was found to be deletions in exon 19.The predominant histopathological pattern observed was lepidic type. Histologic features of lung adenocarcinoma may play a role in predicting the outcome in patients with *EGFR* mutation who are treated with TKIs. Yoshida et al[17] have shown that patients with *EGFR* mutations with the predominant solid pattern of adenocarcinoma have significantly worse overall response to TKIs. Others[18,19,] have shown that the new classification correlates with

Table-1: EGFR mutations and their relationships to demographic patterns and clinical parameters

		EGFR	
		Positive	Negative
Sex	Female	4	16
	Male	7	26
Smoking Status	Present	6	22
	Absent	5	20
Carcinoma in first degree relatives	Present	1	1
	Absent	10	41
Metastasis	Present	4	2
	Absent	7	19

Table-2: ALK mutations and their relationships to demographic and clinical parameters.

		EGFR	
		Positive	Negative
Sex	Female	2	18
	Male	1	32
Smoking status	Present	0	28
	Absent	3	22
Carcinoma in first degree relatives	Present	0	2
Metastasis	Absent	3	48
	Present	0	24
	Absent	3	26

patient prognosis and provides important prognostic information.

Our present study got similar results with that of [20]Rodig SJ et.al. In the study conducted by Rodig SJ et.al, with sample size of 358, 5.6% showed ALK mutations, more common in younger age group and among non-smokers. The predominant histopathological pattern observed was of solid type. Since their discovery in Anaplastic Large Cell Lymphoma, *ALK* rearrangements or mutations have been identified in inflammatory myofibroblast tumors, a subset of diffuse large B-cell lymphomas [21,22], and a subset of neuroblastomas[23,24]. These findings have prompted the development of inhibitors of ALK enzymatic activity for therapeutic use [25]. With the discovery of *ALK* rearrangements in NSCLC[26], the number of potential patients who might benefit from such drugs has increased dramatically. These patients typically present in late stages not amenable to surgical resection and are therefore candidates for aggressive and novel chemotherapeutic regimens that target the mutated ALK protein.

In the study by H.Go et.al, with the sample size of 451, 1.8% of cases showed positive ROS1 mutations. The mutations were more common among women and the predominant histopathological pattern observed was that of mixed type. Our present study got similar re-

Table-3: ROS-1 mutations and their relationships to demographic and clinical parameters

		ROS1	
		Positive	Negative
Sex	Female	1	19
	Male	0	33
Smoking status	Present	0	28
	Absent	1	24
Carcinoma in first degree relatives	Present	0	2
	Absent	1	50
Metastasis	Present	0	26
	Absent	1	26

Table-4: EGFR, ALK and ROS1 mutations and histopathological patterns

	Acinar	Lepidic	Papillary	Solid	Mixed
EGFR Positive	8	2	0	0	1
EGFR Negative	16	2	2	9	13
ALK Positive	0	0	0	2	1
ALK Negative	13	3	2	9	23
ROS1 Positive	0	0	0	0	1
ROS1 Negative	16	3	2	10	21

sults with that of [27] H.Go et.al. Bergethon et al.[28]suggested that *ROS1* rearrangements define a unique molecular classification of lung cancer with distinct clinical characteristics, such as a response to crizotinib. They also showed that *ROS1* rearrangements were detected in the 1.7% of NSCLC patients, and the patients tend to be younger nonsmokers with adenocarcinomas. In a review by Oliver et al, the techniques used to discover each of these candidate oncogenes, their prevalence in nonsmall cell lung cancer, and briefly outline the epidemiological features of the major oncogenes and ways in which their identification can determine therapeutic strategies has been discussed.[29,30]

The study could be continued forward and a larger sample size can be assessed in the future to look for any association between these mutations (EGFR, ALK and ROS1) with demographic patterns and histopathological features. Treatment response to these mutations can be followed up

Conclusions

Among the 53 cases detected of adenocarcinoma lung, 20.7% were positive for EGFR mutations, 5.6% for ALK mutations and 1.8% for ROS1 mutations. The commonest mutation detected is EGFR, which has therapeutic implications. The most common mutation identified among EGFR mutations were deletions in exon 19. EGFR mutations were more common in males, in smokers belonging to the age group 50-70.This contradictory result in our study may be due to less sample size included during the study period. But EGFR mutations were more negative amongst smokers. No distant metastases were noted in ALK and ROS1 positive cases, while it was present in 36.3% of EGFR positive cases. The most common histopathological pattern noted in EGFR mutations was acinar type. However, there were no statistically significant association between these mutations (EGFR, ALK and ROS1) with demographic and histopathological features.

Acknowledgment

Med genome laboratories

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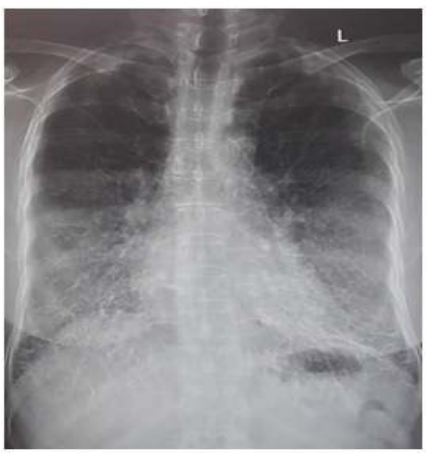
V. Kesavan Nair[1], Sharada Nair. C [2]

Case summary

A 45 year old lady working as housemaid in the U.A.E, presented with chronic cough lasting for more than one year. There was no seasonal or postural variation of cough .The cough was mainly dry with occasional scanty mucoid sputum. There was no hemoptysis or purulence. The cough did not interfere with her work and because she was working in gulf, she did not get it investigated. She had no asthma or whooping cough during childhood. One of her brothers also had chronic cough but he had died in his early 20s, due to suspected pneumonia. Another brother did not have cough and was working in Gulf.

Examination revealed a moderately built lady with dry irritant cough but with no wheeze. The routine blood reports, blood sugar, hepatic and renal function tests, TSH etc. were normal. Her chest X-ray showed some classic findings and subsequently CT thorax was done for confirmation of the diagnosis. It is a very rare case in which the diagnosis is radiological.

Figure 1. Chest x- ray



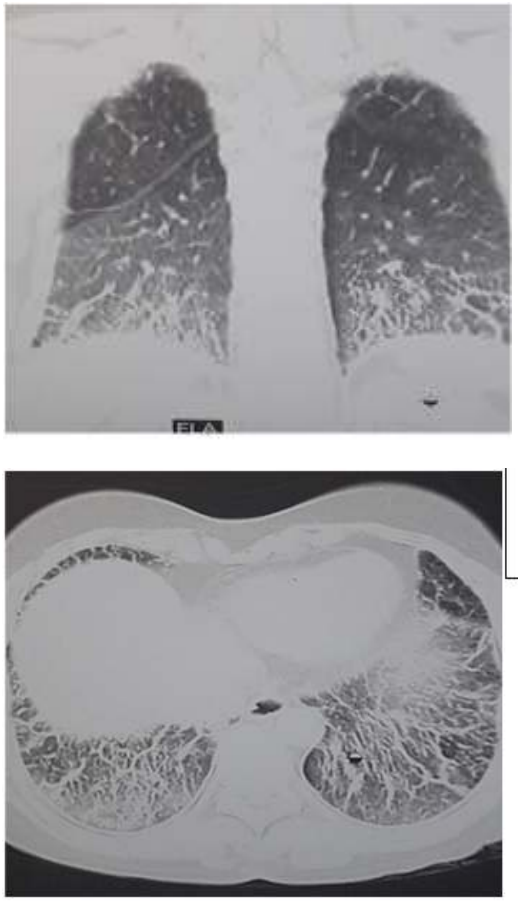


Fig 2 CT Thorax

What is the diagnosis?

Answer

Pulmonary alveolar microlithiasis

Pulmonary alveolar microlithiasis (PAM) is a rare disease, characterized by presence of diffuse innumerable minute calculi called microliths (calcospherites) in the alveoli of the lungs. Very few cases are reported in literature

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from different regions of India till date.[1] Worldwide roughly around 1022 cases are reported so far.[2] Calcification due to PAM has been reported involving extrapulmonary sites including liver, kidney, pancreas, intestines, testes, ovaries and heart valves also.[3]

The definite etiology and pathogenesis are not clearly known. The most accepted etiology is that it is an inherited abnormality involving the enzyme carbonic anhydrase, which promotes alkalinity of the alveolar surface and subsequent precipitation of calcareous salts. Inactivating mutations in the SCL 34A2 gene have also been reported in familial cases.[1] Type II alveolar cells express this gene which encodes a type IIb sodium-phosphate co transporter. When this co transporter is dysfunctional, the alveolar cells are unable to clear the phosphate ion from the alveolar space, resulting in the formation of microliths[4]

The calculi contain calcium and phosphate combinations in a ratio of 2:1. Grossly the lungs are firm to hard in consistency. Numerous tiny calculi (calcospherites), ranging from 0.01 to 3.0 mm, are present within the alveoli. During late stage of disease, fibrosis, interstitial thickening and giant cells may be noticed. Apical blebs and bullae may be present which may lead to recurrent pneumothorax.

In most cases, the first diagnostic clue is the characteristic chest radiograph findings. PAM is a typical example of clinico-radiological dissociation.[5] X-ray chest shows infiltrates in the form of fine sand-like calcific micronodules (sandstorm lung) diffusely involving both lungs, usually more marked in middle and lower zones which often obliterates the mediastinal and diaphragmatic outline.[6]

Characteristic chest CT findings include calcifications which may be seen in peripheral, mediastinal, fissural and subpleural regions. Each lobe is surrounded by a fine dense outline, giving the overall appearance of a 'stony lung'. At advanced stage, the opacities may be so dense so as to make the lungs appear almost uniformly white. The heart border and the diaphragm may be obliterated. Felson first reported a linear radiolucency in the area of the lateral pleura on chest radiographs and called it a 'black pleural line'. High-resolution CT shows that this is caused by thin-walled subpleural cysts.[7]

Young patients may lack clinical symptoms and may have normal pulmonary functions, or a slightly decreased diffusing capacity. In the 40s, they may develop features of cor pulmonale. Progressive respiratory insufficiency is the most common cause of death in these patients.[8]

Serum calcium and phosphate levels, hepatic, renal and parathyroid functions are usually normal. The identification of the SLC34A2 gene mutation (where available) and serum concentrations of the surfactant proteins A and D may be elevated in the patients with PAM, and can be the markers to monitor the activity and progression of the disease.[9]

The diagnosis can be confirmed by lung biopsy (trans-bronchial or open). The characteristic finding is intraalveolar lamellar microliths. The demonstration of

microliths in the bronchoalveolar lavage (BAL) fluid is also possible [10]

Presently, there is no definite treatment for PAM. Systemic corticosteroids have been shown to be ineffective and home oxygen therapy is needed for patients with respiratory insufficiency. Disodium etidronate, which inhibits the micro crystal growth of hydroxyapatite, has been used at a dose of 10 mg/kg per day orally for as long as one year and has been shown to lead to regression of the calcific densities.[11] Bilateral sequential lung transplantation or unilateral lung transplantations have been performed in a few patients worldwide, but their long-term survival is yet to be proved.[12]

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Case Summary

A 65 year old lady with no medical comorbidities presented with cough and weight loss of 2 months. She also had gradually progressive shortness of breath for the last 2 months. She had no history of fever or loss of appetite. She had no allergic or atopic background. Chest Xray- posteroanterior projection (Fig 1) as well as representative images of CT chest (Fig 2 – 5) are shown below. What is the diagnosis?

