
6 Deep Learning to Classify Pulmonary Infectious Diseases

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6.1 INTRODUCTION

Pulmonary infectious diseases, such as COVID-19 and tuberculosis (TB), are a significant cause of morbidity and mortality worldwide. Timely and accurate diagnosis is critical to ensure appropriate treatment and prevent disease transmission. Deep learning, a subset of machine learning, has shown promise in various medical

applications, including image analysis and classification. In recent years, there has been increasing interest in using deep learning techniques to classify pulmonary infectious diseases using chest radiographs (CRX) or computed tomography (CT) scans. By training deep learning algorithms on large datasets of annotated medical images, these models can learn to accurately classify different types of pulmonary infections, allowing for more efficient and accurate diagnosis. This approach has the potential to improve patient outcomes, reduce healthcare care costs, and improve public health efforts by facilitating early detection and treatment of lung infections.

Deep learning models can be trained on large data sets of annotated medical images, allowing them to identify patterns and features that are characteristic of different types of lung infections. For example, a deep-learning model can learn to recognize the presence of infiltrates, nodules, or cavities in CRX or CT scans that are indicative of specific types of pulmonary infections. Furthermore, deep learning models can continuously learn and improve their diagnostic accuracy over time as they are exposed to more data. This means that as more medical images are available for training, deep-learning models can become even more accurate in their diagnosis of pulmonary infections.

6.2 COUGH MONITORING AND ANALYSIS

Coughing is considered a defense mechanism by which the body expels secretions or any blockage that restricts the passage of air in the upper airways. The causes of coughing can be environmental (dust, smoke), bacteria, viruses, or some chronic or acute health condition [412]. In several respiratory diseases, cough is one of the main symptoms, and depending on the type of cough, it is possible to obtain a clinical diagnosis. The cough has different characteristics that can give information about the severity of a certain disease, including disease identification. Among the main attributes of cough are intensity, frequency, duration, and pattern [489]. In patients with TB, for example, the pattern of coughing depends on the amount of *M. tuberculosis* present in the lungs [733]. Also, it has been possible to diagnose COVID-19 in asymptomatic patients using Artificial Intelligence (AI) on the record of a forced cough [327].

There are some criteria to classify cough. According to the resistance, which indicates the duration of the cough, it is classified as acute (less than 3 weeks), subacute (3-8 weeks), and chronic (more than 8 weeks). According to its sound, which is due to the sudden expulsion of air, the cough is classified as dry or wet. In patients with TB and chronic obstructive pulmonary disease (COPD), the majority experience a chronic, wet cough, while in patients with COVID-19, or with exacerbation of asthma, acute and dry cough predominates [262]. It can then be seen that the cough produces characteristic sounds that help identify some respiratory diseases.

The cough is compounded by two sounds and an intermediate stage between them. The first sound provides information about the peripheral airways at the level of the tracheal bifurcation, the second sound provides information about the larynx, and the intermediate zone reflects the processes in the trachea [313]. The duration and intensity of each of these stages will depend on the respiratory health condition of the subject, as shown in Figure 6.1.

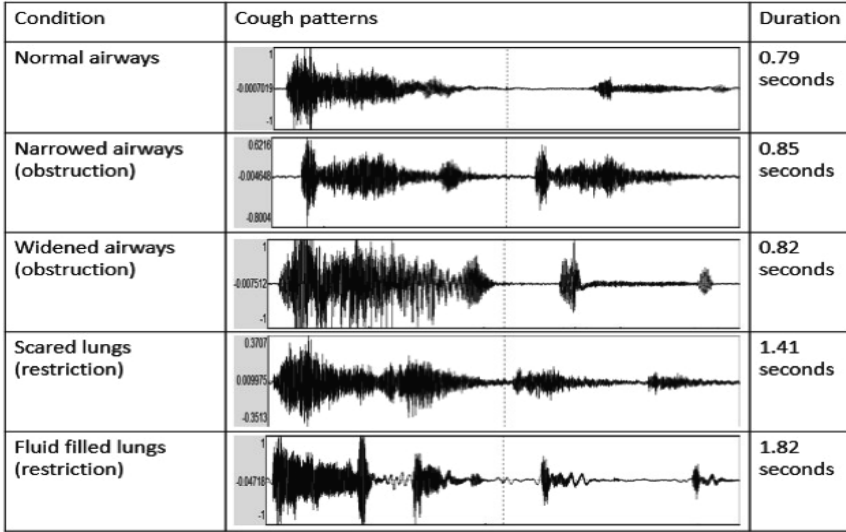


Figure 6.1 Pattern of cough sounds. Reprinted from (Rudraraju et al., 2020) with permission from Elsevier.