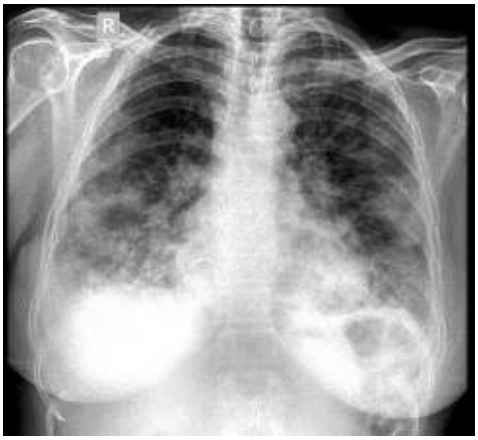

Fig 1

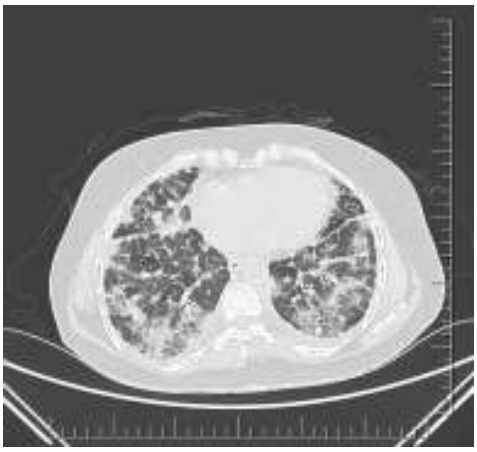

Fig 2

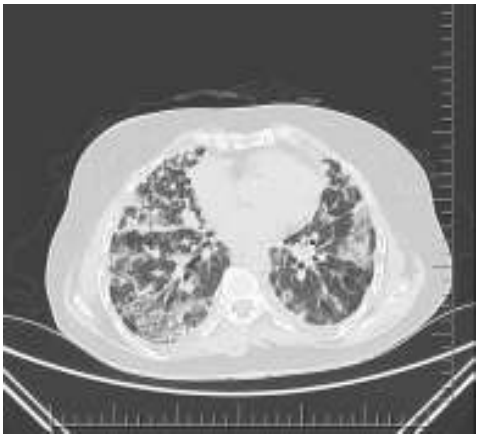

Fig 3

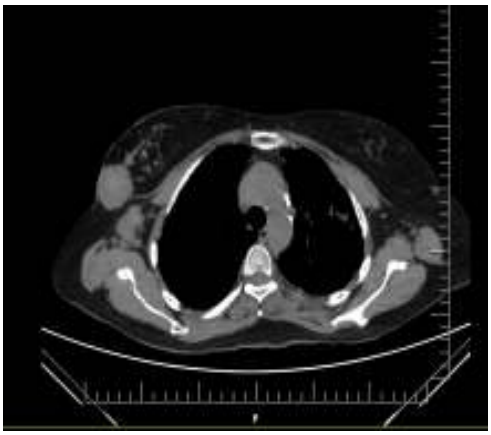

Fig 4

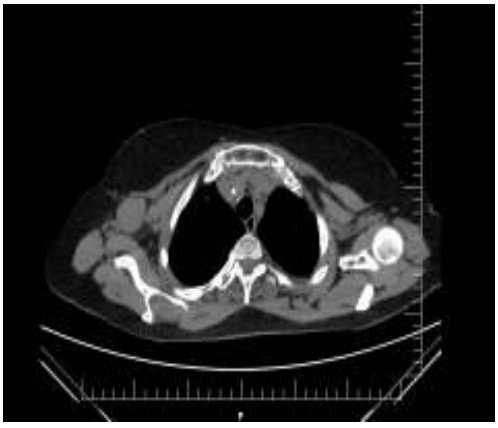

Fig 5

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Diagnosis

Probable carcinoma right breast, right axillary metastatic lymphadenopathy, bilateral pulmonary metastatic lesions and lymphangitic carcinomatosis

Discussion

Chest radiograph reveals bilateral mid zone and lower zone reticulo-nodular opacities with surrounding bilateral patchy consolidation (Fig 1). Representative images of HRCT chest reveal multifocal intralobar and interlobar interstitial thickening, with some areas showing fissural nodules giving rise to a beaded septum appearance (Fig 2). Multiple soft tissue density nodules distributed in a random fashion are also noted in both lung parenchyma, the largest measuring 15 x 13 mm in posterior basal segment of left lower lobe. Multiple patchy foci of ground glass opacities and consolidations were also noted in both lungs (Fig 3). The mediastinal window images show a well-defined soft tissue lesion in the upper outer quadrant of the right breast measuring 3.5 x 3.2 cm reaching up to the skin surface with a calcific foci within (Fig 4). Multiple enlarged axillary lymph nodes are also seen, the largest measuring 2.7 x 2.6 cm (Fig 5). The overall appearance is consistent with a malignant lesion of right breast, right axillary metastatic lymphadenopathy with bilateral pulmonary metastatic lesions and lymphangitic carcinomatosis. The patchy ground glass opacities could possibly represent secondary infection.

Pulmonary metastatic disease is frequently encountered in clinical and radiological practice. Most pulmonary metastases are parenchymal and take a nodular shape (both micro nodular and canon ball shadows), but a small minority are interstitial. In pulmonary lymphangitic carcinomatosis (PLC) there is diffuse infiltration and obstruction of pulmonary parenchymal lymphatic channels by tumour cells. Primary site of malignancy in such cases include breast, stomach, lung, pancreas, colon, stomach, and prostate[1]. Although any histological subtype can account for PLC, 80% of the cases tend to be from metastatic adenocarcinomas[2].

Imaging with high-resolution computed tomography (HRCT) is a sensitive technique and plays central role in the detection of PLC. A highly suggestive radiological appearance with background clinical information may clinch the diagnosis of PLC. HRCT findings have been well correlated with pathologic features[3]. Usual HRCT features include nodular and irregular interlobular septal thickening, although a smooth septal thickening may be encountered infrequently. A smooth bilateral septal thickening, bilateral pleural effusions and cardiomegaly should, on the other hand, alert the astute clinician of the possibility of pulmonary oedema in the appropriate clinical setting. This septal thickening in PLC is usually associated with parenchymal nodules also. Thickening of the fissures may be visualised due to subpleural interstitial lymphatic involvement. Preservation of normal parenchymal architecture at the level of the secondary pulmonary lobule is the norm in PLC and architectural distortion should alert the radiologist of the possibility of a fibrosing ILD like IPF, end stage sarcoidosis or fibrosing NSIP[4]. Another appearance described is polygonal arcades with prominence of the centrilobularbronchovascular bundle in associa-

tion with interlobular septal thickening (50%). Intrathoracic lymphadenopathy is seen in 30-50% cases and pleural effusions in about 30% subjects. Findings may be unilateral or bilateral, focal, or diffuse, and symmetrical or asymmetrical. Focal, unilateral disease accounts for 50% of cases. This pattern is usually associated with underlying bronchogenic carcinoma.

The differential diagnosis of PLC includes other interstitial lung diseases like IPF, NSIP, sarcoidosis, chronic hypersensitivity pneumonitis and occupational lung diseases. A focused clinical history can exclude many of these differentials. The relative absence of polygons and the presence of architectural distortion at the level of the secondary pulmonary lobule suggest an alternative diagnosis rather than PLC. Parenchymal involvement in sarcoidosis is typically more central and/or perihilar, as well as more bilaterally symmetrical, than in PLC. Honeycombing almost never occurs in PLC. The presence of a possible malignant lesion (lung, breast, upper abdomen etc) may be evident in the CT chest, as in this case, rendering the diagnosis obvious.

Our patient was evaluated with a trucut biopsy of right breast lesion which revealed an invasive ductal carcinoma, SBR grade 3.

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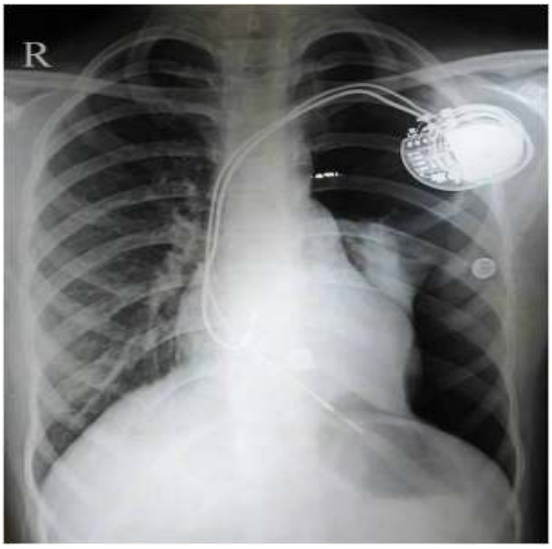
Vishnu Sharma. M.

Question 1:

This patient became breathless immediately after cardiacpacemaker implantation.



Chest x ray was taken

What is the **MOST LIKELY** cause for breathlessness?

1. Massive pulmonary embolism left side
2. Cardiogenic pulmonary edema
3. Left sided pneumothorax
4. Collapse of left lung
5. Misplaced leads leading to cardiac arrhythmia

Answer 1:

Left sided pneumothorax

Chest x-ray shows hyper translucent lung field on left side which is devoid of bronchovascular markings, medially bounded by collapsed visceral pleural line which is diagnostic of pneumothorax. Pace maker is seen on left side. Intercostal tube was inserted on left side and patient improved.

Second x ray shows intercostal tube on left side and left lung is expanded.

Incidence of pneumothorax after subclavian vein access for pacemaker implantation varies from 0.6-1% to 5.2% with an average of 2 %[1]. Pneumothorax usually occurs on the same side of subclavian puncture. Contralateral pneumothoraces are rare, reported in the literature, can occur due to perforation caused by the endocardial atrial lead. The screw in atrial lead increases the risk of perforation through the wall of the right atrial appendage. Hence operators must be very careful of the anatomy of the right atrial wall and avoid over screwing the screw in leads, so the complication of pneumothorax is eliminated. Pneumothorax could also be associated with pneumopericardium, pneumomediastinum, and subcutaneous emphysema.

Clinical signs of pneumothorax after cardiac lead placement include shortness of breath, hypoxia, pleuritic pain, and hypotension. If the pneumothorax occurs during the implantation procedure, then the symptoms are sudden; sudden chest pain, respiratory distress, air aspiration during subclavian vein puncture and sudden hypotension. If any one or more of the above symptoms or signs are present, then an urgent fluoroscopy of the upper lung and critical monitoring should be performed[1]. If the oxygen satu-

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ration is less than 90%, and/or patient develops hypotension, then the procedure should be terminated. Sometimes a small pneumothorax could be asymptomatic and may be seen on routine chest radiography done after the procedure.

Question 2:

Which of the following is **NOT** associated with increased risk of pneumothorax as complication in pacemaker implantation (PMI) ?

1. Female patients.
2. Dual chamber PMI
3. Associated chronic obstructive pulmonary disease (COPD)
4. Cut-down technique of the cephalic vein for PMI
5. Longer procedure duration

Answer:

Cut-down technique of the cephalic vein for PMI

The risk of pneumothorax could be eliminated by puncturing the axillary vein or Cut-down technique of the cephalic vein [2]**.** However this technique is not the appropriate one for all cases as it demands extensive skin and muscle dissection. A fluoroscopic guidance of the subclavian vein puncture instead of the blind puncture is also helpful to reduce the risk of pneumothorax. Good knowledge of the anatomy of the patient (aware of any deformation of the clavicle or chest abnormality or anatomical variations) and careful handlings are essential for the safe accomplishment of the implantation. Dual-chamber pacing needs a second subclavian puncture, for the passage of two leads and that is the reason for the higher risk of pneumothorax [2]. Rupture of bulla may be the reason for increased risk of pneumothorax in patients with COPD. Longer procedure, which probably means less experienced operators, increases the risk of pneumothorax [2].

Question 3:

What is the **LEAST LIKELY** complication of Pacemaker implantation?

1) Bleeding
2) Hemoptysis
3) Pneumothorax.
4) Infection
5) Perforation

Answer

Hemoptysis

Surgical complications of PMI may occur during the immediate or early post-operative period and can be related to venous access process (pneumothorax, hemothorax, air embolism, hematoma, arterial puncture, wound healing problems, infection, pain), to the pacemaker lead (cardiac perforation, tamponade, malposition or dislodgement of the lead) [3]. Other complications that may occur during the late post-operative period are infections, thrombosis, endocarditis, pulmonary embolism, superior vena cava (SVC) syndrome (due to thrombus formation and/or fibrosis of the pacing wires within the SVC) and pericarditis. Sohail et al in their retrospective review of 189 patients with pacemaker related infections found out that the

most common clinical presentations were generator pocket infection (69%) and device-related endocarditis (23%)[4].

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The first case of Sars Cov-2 (COVID-19)pneumonia treated with anti-retroviral drugs in Kerala,India

Fathahudeen A[1] , Jacob K. Jacob [2] , Ganesh Mohan[3], Vibha Santosh[4], Renimol[5]

Abstract

The first case of SARS CoV-2 was reported in Kerala on January 30th following the outbreak of the novel Corona Virus -19 in Wuhan, China and the government of Kerala took efforts to contain the pandemic and arrest its spread in the state. Here we report the case of a tourist of British origin who presented to our COVIDcentre attached to a Government Medical College, ateaching hospital and a tertiary care centre, with fever, tiredness and mild upper respiratory tract symptoms. This report describes the use of Antiviral cocktail in a COVID-19 positive patient withCOVID Pneumonia in conjunction with hydroxychloroquine and azithromycin.The case affirms the early use of antivirals on an emergency compassionate basis, in the current COVID – 19 pandemic

Case Report

On March 15th 2020, a 57-year-old gentleman from Britain who came as a tourist to Kerala, South India from England via Dubai, was brought to the COVID facility of government medical college, Ernakulam, Kerala. He gave a history of fever and upper respiratory symptoms 3 days prior while he was touring in Munnar and his swab was taken promptly there itself. We received him in our facility with a confirmed COVID-19 report. He was given N95 mask and his oropharyngeal and nasopharyngeal swabs were taken and sent for rRT PCR. He was febrile and had a cough. He did not have breathlessness at rest and was maintaining a normal Oxygen saturation on room air. He was on treatment for systemic hypertension and hyperuricemia. On examination in the COVID-triage, he was obese with a BMIof 27kg/m2, with stable hemodynamic parameters. He was admitted into the special COVID isolation room adjacent to COVID-ICU and was connected to a multi-para monitor for continuous hemodynamic and oxygen saturation monitoring. He was continuously monitored round the clock by duty physicians and nursing staff. A portable Chest X-ray was showing an infiltrate in the left lower zone. Since his blood count showed a viral picture with neutrophil: lymphocyte ratio (NLR)of 1.77, we thought he was stable and continued with the usual care and monitoring.

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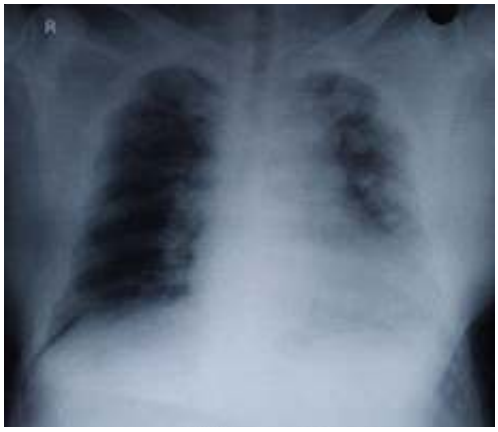

Figure 1. Portable chest radiograph on admission (PA view)

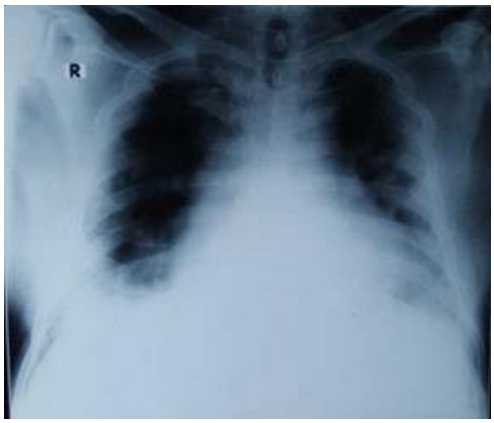

Figure 2. Portable chest radiograph on third day of admission showing new onset infiltrates on the right lower zone.

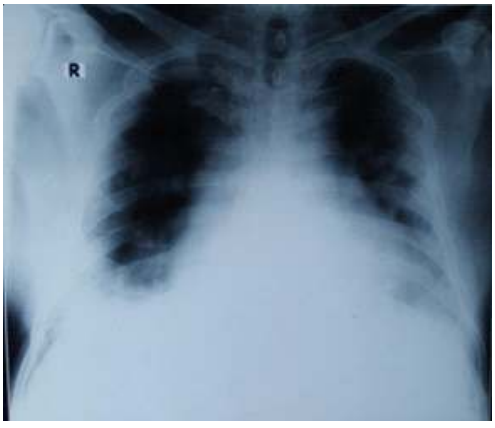

Figure 3 . Portable chest radiograph at discharge showing near complete resolution of Covid pneumonia.

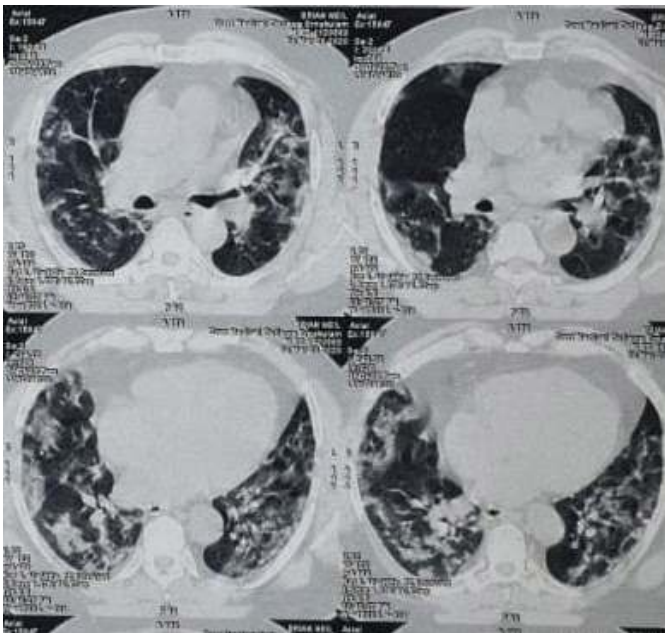

Figure -4. CT Thorax films

Azithromycin, Oseltamavir, antibiotics and supportive measures including deep vein thrombosis prophylaxis with low molecular weight heparin were promptly given and his nutrition and emotional status were also taken care of well by psychiatrists. A repeat chest X-ray the very next day morning showed the same findings and all his hemodynamic and respiratory parameters were remaining normal. He had a few episodes of loose stools which subsided with conservative management but fever persisted. His total count was 4000cells/c.cm and there was no lymphopenia or thrombocytopenia. Blood sugar, liver and renal function tests, electrolytes, prothrombin time/INR, ECG, ECHO were normal. CRP was raised and Serum Ferritin was 859 ng/ml (Normal is 20-322 ng/ml).His repeat swabs were sent after 24 hours. On the third day, he continued to be febrile and a third repeat Chest X-ray showed progression of infiltrates in the left lower zone. A repeat white blood count showed a rise in neutrophil: lymphocyte ratio to 3.71. His SpO2 dropped to

93% on room air and was shifted to adjacent COVID-ICU and was started on 3 litres of Oxygen via nasal prongs and SpO2 picked up to 97%. He continued to be Oxygen-dependent and febrile. Meanwhile, HRCT chest was taken with standard infection prevention and control protocol and he was moved out in a PPE and subsequently, the CT room and the CT machine were fumigated. CT chest showed bilateral extensive peripheral patchy consolidation with few areas of ground glassing at sub-pleural locations.

Considering the clinical scenario, with the concurrence of the State Medical Board, the IMB decided to put him on Lopinavir/Ritonavir combination therapy on an emergency compassionate basis along with hydroxy-chloroquine after taking informed consent in the format sent by the state medical board. He and his wife were counselled before starting the antiviral medications. However, he continued to have fever spikes and worsening hypoxia with tachypnoea and tachycardia but was fully conscious and oriented. Despite on nasal oxygen supplementation up to 10 L/mt, his SpO2 remained 88 % and the respiratory rate rose to 36/mt. ABG showed type 1 respiratory failure with respiratory alkalosis and P/F ratio was suggestive of early ARDS. He was started on NIV on CPAP an ICU through ventilator with non- vented mask with a viral filter attached at the end of the expiratory port. His CBC, serum amylase, LFT, CRP, were repeated every 48 hours. ECG was repeated every day and QTc was constantly monitored by the cardiologist. Echocardiogram was repeated to rule out any evidence of early myocarditis. His saturation picked up to 94 % with a FiO2 of 0.6 in the next 8 hours and his respiratory rate dropped down to 27/mt and he tolerated the NIV well. He continued to have an unremitting fever and remained tachypneic.However, with the continued close observation and meticulous CPAP adjustments, he slowly got stabilized over the next few days.Hemodynamic parameters remained stable throughout. His ECG showed prolonged QT interval on the 5th day of HCQ and hence, HCQ was stopped. SpO2 rose to 98% on 0.4 FiO2.Fever subsided on the 7th day of anti-viral therapy and tachypnoea reduced.By this time he was stabilized on intermittent CPAP support and he was weaned off completely and continued with nasal prongs 2L Oxygen for another 48 hours. Two days before discharge he was off Oxygen and maintaining a saturation of 97% on room air.

His two throat swabs taken at 24 hours interval for SARS Cov-2 (Covid-19) was reported to be NEGATIVE.