There are medical devices that detect cough in hospital environments, such as spirometers, and pneumotachographs, among others. However, these devices require special skills to operate and use procedures that are performed in controlled environments, giving short-term information, which makes it difficult to observe the evolution of the disease or the effectiveness of treatment. Carrying out continuous or periodic measurements on an outpatient basis, and in real-time, would allow coughing to be recorded for prolonged periods, helping to assess the progress of the disease. In this sense, there are ambulatory cough monitoring systems that allow 24-h records to be obtained. Among them are the Leicester monitor [408] and the Cayetano monitor [490]. These devices have a microphone and an audio recorder, which stores the data of the cough sounds so that they can be analyzed later. In the case of the Leicester monitor, the data is stored in .mpeg format, while in the Cayetano monitor the data is stored in .mp3 format.

Some proposals detect cough in non-hospital conditions for longer than 24 hours using the wearable paradigm. Because coughing causes a turbulent flow of air in the airways, the vibrations that this flow produces in the upper airways can be measured. In this sense, some proposals use piezoelectric or piezoresistive flexible films, which are attached to the subject’s throat and thorax to measure the vibrations produced by coughing [31, 373]. Although it is a proposal allowing real-time measurements, its long-term use can cause discomfort in the subject because an object must be attached to the skin at the throat or thorax level. There are proposals that detect the sounds of coughing more comfortably. One of the best-known systems is the LifeShirtTM (Vivometrics) [577], which is a jacket with multiple sensors that allow the detection of several cardiac and respiratory parameters. This system can detect cough sounds,

thanks to the addition of a microphone placed near the throat. LifeShirtTM has been used to detect cough in COPD outpatients for 24 hours with a sensitivity and specificity of 78.1% and 99.6%, respectively [133]. Other simpler approaches use microphones incorporated into wearable electronic stethoscopes [373] or use microphones that are integrated into smartphones [641]. Smartphones have been used to detect biomarkers related to COVID-19, and lower respiratory tract infections [65]. Also, to discriminate TB coughs from COVID-19 and healthy coughs [464]. However, the drawback of using microphones is that cough sounds are superimposed on environmental sounds, which often require complex algorithms to reliably extract cough characteristics.

From the signal point of view, cough sounds have frequency components that can vary according to the state of health of the respiratory tract. In healthy subjects, cough sounds range from 300 Hz to 500 Hz; however, these frequencies may increase up to 1200 Hz in subjects with bronchitis [313]. Cough sounds can be analyzed in the time domain using their amplitude-time characteristics, or in the frequency domain. The spectrogram of cough sounds has also been used to extract their characteristics using AI algorithms [733].

There are several characteristics of cough sounds that provide information about various respiratory diseases, but these characteristics are not audible to the human ear. In that sense, various AI-based algorithms have been developed that analyze these characteristics. Among the most widely used spectral characteristics are the Mel-Frequency Cepstral Coefficients (MFCC) [28, 69, 116, 412], Mel-Scaled Spectrogram, Tonal Centroid, Chromagram, Spectral Contrast [116], and Log Spectral Energies [69]. Regarding the classifiers, there are different points of view about their performance. In TB patients, the rapid increase in signal energy has been used to be able to differentiate the cough from the voice signal [620]. The algorithm detected cough and non-cough events using classifiers based on machine learning (ML) algorithms. Multilayer perceptrons (MLP), machine support vectors (SVM), and minimum sequential optimization (SMO) were compared. They chose SMO for its simplicity, obtaining a sensitivity of 81% and false positives of 3.3/hour. The algorithm was able to detect a reduction in cough events in 28 patients with drug-sensitive TB. Some works have compared the Log Spectral Energies with MFCC in short-term recordings of cough in subjects with TB, to then apply classifiers through statistical models using linear regression, Hidden Markov Models (HMM), and Decision Trees [69]. In this study, cough could be distinguished between TB and healthy subjects with an accuracy of 80% and a specificity of 95%. MFCCs discard information that is useful for classifying sounds, so their accuracy was only 63% and their specificity was 80%. Linear regression has been used as a classifier to differentiate cough from non-cough in various respiratory diseases, including COVID-19 and TB, and to differentiate the cough from other sounds present in the environment. Artificial neural networks (ANN) and Random Forests (RF) have been also used as classifiers in cough sounds detected by spirometry [28]. In patients with COVID-19, it has been possible to diagnose the disease from the sounds of coughing in symptomatic and asymptomatic subjects. Chowdhury et al. (2022) used different ML classifiers to

distinguish patients with COVID-19. The classifiers based on Extra-Trees, HGBost, and RF showed the best performance, obtaining accuracies of the order of 87%