Discussion

With SARS Cov-2 reaching a highly explosive global proportion anda large number of deaths being reported from all over the world particularly in Europe and the US, desperate attempt to find a curative treatment is underway. A wise approach than inventing a new drug molecule is to test whether the existing drugs are useful in related viral infections such as SARS-CoV-2. In this background, reports of treating COVID-19 patients with anti-HIV drug cocktail along with hydroxychloroquine and azithromycin from China, Singapore and South Korea have encouraged the rest of the world to try this antiviral cocktail.Hence, we in our COVID Centre,

Table 1. Clinical Lab Results

Measure	Reference Range	Day 1 (15/03)	Day 4 (18/03)	Day 6 (20/03)	Day 8 (22/03)	Day 9 (23/03)	Day 16 (30/03)	Day 16 31/3
White-cell count	3800-11000	4158	4705	5690	8210	7813	8500	7853
Absolute Neutrophil Count	1900-7400	2618	3682	3564	6321	5913	6375	5840
Absolute Lymphocyte count	1000-3900	1540	948	1126	1889	1900	2125	2013
NLR	>3.5	1.77	3.8	3.16	3.34	2.	3.0	2.9
Platelet count	150,000-400,000	150,000	-	-	222,000	334000	-	354000
Haemoglobin(g/dl)	13.2-17.0	14.6	13.3	12.0	13.1	11.3		11.1
ESR mm/1st hour	25	-	-	97	-	108	-	110
Sodium(mmol/L)	136-145	140	140	137	136	136	138	137
Potassium(mmol/L	3.5-5.1	4.5	4.5	3.2	3.5	3.7	3.9	4.6
Calcium(mg/dl)	8.7-10.4	9.3	9.4	-	-	-	9.4	-
Glucose(mg/dl)	65-140	126	128	124	96	124	128	130
Blood Urea (mg/dl)	9-23	26	28	28	26	29	22	19
Creatinine(mg/dl	0.7-1.3	1.2	1.1	1.20	1.10	1.10	0.80	0.8
Total Protein(g/dl)	5.7-8.2	5.8	5.8	5.9	5.7	5.10	5.90	5.5
Albumin(g/dl)	3.4-4.8	3.4	3.3	3.3	3.2	2.90	3.10	3.2
Total Bilirubin(mg/dl)	0.3-1.2	0.6	0.8	0.8	1.0	1.00	0.50	0.6
Procalcitonin(ng/ml)	< 0.05	0.04	-	0.05	0.04	-	-	-
SGPT (U/L	10-49	47	46	43	40	38	31	31
SGOT(U/L	<33	32	32	32	27	28	35	38
ALP(U/L	46-116	39	39	38	39	30	37	36
Fibrinogen(mg/dl)	150-450	160	169	-	-	-	170	-
PT (Sec)	12.2-14.6	14.2	-	16.55	20.2	-	16.7	16.7
INR	0.4-2.0	0.98	-	1.18	1.51	-	1.19	1.19
Creatine KinaseU/ L	62-325	235	241	240	-	-	243	-
Serum Ferritin ng/L	12-300	859	-	874	-	-	873	-
LDH U/L	140-280	435	454	754	743	760	756	415
CRP mg/L	< 3	9.6	12	76	75	56	44	10.8
S. Lipid profile	Normal	N	N	N	N	N	N	N
D-Dimer	<0.5	347	348	356	378	388	368	368
Viral markers	Negative							
Troponin I ng/ml	<o.04	0.016	-	0.013	-	-	-	-
APTT Seconds	30-40	34	-	35	-	35	-	34
S. Amylase 23-85 U/L	23-85	105	110	117	116	118	114	115
Other Fever markers	Negative							
S.Lactate mmol/L	0.5-1	3.1	-	3.1	-	-	-	-
G6PD	Negative							

after proper institutional clearance and informed consent, started this gentleman with severe COVID pneumonia in respiratory failure, on lopinavir, ritonavir along with hydroxychloroquine and azithromycin.

The clinical criteria that we followed in our centre to start anti-viral cocktail along with hydroxy-chloroquine on the compassionate ground were progressive pulmonary infiltrates and drop in oxygen saturation. The pre-treatment protocol that we have followed in our COVID centre is:

1. An informed consent which is countersigned by the institutional medical board (format attached)

2. A thorough evaluation of the drug history of the patient to avoid possible drug interactions.

3. Baseline tests for Hepatitis C, B and HIV besides routine parameters, LFT, Serum amylase, PT/INR, platelet count, serum electrolytes, G6PD levels, ECG with QTc measurement.

4. Serial monitoring of above parameters during treatment.

We believe that one of the crucial factors which altered the course of illness in our patient is the timely introduction of this anti-viral cocktail along with hydroxy-chloroquine and azithromycin sufficiently early with the onset of pneumonia before the patient starts desaturating. In all COVID positive patients, meticulous monitoring to pick up early signs of clinical worsening, like tachypnoea, drop in SpO2, rise in NLR are of utmost importance. We have made it our standard clinical protocol to do HRCT chest to pick up pulmonary infiltrates early in the disease and also to delineate the extent of the infiltrates in the lungs. We found that doing CT in COVID patients suspected of pneumonia is the most sensitive imaging modality of choice than conventional X-ray chest. However, these observations will need a randomized control study to establish an association and correlation.

We thank our patient and his wife for giving us the consent in this emergency. We also thank the Hon. Chief Minister of Kerala, Hon. Health Minister of Kerala, Health secretary, DME Kerala, Kerala State Medical Board, Principal GMC, Ernakulam, Institutional medical board of Government medical college, Ernakulam, all the doctors, nursing and paramedical staff and other health care workers who took care of the patient in our COVID centre.

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Table 2 :Time-line showing the various symptoms and days of illness

12th March to April 1st 2020	Mar-12	Mar-13	Mar-14	Mar-15	Mar-16	Mar-17	Mar-18	Mar-19	Mar-20	Mar-21	Mar 22	Mar-23	Mar	Apr-24	Mar-31	Apr-01
	Tour			Hospital												
Day of illness	1	2	3	1	2	3	4	5	6	7	8	9	10	14	18	19
Fever																
Cough																
Dyspnoea																
Diarrhoea																
Antivirals																Discharge
Chest Xray																

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Informed concent form for compassionate use of LOPINAVIR-RITONAVIR for COVID-19 virus

Institutional medical board has informed me that I/ my relative have been diagnosed with COVID-19 infection. They have clearly explained to me that there is no effective and approved medication against COVID-19 infection.They have explained to me in detail that, there is some scientific evidence regarding the effectiveness of using LOPINAVIR-RITONAVIR in people affected by COVID-19.They have explained to me that LOPINAVIR-RITONAVIR has been used in treatment of HIV, even in children for more than 10 years in India with an acceptable adverse effect profile.

The team of doctors informed me that, I have developed Pneumonia due to COVID-19, I might benefit by the restricted compassionate use of LOPINAVIR-RITONAVIR. They have clearly explained to me that LOPINAVIR-RITONAVIR has not been approved for the definitive treatment of COVID-19. They have explained to me in detail that there are no approved drugs for COVID-19, and as there is a risk of progression to acute respiratory distress syndrome, LOPINAVIR-RITONAVIR may be used. They have explained to me about the probable side-effects of LOPINAVIR-RITONAVIR like diarrhea, hypersensitivity, pancreatitis, gastritis and hepatitis. They have made it clear that the standard treatment for COVID-19 infection will be continued irrespective of my decision regarding the compassionate use of LOPINAVIR-RITONAVIR. Knowing that LOPINAVIR- RITONAVIR is not an approved medication, for the treatment of COVID -19 infection, I fully agree to the restricted public health emergency

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Hisham Momattin Khurram Mohammed,AlimuddinZumla, Ziad A. Memish,and Jaffar A. Al-TawfiqTherapeutic Options for Middle East Respiratory Syndrome Coronavirus (MERS-CoV) –

Case report

Chronic Graft versus host disease presenting as pneumothorax

Melcy Cleetus[1], Rajesh V[2], Rinett Sebastian[3], Mobin Paul[4], Teena Sleeba[5], Jolsana Augustine[6], Divya R[6]

Abstract

Pulmonary manifestations or complications occur in 25-50% of allogeneic haematopoietic -stem cell transplant (HSCT) recipients, and can account for approximately 50% of transplant related deaths. Pulmonary involvement and disease severity ranges from subclinical pulmonary function test impairment to respiratory insufficiency secondary to bronchiolitis obliterans. Bronchiolitis obliterans syndrome is the characteristic pulmonary manifestation of chronic graft-versus-host disease (GVHD), which is a well described late complication of hematopoietic stem cell transplantation (HSCT). Pulmonary chronic GVHD can present with obstructive and /or restrictive changes.

We describe the case of a 22 year lady with history of acute myeloid leukaemia at 16 years of age treated with allogenic stem cell transplantation, following which she attained remission. She had no diagnosed transplant related chronic complications till her presentation to us. She reported to Emergency Medicine department with acute breathlessness and was detected to have large left sided pneumothorax. She was managed with tube thoracostomy. Further evaluation following admission revealed features of cGVHD. She had prolonged air leak which spontaneously sealed off in 3 weeks. Appropriate management of cGVHD was undertaken by a multidisciplinary team and she continues to be in out-patient follow up.

Key words

Chronic graft versus host disease, hematopoietic stem cell transplant, thoracic air leak syndrome, bronchiolitis obliterans syndrome

Introduction

Hematopoietic stem cell transplantation (HSCT) remains the treatment of choice for many hematologic malignancies. Many severe congenital or acquired disorders of hematopoietic or immune system may also need HSCT at some stage of their course. HSCT recipients are at high risk for a variety of pulmonary complications. Pulmonary complications occur in 25-50% of allogeneic HSCT recipients, and can account for approximately 50% of transplant related death[1].

Chronic graft versus host disease (cGVHD) is a common cause of late morbidity and mortality following allogeneic HSCT. Though the pathophysiology of chronic graft versus host disease

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(cGVHD) remains poorly understood, some of the most severe organ manifestations are secondary to end organ fibrosis. Pulmonary complications significantly contribute to late mortality after allogenic HSCT. In patients surviving longer than 2 years, Bathiaet al [2] found a 15 fold increased risk of late mortality because of pulmonary dysfunction compared with the general population.

Thoracic air-leakage syndrome (TALS), which was defined by Franquet et al as the presence of intrathoracic extra-alveolar air, includes spontaneous pneumothorax, pneumomediastinum, pneumopericardium, subcutaneous emphysema and interstitial emphysema[3]. These subjects frequently have bronchiolitis obilterans syndrome. Thoracic air-leakage syndrome (TALS) is associated with a poor prognosis with majority of the patients progressing to fatality if not aggressively cared for[4].

Case report

A 22 year old lady student, resident of central Kerala, presented to Emergency department with shortness of breath and left sided chest pain of 3 days duration. Her past history was remarkable for acute myeloid leukaemia diagnosed at 16 years of age which attained remission with allogeneic stem cell transplantation. She had further follow up from the institution where she underwent the HSCT and the last visit was a couple of years back. Review of records at last visit showed no evidence of disease relapse or therapy related complications. On presentation to us, she denied any fever, cough, strenuous exercise or chest trauma prior to the worsening of symptoms. She had history of progressively increasing dyspnoea on exertion for which she was on regular inhaled steroids and bronchodilators for past 8 months as per the advice of a general practitioner. However, there was no documented chest radiograph or spirometry done prior or after initiation of inhalers. A weight loss of 4 kg was noted over the last 6 months and interrogation revealed dryness of eyes, drooping of left eye lids and dysphagia.

She was tachypnoic and hypoxic at presentation. Chest examination revealed features consistent with large left sided pneumothorax. ECG showed sinus tachycardia. Chest radiograph postero-anterior projection (Fig 1A) and computed tomography (Fig 1B) showed large left sided pneumothorax with features of tension pneumothorax.

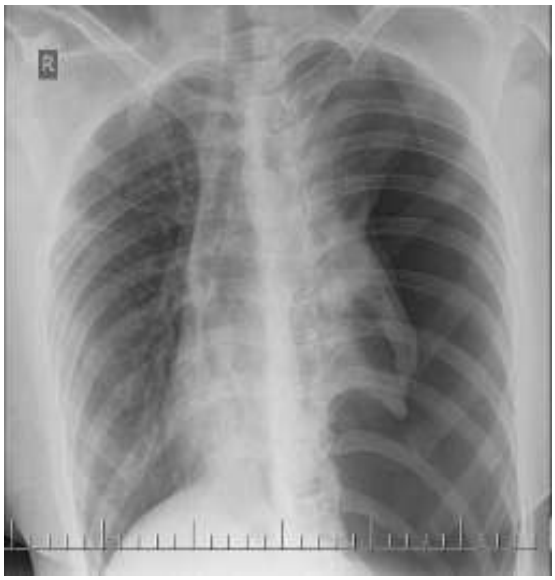

Figure IA-Chest radiograph at presentation showing large left tension pneumothorax with left lung collapse, mediastinal shift and left diaphragmatic depression.

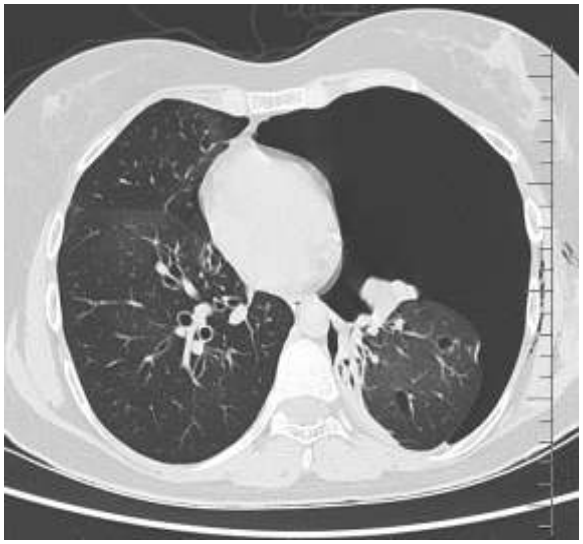

Figure 1B – CT thorax – representative image showing large left pneumothorax and collapsed lung

Tube thoracostomy was undertaken on an emergency basis which relieved her distress. CT chest (Fig 2A-D) repeated after tube thoracostomy showed expanded left lung with minimal pneumomediastinum, fibrotic changes with traction bronchiectasis in left upper lobe, cylindrical bronchiectasis in superior segment of left lower lobe, minimal fibrotic changes in right apex and bilateral mosaic attenuation consistent with air trapping. The CT chest findings were consistent with bronchiolitis obliterans syndrome. Given the clinical setting, the findings were consistent with pulmonary involvement due to chronic graft versus host disease.

A focused history revealed that patient had persistent irritation of the left eye for the past 9 months. Ophthalmic evaluation revealed a normal right eye with left eye showing moderate ptosis, dry ocular surfaces and mydriasis. Vision test also showed a decrease in visual acuity in left eye (6/24). Schirmer's test findings

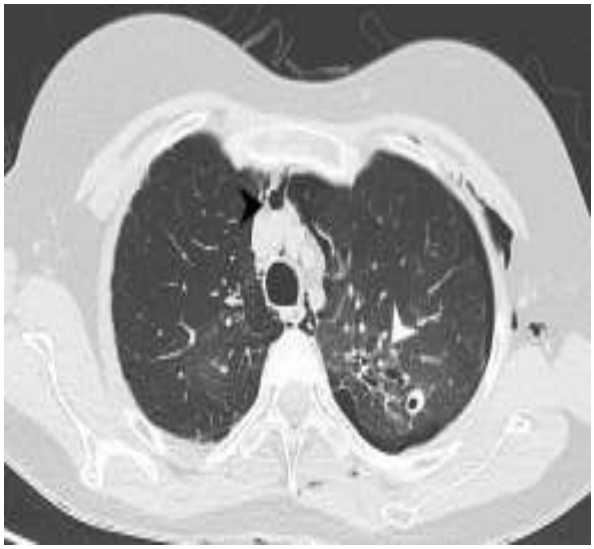

Figure 2A: Axial CT section showing minimal pneumomediastinum (black arrow) and fibrotic changes with traction bronchiectasis in the left upper lobe (white arrow)

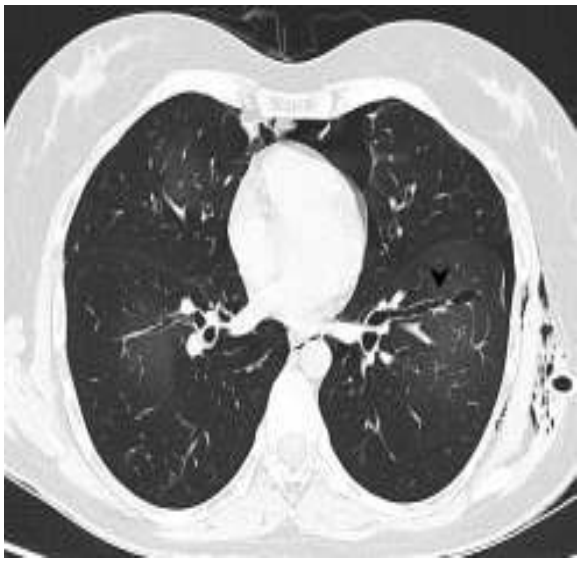

Figure 2B: Axial CT section showing cylindrical bronchiectasis in the left lower lobe (black arrow)

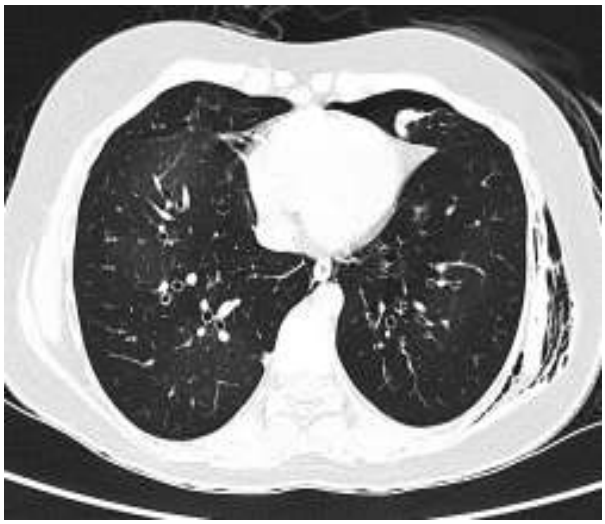

Figure 2C: Axial CT section showing dilated segmental bronchi in both lower lobes

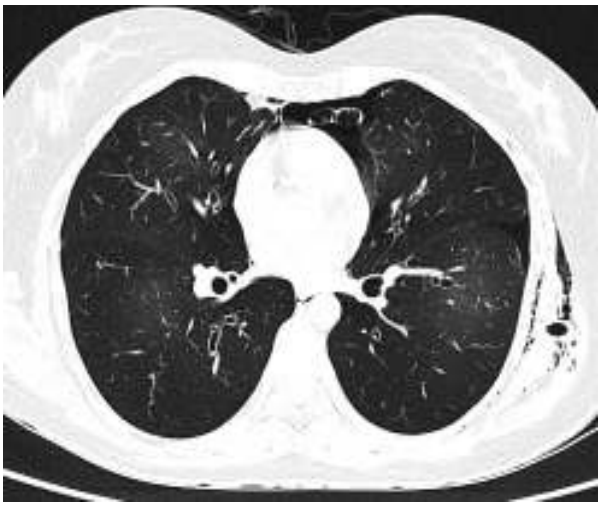

Figure 2D: Axial CT section showing mosaic pattern secondary to air trapping.

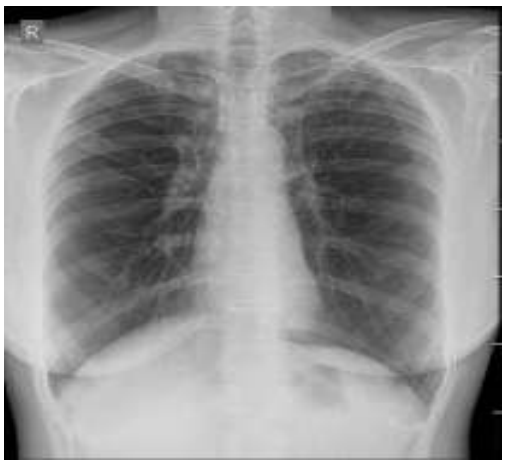

Figure 3 – chest radiograph at discharge

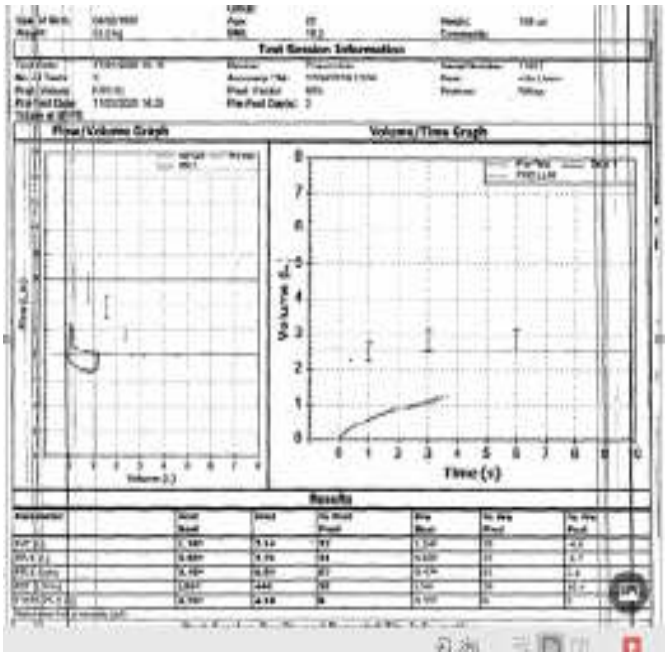

Figure 3 – Spirometry showing moderate obstructive ventilatory defect

were consistent with dry eye syndrome. All the findings were suggestive of ocular graft versus host disease. Skin and oral mucosa examination revealed hand and foot eczema, generalised xerosis of the extremities and alopecia. Oral mucosa showed lichen planus like lesions. Liver function tests were normal. Spirometry was done after reasonable lung expansion which showed moderate to severe obstructive ventilatory defect (despite the presence of small pneumothorax), a finding significantly suggestive of BOS. (Figure 4). Overall, she fulfilled the diagnostic criteria for chronic GVHD and the need for a biopsy confirmation was obviated.

She had persistent airleak from the thoracostomy tube even after 10 days of thoracostomy tube insertion, for which thoracic surgery opinion was sought. Fibre optic bronchoscopy and BAL microbial evaluation (bacterial cultures, CB-NAAT, fungal culture and cytology

for PCP) did not yield any infective agent. Haematologist opinion was also sought considering her past history and present diagnosis of chronic GVHD. After multidisciplinary discussion with Pulmonologist, radiologist and thoracic surgeon it was decided to continue conservative measures as the airleak was not worsening and surgical therapy carried high risk. The airleak sealed spontaneously after 3 weeks of conservative approach and Intercostal drain was successfully removed on the 25th day of presentation. Chest radiograph post removal of ICD is depicted (Fig 3). Spirometry after thoracostomy tube removal was not attempted due to fear of rupturing any bullae with potential recurrence of pneumothorax. Aggressive management of cGVHD was initiated and follow up was undertaken from the institution of her choice. She remains under clinical follow up from the institution where HSCT was done, to determine the long term outcome.

Discussion

Chronic GVHD is the most common non-relapse complication in patients with hematologic malignancies treated with HSCT, occurring in approximately 60% to 80% of long term survivors of allogeneic HSC transplant.[5] Chronic GVHD may mimic autoimmune and other immunologic disorders. The pathophysiology of the chronic GVHD syndrome may involve inflammation, cell-mediated immunity, humoral immunity, and fibrosis.