6.3 ELECTROCARDIOGRAM MONITORING AND ANALYSIS

The electrocardiogram (ECG) is the representation of the bioelectric potentials of cardiac cells. It is composed of several waves (P, Q, R, S, T) that describe the depolarization (electrical activity before contraction) and repolarization (recovery after depolarization) of the atria and ventricles. In summary, the P-wave represents the depolarization of the atria, the QRS complex represents the depolarization of the ventricles and repolarization of the atria, and the T-wave represents the repolarization of the ventricles [116]. In addition, some intervals also provide information about the bioelectrical functioning of the heart. These intervals represent the time that elapses between two ECG waves. Said intervals are, RR, PQ, and QT. The RR interval provides information about the heart rate since it is the time that elapses between one beat and another. The PQ interval represents the time between the onset of atrial depolarization and the onset of ventricular depolarization. The QT interval is the time it takes for the ventricle to start to contract and to finish relaxing and is measured from the beginning of the Q wave to the end of the T wave [116]. The amplitude and duration of each of these waves, and the duration of the intervals provide information about the heart condition of a subject, therefore, any alteration in the ECG is an indication of a cardiac problem.

ECG detection for diagnostic purposes is carried out using 12 leads that are widely known in the literature [666]. These leads are I, II, III, aVR, aVL, aVF, and V1-V6. To do this, 10 electrodes are attached to the surface of the skin at different locations on the body. The 12-lead ECG is measured in clinical settings and is performed by qualified personnel; however, under these conditions, patterns or abnormalities in the ECG that can appear during daily activities are often not detected. In this sense, there are Holter systems that record the ECG continuously for 24–48 hours on an outpatient basis. However, they also require various electrodes that cause discomfort and need a specialist to place them on the subject. The importance of ECG detection during activities of daily living has driven the development of portable and wearable systems that detect long-term ECG comfortably and simply, and without the need for specific knowledge to use them. There are systems based on smartwatches [199], which detect lead I when used in the standard way, however, it has also been possible to detect leads II and V2 using a different configuration [580]. There are also systems based on mobile applications, such as the KardiaMobile® (ALIVECOR), which can obtain 6-lead medical grade records of the ECG (<https://www.kardia.com/>). Other wearable technologies, but less conventional, are embedded in objects such as glasses, bands, and patches [199, 540]. Figure 6.2 shows various wearable and portable systems capable of detecting multiparameter, including ECG in daily life.

There is evidence that some respiratory diseases produce ECG abnormalities. In subjects with COPD, the most common abnormalities are a rightward P wave axis ($\geq 70^\circ$) and a rightward QRS axis ($\geq 90^\circ$), as well as transient atrial and ventricular arrhythmias [516]. The presence of P pulmonale (a peaked P wave in lead II)

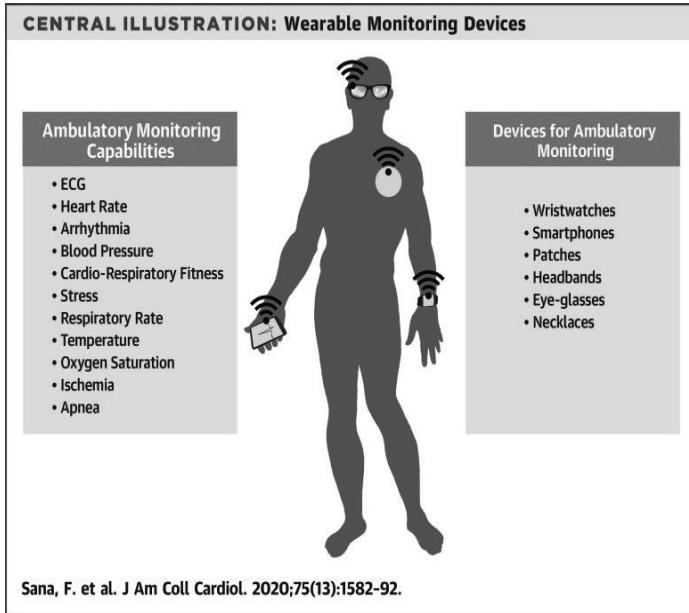


Figure 6.2 Depiction of wearable and smartphone-based solutions for continuous ambulatory cardiac monitoring, along with a summary of potential use cases. Reprinted from Sana et al. (2020) with permission from Elsevier.