Clinical manifestations are almost always present within the first year after transplantation, but some cases develop many years after HSCT. The disease can virtually involve any organ although skin, oral mucosa and liver are commonly involved. Diagnostic criteria for chronic GVHD has been laid down by the national institute of health and severity scores are available.[6]

Although BOS is the classic pulmonary manifestation of cGVHD, lung involvement in chronic GVHD can be associated with restrictive lung function impairment, late interstitial pneumonitis (IP), cryptogenic organizing pneumonia (COP) etc. Although the occurrence of BOS as a complication of chronic GVHD has been reported in 2% to 14% of allogenic-SCT recipients who survive for more than 3 months, the incidence of thoracic air leak syndromes in allogenic -SCT recipients with chronic GVHD and BOS is between 0.8% to 2.3 %[7]. Clinical presentation of bronchiolitis obliterans syndrome (BOS) may include dyspnoea on exertion, cough, or wheezing although milder cases may be asymptomatic. Evaluation mandatorily requires spirometry and high-resolution computed tomography. BOS is characterized by the new onset of an obstructive ventilatory lung defect. Screening PFTs are recommended at day 100 post-transplant, at initial diagnosis of chronic GVHD, at one year after transplant, and at 6-monthly intervals for the first two years after the initial diagnosis of chronic GVHD. More frequent PFT monitoring is recommended in patients diagnosed with BOS and in those with significant decline in lung volumes. Bronchoscopy may be undertaken for obtaining bronchoalveolar lavage samples, which might be essential to rule out infectious complications as they can have similar clinico-radiological picture. In challenging cases, bronchoscopy also

aids in procuring transbronchial tissue specimen for histology.

National Institute of Health, USA, has provided a definition for BO/ BOS, which is primarily based on PFT and HRCT, and requires the following criteria[8] in order to characterize BO/ BOS as a manifestation of chronic GVHD

(1) FEV1 <75% of predicted normal and FEV1 / FVC <0.7

(2) Either signs of air trapping by PFT (RV >120% of predicted normal) or signs of air trapping, small airway thickening or bronchiectasis in expiratory HRCT or pathological confirmation of constrictive bronchiolitis.

(3) Absence of active respiratory tract infection.

(4) In case of lacking histological proof of BO, at least one other distinctive manifestation of cGVHD in an additional organ system is required.

Our case had characteristic lesions in skin, oral mucosa and eyes, thus obviating the need for histological confirmation of BOS.

Pneumothorax in GVHD

TALS is caused by alveolar rupture, leading initially to pulmonary interstitial emphysema and then travelling centrally along the bronchovascular sheaths into the pleural space and mediastinum. This pathogenetic mechanism is called the Macklin effect.[9] In BOS, localized or regional peripheral obstruction of distal airway results in a ball-valve effect, leading to alveolar over distension, alveolar bursting and entry of air into the bronchovascular sheath. Other possible causes of TALS include infections such as aspergillosis, candidiasis and pneumocystis pneumonia.[10] The emphysema may result from lung injury, caused by pretransplantation chemotherapy, total-body irradiation, GVHD, or immunosuppressant drugs. The risk factors for thoracic air leak syndromes following HSCT include development of chronic GVHD, second or subsequent SCT, male sex, an age of less than 38 years, tacrolimus-based GVHD prophylaxis etc.

The occurrence of TALS in a patient with cGVHD is per se considered to be associated with poor outcome. According to previous reports, the response of cGVHD after the occurrence of TALS was poor and the mortality rate was 66.7-100%.[11] TALS in subjects with cGVHD was not easily amenable to treatment in usual lines and poor prognosis with pulmonary complications was the norm.

Treatment of pulmonary chronic GVHD follows similar lines as that of cGVHD involving other sites. Topical treatment with combination of inhaled corticosteroids and bronchodilators may be tried for early and mild disease with limited success. Systemic immunosuppressive therapy is preferred in non responders; systemic therapy may be started upfront in those patients initially presenting with moderate-to severe clinical symptoms, a lung function score (LFS)>II, a FEV1 <75% in combination with a FEV1/FVC <0.7 or when air-trapping is present by PFT or HRCT.[12] Corticosteroids form the mainstay of systemic therapy. Calcineurin inhibitor (tacrolimus), mammalian target of rapamycin inhibitors (such as sirolimus and everolimus), or mycophenolate mofetil,

macrolide antibiotic like azithromycin and tyrosine kinase inhibitors like imatinib mesylate are also tried for the treatment of pulmonary graft versus host disease with variable success rates.[13]

Conclusion

To summarise, we have shared the case of a young lady with history of acute leukaemia, post allogenic HSCT, who presented with pneumothorax secondary to chronic pulmonary GVHD. The case deserves attention of practising clinicians on account of multiple reasons.

1.A significant majority of chronic GVHD subjects have some manifestations by the end of first year of engraftment. Late presentation, as in our case, is infrequently reported.

2.Pneumothorax as the presenting feature of cGVHD is distinctly uncommon.

3.Thoracic airleaks in pulmonary GVHDare traditionally poorly responsive tostandard therapy. Our case had a successful outcome in contrary to majority of cases encountered in literature.

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Thoracic endometriosis syndrome - a case series

V. Kesavan Nair[1] , Rohith.S [2], Arjun Somachandran[3] , Sajan J M[4]

Abstract

We are presenting four cases of Thoracic endometriosis syndrome presenting as haemothorax or hemopneumothorax which were different in presentation, management and outcome.

All the cases were detected to have pleural effusion while being investigated for infertility. The pleural fluid had the colour of altered blood and a diagnosis of catamenial haemothorax was suspected because of the presence of endometriosis elsewhere in the body. The cases were managed differently; by decortications in two cases, intercostal tube drainage in the third and conservatively in the fourth. There were no adverse events during the management or afterwards.

These rare cases are being presented to highlight the importance of this differential diagnosis in females presenting with recurrent haemothorax or hemopneumothorax. The diagnosis is often delayed as the connection with menses is either missed by the patient or the doctor.

Key words: Catamenial Haemothorax, infertility, pleural endometriosis.

Case report 1

A 27 year old lady was referred to the Dept. of Respiratory Medicine with complaints of progressive exertional dyspnoea. She was detected to have right sided pleural effusion by a physician while being investigated for primary infertility. She was given Anti tuberculous treatment suspecting tuberculous pleural effusion. As the fluid did not subside after 3 months she was referred. Examination showed moderate effusion, the pleural fluid was dark red in colour, and did not show any cells. The anti tuberculous treatment was stopped and the patient was kept under observation for reaccumulation of fluid .The fluid slowly increased in volume over a period of six months. Though aspiration improved the dyspnoea she consented for decortication of pleura to relieve her discomfort. Decortication was done by thoracic surgeon and the pleural tissue biopsy showed endometrial tissue (figure 1).

The diagnosis of pleural endometriosis leading to catamenial haemothorax was thus confirmed. She later conceived and delivered a healthy boy afterwards.

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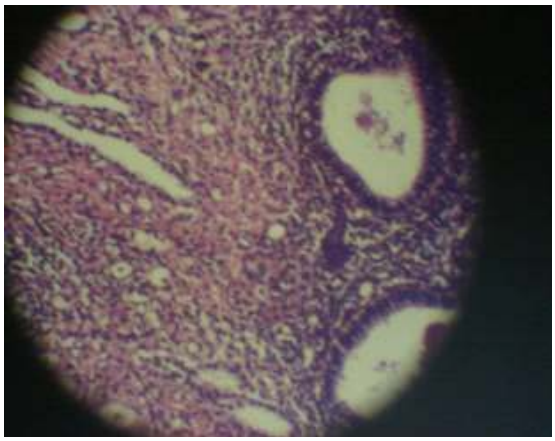

Figure 1: Case 1- Histopathology of the pleural tissue

Case report 2

This was a teacher who was having right sided chest discomfort and pain over 6 months which worsened during each menses. Examination showed diminished air entry on the right side and the Xray chest and CT thorax showed a thin rim of right sided pneumothorax and minimal pleural effusion. (Figure 2)

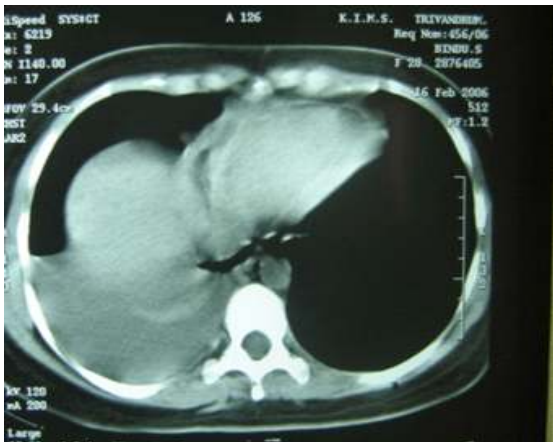

Fig 2 – CT thorax showing hydropneumothorax

There was another line of pleural thickening above the present one indicating recurrence of effusion. Needle aspiration attempted, it was a dry tap. Patient improved with conservative management alone. The lady had earlier undergone investigations for secondary infertility as the first child birth was 11 years back. Though she continued to have chest discomfort off and on during menses, there was no recurrence of pneumothorax or effusion at 6 months follow-up.

Case report 3

31-year-oldlady, presented with exertional dyspnoea, right sided chest pain of one-week duration. She was diagnosed to have endometriosis at the age of thirteen years. She had endometrial cyst and umbilical nodule containing endometrial tissue and were removed in 2008 and 2015 respectively. She used to get right shoulder pain during menstruation. She was on treatment for infertility. Clinical examination showed features of right sided pleural effusion and 250 ml of dark coloured fluid was aspirated (figure 3 & 4).

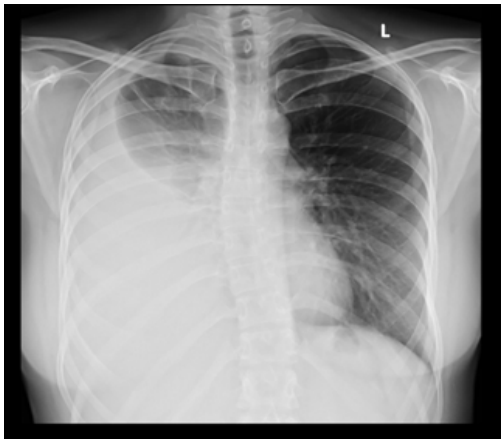

Fig 3: Right sided pleural effusion

Decortication was done by thoracic surgeon and part of the pleural tissue showed suspicious endometrial tissue.

Case report 4

A 30-year-old lady presented with right sided chest pain of two weeks duration. She also complained of an inguinal swelling which became

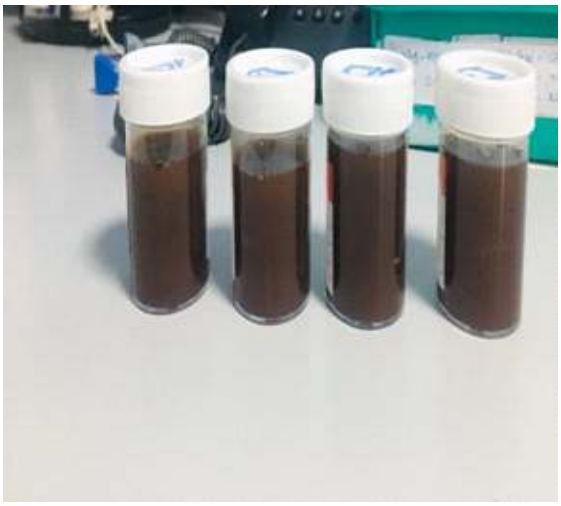

Fig 4- Dark coloured pleural fluid aspirated

painful during every menstrual cycle and surgery was planned elsewhere for the same. Clinically and radiologically, she had right sided hydropneumothorax at presentation here, for which Intercostal tube drainage was done. The fluid which was initially sero-sanguineous changed to deep red colour during hospital stay when she had her menses. She was not willing for decortication. Once lung expanded, intercostal tube was removed. Later she underwent excision of her inguinal swelling elsewhere, and biopsy confirmed endometriosis.

Discussion

The presence of endometrial-like glands and stroma outside the uterine cavity is called endometriosis. Thoracic involvement is the most frequent extra-pelvic location of endometriosis[1].

Thoracic endometriosis syndrome (TES) has been used when one or more clinical manifestations of thoracic involvement is present in association with menstruation, but without histologic confirmation. It consists of four distinct clinical entities: catamenial pneumothorax (70%), catamenial hemothorax (14%), (when the ectopic endometrial tissue is located on the pleural surface), catamenial hemoptysis (7%) (when it is located in the tracheobronchial tree), and pulmonary nodule (6%) (when it is located in the pulmonary parenchyma) and isolated catamenial chest pain, and catamenial pneumomediastinum[2].

Endometriosis of the lung parenchyma was first characterized by Schwarz in 1938. The pathogenesis of TES is unclear even till date and the mechanisms suggested are as follows: tissue migration through pelvic vessels and the reflux of endometrial tissue via fallopian tubes into peritoneal cavity, then leading into thoracic cavity through diaphragmatic fenestrations/ defects. The most commonly described sites of lesions are the thoracic diaphragm and visceral diaphragm (38.8% and 29.6%, respectively), with the parenchyma being less commonly involved[3].

Patients usually have catamenial symptoms, which start in the first 24 to 48 hours from onset of menses. The common symptoms of the patient are chest pain (90%), followed by dyspnea (73%), hemoptysis (7%), and cough (rare)[4].

Catamenial hemothorax is the recurrent pleural effusion in relation to menses due to the presence of endometrial tissue in the pleura. The pleural fluid appears bloody and is usually acellular. There is a report showing increased value of ADA in pleural fluid[5]. But none of the Indian reports mention about such a finding. Level of CA-125 may be elevated in the serum and body cavity fluid of these patients[6]. There have been reports of catamenial

hemothorax associated with hemo- pericardium. Haemothorax may also present bilaterally[7].

The diagnosis is often delayed or missed by clinicians, which can result in recurrent hospitalizations and other complications. Thoracic endometriosis syndrome is typically a diagnosis that is made clinically and, although histopathologic confirmation is preferred, it is not always necessary. The cases presented here were also missed initially as the fluid accumulation was slow, and the connection with periods was not appreciated by the concerned physicians. This has been the reason for delayed diagnosis all over the world according to literature.

The treatment is by removal of the endometrial tissue by decortication as done in the first case presented here. Video Assisted Thoracoscopic Surgery (VATS) may be used both for the diagnosis and for curative excision of pleural/diaphragmatic endometrial tissue. The options for medical therapy include oral contraceptives, pregestational agents and gonadotropin-releasing hormone analogs. In women who desire to preserve fertility, it is often better to perform an operation to remove the offending parenchymal endometrial tissue rather than long-term hormone therapy as it may suppress normal menses and alter secondary sexual characteristics[8].

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Eosinophilic pleural effusion – key to the Pandora's box!

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Abstract

Background :The aetiology of pleural effusion remains uncertain in nearly 20% of cases.Pleural fluid with eosinophil values greater than 10% is seen in around 10% of effusions.Pulmonary thromboembolism may rarely present as pleural effusion also. Anticoagulation, primary thrombolysis, surgical embolectomy, or percutaneous intervention is all dictated by patient's hemodynamic status.

Case presentation: We present a case of a 43 year old man, with eosinophilic pleural effusion. After investigations, including plain computed tomography chest, no definite cause could be found. He progressed to have a myeloproliferative disorder and was referred for recurrent pleural effusion. CT Pulmonary angiogram showed bilateral pulmonary embolism with right lower lobe pulmonary infarct. Pleural effusion cleared on treatment of embolism with anticoagulation.

Discussion :The incidence of eosinophilic pleural effusions is approximately between 5% and 16% of all pleural effusions.Pleural effusions secondary to pulmonaryembolism are maximal by the third to fourth day. We suggest that a CT pulmonary angiogram is worth considering in all cases of breathlessness with eosinophilic pleural effusions especially in hyper-coagulable states.

Key words: Pulmonary thromboembolism , eosinophilic pleural effusion

Introduction

A pleural effusion may be related to disorders of the lung, pleura, or to a systemic disorder. It can pose a diagnostic dilemma to the treating physician. However, the aetiology of pleural effusion remains uncertain in nearly 20% of cases[1]. Ninety per cent cases of pleural effusion in the western countries are reported to result from mostly five diseases i.e. congestive heart failure, pneumonia, malignancy, pulmonary embolism and viral infections[2]. The aetiology in few cases becomes evident only on subsequent follow up. In India, unlike the western countries, tuberculous pleural effusion is fairly common[3].

Pleural fluid with eosinophils values greater than 10% is seen in around 10% of effusions. The presence of eosinophilia makes tu-

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berculous aetiology unlikely and also reduces possibility of progression of para pneumonic effusion to an empyema[4].

One of the described causes of eosinophilic pleural effusion is pulmonary thromboembolism, which may present as dyspnoea, chest pain, haemoptysis, syncope, pleural effusion, hypotension or shock. Anticoagulation, primary thrombolysis, surgical embolectomy, or percutaneous intervention is all dictated by patient's hemodynamic status and comorbidities[5].

In summary, pleural fluid analysis, in combination with a focussed history, complete physical examination, and appropriate imaging, allows us to make a confident diagnosis in majority of patients[2].

Case report:

We present a case of a 43 year old gentleman, who came to our OPD with breathlessness. A chest radiograph done as part of initial workup of breathlessness showed left sided pleural effusion (Fig.1)

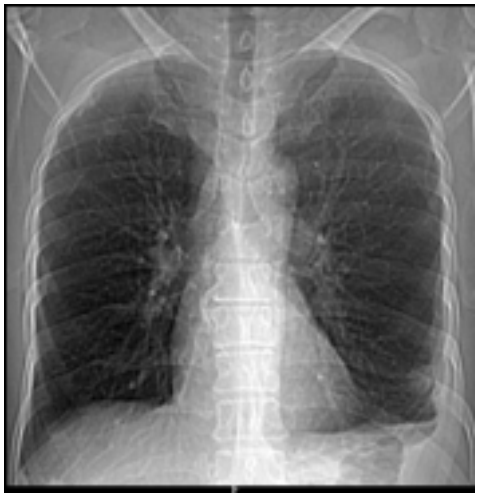

Fig.1. Chest X-ray showing blunting of left costophrenic angle.

Upon further evaluation, we found that blood counts showed peripheral eosinophilia (11%). Ultrasound guided pleural tapping was done, which revealed ~ 20 % eosinophils. Rest of the pleural fluid analysis showed exudative effusion with low adenosine deaminase (ADA). Plain CT chest revealed only a left moderate pleural effusion. (Fig 2)

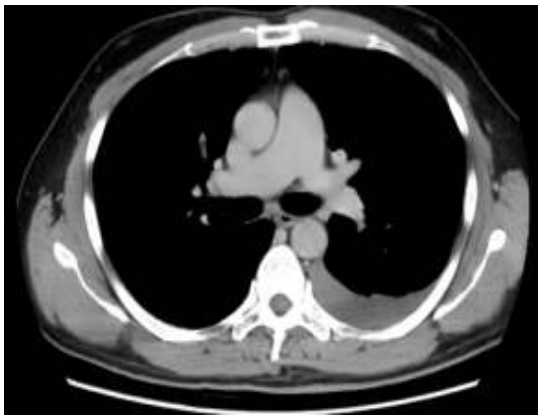

Fig.2. Computed tomograph Chest confirming left pleural effusion.

To find the cause of eosinophilic effusion, an autoimmune profile consisting of Antinuclear antibody (ANA) profile and anti-neutrophil cytoplasmic antibody (ANCA) profiles were sent, which were all negative. Ultrasound abdomen was unremarkable. Relevant drug history was ruled out. A presumptive diagnosis of tropical pulmonary eosinophilia was made and was started on diethyl carbazine at recommended doses was given for 21 days. The patient was then lost to follow up. Few months later he came back as a referral from the haematologist; when he was diagnosed to have myeloproliferative disorder with pleural effusion.

In view of breathlessness not subsiding and persistent hypoxia in the background of my-

eloproliferative disorder and a Wells score of 5.5 (Moderate risk); a CT Pulmonary angiogram was done (Fig 3 & 4). It showed pulmonary thromboembolism with peripheral wedge-shaped opacities in the posterior segment of left lower lobe and anterior and posterior segments of right upper lobe - likely to be a pulmonary infarct. Pulmonary arterial hypertension with right-sided cardiac strain and left pleural effusion was present. Rest of lung parenchyma was grossly normal. There was no evidence of deep venous thrombosis on lower limb colour doppler.

Patient was managed with adequate anticoagulation, hydration and supportive therapy. The patient eventually improved and was discharged on lifelong anticoagulation.

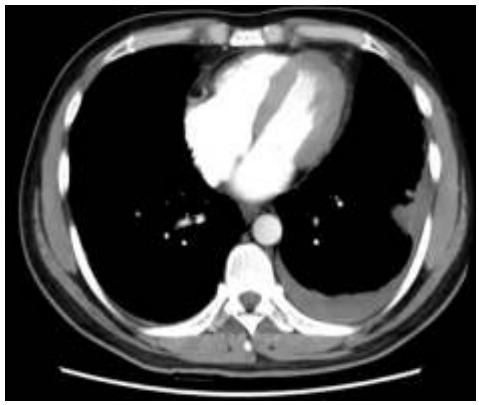

Fig. 3. Second computed tomograph chest showing possible left lower lobe pulmonary infarct.

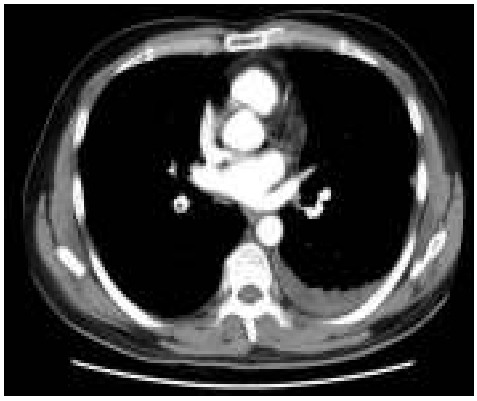

Fig.4. CT Pulmonary angiogram showing right lower lobe pulmonary artery "doughnut" sign (filling defect) suggestive of pulmonary infarct. Along with left pleural effusion and paucity of contrast on left side too.

Discussion:

Eosinophilic pleural effusion (EPE) is a pleural effusion that contains at least 10% of eosinophils. The incidence of EPE is approximately between 5% and 16% of all pleural effusions. Since pleural fluid eosinophilia is uncommon, our knowledge concerning EPE is based on infrequent case reports. In probably one of the most extensive studies on eosinophilic pleural effusions, the most common condition associated with pleural fluid eosinophilia was malignancy (34.8%) followed by infections (19.3%) ,chest trauma (8.9%) and rest due to various surgical procedures. In around 14%, the aetiology remained obscure despite extensive evaluation[6].

Few studies suggest that pleural effusion caused by pulmonary embolism is associated with more severe disease. High NT-pro BNP levels along with right ventricular dilation were seen in the effusion group with central embolism[7]. The exact pathogenesis of pleural effusions associated with embolism remain unknown. However, two mechanisms that can be postulated are raised capillary pressure in the parietal pleura and increased pulmonary vascular permeability[8]. There are pulmonary opacities suggesting pulmonary infarction, in up to 61% of the patients[9].