is indicative of the severity of COPD even without the need for spirometry. COPD does not cause changes in the duration of the P wave, nor in the amplitude of the QRS complex [16]. On the other hand, there is evidence that P wave dispersion is a good predictor of COPD exacerbations. In the case of subjects with TB, although the disease itself does not cause significant changes in the electrocardiogram, certain drugs used for the treatment of TB cause a prolongation of the corrected QT interval (QTc) [227]. The QTc is used since the QT interval changes with the heart rate, so, this effect must be corrected. For this, several formulae have been proposed, namely, Bazett, Fridericia, Framingham, Hodges, and Rautaharju [636]. A prolonged QTc (450 ms in men, 460 ms in women) is an indicator of risk for Torsade de Pointe [202], which is a rare ventricular tachycardia and is also a potential indicator of sudden death. Therefore, long-term ECG monitoring is considered good practice in subjects receiving treatment for multidrug-resistant tuberculosis. Some ECG abnormalities have also been found in subjects with COVID-19. These include T-wave inversions [373,410] and sinus tachycardia [379]. Prolongation of the QTc interval has also been found, but it is mainly caused by the different pharmacological treatments [410]. Some studies reveal alterations in the ST segment in subjects with COVID-19, specifically an elevation of this segment, which is due to coronary artery disease or heart muscle disease (myocarditis) [373]. ST segment deviations are also considered a predictive indicator of mortality risk in subjects with COVID-19 [330].

From all the ECG alterations caused by respiratory diseases, one of the most studied is the prolongation of the QTc interval due to the implications it has on the subject's life. Accurately estimating the QTc is not easy since it is necessary to identify the point where the end of the T-wave coincides with the isoelectric line. In situations where there are motion artifacts, this can be a difficult task. Commonly, the QTc is measured manually by a trained personal using printed or digital ECG recordings. QTc should be measured using leads II, V5, or V6, and should be estimated from the average of at least 3 beats [206]. Artificial intelligence for the QTc from the ECG recorded using the KardiaMobile® device (ALIVECOR) has been proposed. Using deep neural networks (DNN), it was possible to estimate the QTc with a specificity of 94.4%, making it an effective method to estimate congenital or acquired prolongation of the QTc [202]. The algorithm was trained, tested, and validated on more than 1.6 million 12-lead ECG records from 538,200 subjects. These types of tools can be helpful for subjects with TB and COVID-19 who are under drug treatment. There are other proposals that use the single-lead ECG, which is the one normally detected by smartwatches. Maille et al. (2021) used a deep convolutional neural network, composed of 11 U-net architectures with 11 convolutional layers and six residual blocks. The results showed a great similarity with the QTc measured manually in ECG recordings in leads II, V5 and V6, where a difference of 50 ms was obtained in 98.4% of the 85 patients who participated in the study.

There is a wide variety of artificial intelligence algorithms that have been used to detect abnormalities in the ECG recordings, especially arrhythmias. According to Saini and Gupta, the most popular are ANN, Fuzzy Systems, Neuro-fuzzy Systems, Genetic-Fuzzy Systems, Probabilistic Neural Networks (PNN), CNN, Support Vector Machines (SVMs), and Linear Discriminants (DNs) [537]. However, the most used in the extraction and classification of ECG characteristics are the CNNs due to their self-learning capacity.

Among the characteristics of ECG recordings, the most studied are the following: time-domain, frequency-domain, time-frequency domain, statistics features, and non-linear features. Commonly, these features are extracted from the raw ECG data, however, the generated ECG image has been used instead of the raw data to apply neural networks. The advantage of using images of ECG traces is that they can be captured by smartphones. Using heat maps helps the algorithm to focus on a certain area of the image to make decisions, which helps the clinician to find abnormalities in the ECG that may be indicative of COVID, TB, and other cardiac diseases [223,500]. Figure 6.3 shows the image of an abnormal ECG trace of a subject with COVID-19, where those areas where the abnormalities are highlighted, and that is where the algorithm should focus on making decisions.

6.4 CHEST RADIOGRAPHY AND DEEP LEARNING

There are numerous diagnostic methods for TB, each with advantages and limitations. Chest radiography (CXR) is one of the most used diagnostic tools to detect pulmonary TB [443]. We will discuss the benefits and disadvantages of using CXR for pulmonary TB diagnosis compared to other diagnostic techniques and how the combination of CXR with AI addresses its limitations.