In one series, pulmonary embolism was

the fourth leading cause of pleural effusion[10]. It is theoretically possible that many cases of undiagnosed pleural effusion may well be due to pulmonary embolism because the diagnosis is not suspected[11]. Pleural effusions secondary to PE are maximal by the third to fourth day and progression of effusions after this period generally indicate recurrent embolism or empyema. Pleural effusions caused by PE usually occupy less than one-third of the hemithorax although occasionally there may be a large amount of fluid. In one study, the mean size was about 15% of the hemithorax[12]. However, in the PIOPED study, approximately 86% of the pleural effusions manifested only by the blunting of costophrenic angle[13].

Myeloproliferative disorders are hypercoagulable states and can lead to pulmonary embolism. Formation of intra-pleural membranes is thought to be the reason for loculation. It is important to recognise loculations as a manifestation of PE, as in one study there was a delay of more than two weeks between the time the patient first became symptomatic and the diagnosis of PE. Interestingly, the multiple collections of loculated pleural fluid responded to systemic anticoagulation therapy[14].

In our case pleural fluid eosinophilia was related to the myeloproliferative disorder (hypercoaguable state) which made us to consider a CT pulmonary angiogram which clinched the diagnosis of pulmonary thromboembolism as a cause of pleural effusion.

Lessons learnt & conclusion

This case highlights the need for in-depth and follow up evaluation of all cases of pleural effusion, beyond the narrow perspective of just ruling out tuberculosis in our country. This is more so in cases of eosinophilic effusions. We suggest that a CT pulmonary angiogram is worth considering in all cases of breathlessness with eosinophilic pleural effusions.

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Risk factors for respiratory distress among preterm infants in a backward district of Kerala - a retrospective study

Ravindran Chetambath[1],Charitha Puvvada[2],Manoj Narayan K A[3]

We would like to bring to the attention of the readers that, lung diseases in adults, especially COPD is etiologically linked to lung growth in fetal life and lung health in early childhood. So it becomes important that we identify the risk factors leading to respiratory morbidity in neonatal period which will allow avenues for optimal corrective measures. Here we present our observations from a retrospective analysis of various maternal and fetal risk factors for respiratory distress in preterm infants.

Introduction:

Preterm infants are at a relatively higher risk of morbidity and mortality when compared to term babies especially if gestational age at birth is below 34 weeks. About 6.7% of new born infants are affected by respiratory distress[1]. Preterm babies had the highest incidence of respiratory distress (30%)[1]. This could be due to hyaline membrane disease, meconium aspiration syndrome, birth asphyxia, pneumothorax, respiratory infections, diaphragmatic hernia and transient tachypnea of new born[2]. Infants born before 28 weeks of gestation, if having multiple congenital anomalies also are at a higher risk for respiratory distress and neonatal asphyxia. It is essential to identify the maternal and fetal risk factors leading to respiratory distress so that appropriate corrective measures can be implemented during antenatal period. This is especially important in a backward district like Wayanad of Kerala state where 18% of its population is tribes with poor access to health care.

Objective:

To study the various fetal and maternal risk factors leading to respiratory distress among preterm babies born below 34 weeks of gestation.

Study methodology:

This is a retrospective study of a cohort of preterm infants born below 34 weeks of gestation and admitted in a neonatal intensive care unit (NICU)of a tertiary care hospital for a period of one year from 1st January 2017 to 31st December 2017. All preterm babies admitted during this period are included and their details are collected from electronic hospital records. Variables are entered in Microsoft excel and significance is established using Chi square test. A p value below 0.05 is considered significant. This study is approved by institutional ethics committee.

Results:

During the study period 124 infants born below 34 weeks of gestation were admitted in the NICU. These 124 babies where included in this

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retrospective cohort study. Among these, 107 preterm infants (86.29 %) were born in this hospital and 17 (13.71%) were born in peripheral hospitals and referred for neonatal care. Thirteen infants (10.6%) were born between 24-28 weeks of gestation (extreme preterm), 19 (14.9%) were between 29-31 weeks of gestation (very preterm) and 92 (74.5%) were between 32-34 weeks of gestation (moderate preterm). Mean birth weight increased with each increasing gestational week, from 354 gm. at 24 weeks to 2450 gm. at 34 weeks. 71 infants in the cohort were males (57.25%) and 53 (42.74%) were females. The overall incidence of respiratory distress was 34% among this cohort. 64% of the infants less than 28 weeks, 44% of the infants between 29-31 weeks and 27% of the infants between 32-34 weeks developed respiratory distress.

The maternal risk factors (Fig-1) identified were anaemia (64%), pregnancy induced hypertension (PIH -26%), sepsis (21%), delivery by Caesarian section (CS-19%), gestational diabetes mellitus (GDM-14%) and antepartum hemorrhage (APH-7%).

Fetal causes for respiratory distress identified in this cohort were gestational age, birth weight and place of birth. 64% of the infants less than 28 weeks, 44% of the infants between 29-31 weeks and 27% of the infants between 32-34 weeks developed respiratory distress. 55% of the infants with birth weight less than 1000 gm., 45% of the infants with birth weight 1101- 1500 gm., 24 % of the infants with birth weight 1501-2500 gm. had developed respiratory distress. 9 out born preterm infants (52.9%) and 33 inborn preterm infants (30.8%) developed respiratory distress (Fig-2). Other risk factors identified were pneumonia, cardiac defects, neurological diseases and metabolic diseases which were not statistically correlated.

Discussion:

In this study 34% of premature infants developed respiratory distress (RD). RD was reported in approximately 6.7 percent of infants in ear-

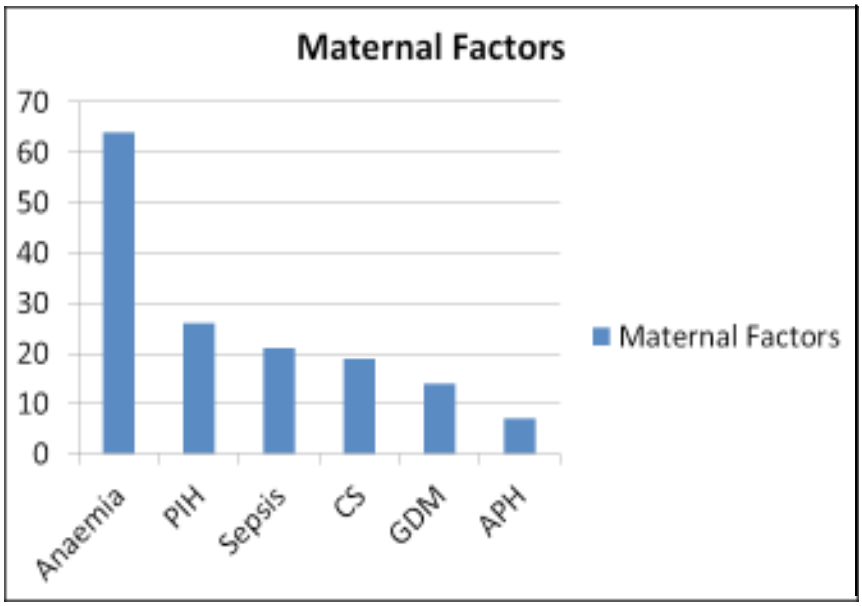

Fig-1: Bar diagram showing various maternal factors identified in respiratory distress

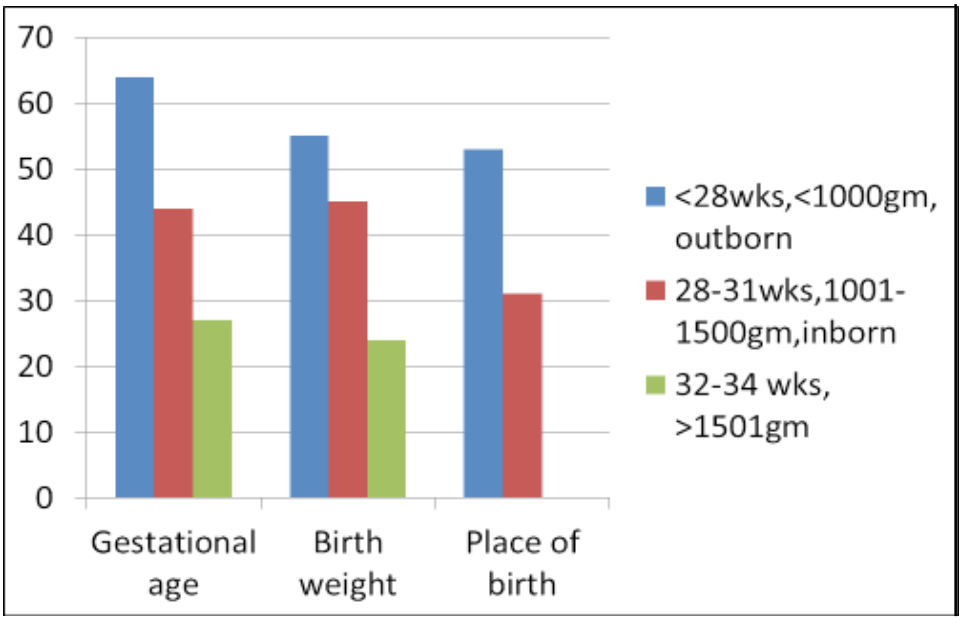

Fig-2: Bar diagram showing the significant fetal factors for the development of respiratory distress

lier studies[1]. Preterm babies had the highest incidence (30%) followed by post-term (20.9%) and term babies (4.2%)[1]. In another study it was reported that 15% of term infants and 29% of late preterm infants admitted to the neonatal intensive care unit developed significant respiratory morbidity[2].

Important maternal factors leading to prematurity are anaemia (64%), PIH (26%), sepsis (21%), Caesarian section (19%), GDM (14%) and antepartum hemorrhage (7%). Among these statistical correlation was established only with anaemia (P value-0.036), sepsis (P value-0.004), and CS (P value-0.047) (Table-1). Earlier studies reported that hypertension was the commonest maternal risk factor followed by oligohydramnios, twin pregnancy, GDM and infection[3].

Important fetal factors deciding respiratory distress were gestational age, birth weight and place of birth (Table-1)[4,5]. 64% of babies born below 28 weeks of gestation and 44% of babies born between 29-31 weeks of gestation developed respiratory distress (p-0.01). Respiratory distress is inversely proportional to birth weight. 55% of infants having birth weight of <1000 gm developed respiratory distress, whereas this was 45% between 1001-15000 gm and 24% between 1501-2500 gm (p-0.02). Apart from gestational age and birth weight, one important factor correlated with respiratory distress is out born babies. 52.9% of out born babies (n=17) presented with respiratory distress. In the inborn infants respiratory distress developed only in 30.8% (n=107) of premature infants (p-0.02). Preterm compared to their term counterparts and low birth weight babies have been observed to be more likely to develop respiratory distress[6].

Conclusion:

This retrospective analysis of respiratory distress among preterm infants in a backward dis-

Table-1: Statistically significant risk factors for respiratory distress.

Variable against respiratory distress	P Value
Out born infants	0.0264
Gestational age	0.0141
Birth weight	0.0218
Maternal anaemia	0.0368
Maternal sepsis	0.0046
Caesarian Section	0.0475

trict of Kerala identified significant maternal and fetal factors which are correctable with optimal antenatal care. The results from the present study may contribute towards early identification of neonates at increased risk of respiratory disease. Identification of risk factors for respiratory distress allows for planning of approaches, both during antenatal and immediate postnatal period.

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