Figure 6.3 Score-CAM visualization of abnormal (COVID-19) ECG. Taken from Rahman et al. (2022) with the permission of Springer Nature BV.

CXR is a relatively inexpensive, non-invasive, and widely available diagnostic tool that can provide valuable information on the presence and extent of TB-related lung abnormalities. It is beneficial for detecting characteristic lung parenchymal changes and the presence of cavitations indicative of active TB disease [322].

CXR has a reported sensitivity of 92% [72]; it can detect TB cases in early stages, even before characteristic symptoms occur, allowing to find individuals at high risk of developing the disease, such as close contact with patients with active disease or people from TB-endemic regions (2016). CXR can be used as a screening method. It can be applied in large groups to find individuals initially considered healthy, leading to rapid treatment and reduced disease transmission (2016). On the other hand, in recent years, there have been significant improvements in the portability of the equipment for taking CXR; currently, there is FDA-approved equipment with a weight of less than 4 kg, which makes them accessible to remote areas where there are no other diagnostic tests Table 6.1.

Although CXR is a useful diagnostic tool for pulmonary TB, it has some limitations that should be considered. CXR reports low levels of specificity; in practice, the CXR is examined and interpreted by a medical radiologist, so the process is subjective to the medical staff's experience. Different diseases show similar radiological patterns to pulmonary TB, leading to a high false-positive rate in people with other lung diseases or conditions, such as COPD or lung cancer [633]. One of the significant problems with CXR is the need for more trained radiologists available in low-resource areas, in conjunction with the absence of other diagnostic techniques, which play a significant role in the prevalence and spread of the disease [496].

Table 6.1
Characteristics of commercially available ultraportable X-ray equipment.

Manufacturer	Fujifilm	Delft
Model	FDR Xair	Light
Price(USD)	\$ 47,000	\$ 66,750
X-ray generator weight (kg)	3.5	7
Weight of complete system (kg)	29.4	33.2
Sockets per battery charge	100	200

AI has the potential to revolutionize medical care by improving diagnostic accuracy, efficiency, and access to care. In recent years, there has been growing interest in using AI to develop computer-aided diagnosis (CAD) systems to detect pulmonary TB [311]. CAD systems use machine learning algorithms to analyze medical images, such as chest X-rays or CT scans, and provide diagnostic results automatically [311].

AI-based CAD systems have several potential benefits for TB diagnosis. First, they can provide accurate and consistent diagnoses without a radiologist or trained medical professional, which is very helpful in resource-limited settings where access to trained medical professionals may be limited [493]. Second, AI-based CAD systems can improve diagnostic efficiency by reducing the time required for image interpretation and diagnosis, increasing the throughput of diagnostic services, reducing waiting times, and improving patient outcomes. Third, AI-based CAD systems can help reduce diagnostic errors and improve the accuracy of TB diagnosis, leading to better patient outcomes [493]. On the other hand, AI-based CAD systems have the potential to be used for active screening of people at high risk for TB, identifying early TB cases, leading to prompt treatment and reduced disease transmission [266]. AI-based CAD systems can also monitor disease progression and response to treatment, providing valuable information for patient management and care [321].

One of the main problems of commercially available CAD systems for pulmonary TB diagnosis is their high cost; an InferRead DR software license costs USD 5,552, including installation service and support, while a CAD4TB license costs USD 28,475, including installation service and support for three years [495]. The high costs of commercial systems make it difficult for them to be acquired and implemented in high-burden countries, so developing new CAD systems to support TB diagnosis is a viable option. An example is the MIA-TB RX UABC system [218], developed by the Autonomous University of Baja California in collaboration with the Tijuana Tuberculosis Clinic and Laboratory, which serves patients in northwestern Mexico. This CAD system was developed by implementing pre-trained convolutional neural networks using CXR radiographic images of patients collected over two years. It can classify radiographs into three categories: TB, normal, or other pathology. The system obtained a sensitivity value of 1.0 and an accuracy of 0.92 for pulmonary TB Table 6.2

Table 6.2**Characteristics of the software currently available on the market.**

Company	Delft Imaging Systems	Infervision	JLK	Lunit	Qure.ai	RadiSen
Country	The Netherlands	Beijing, China	Seoul, Republic of Korea	Seoul, Republic of Korea	Mumbai, India	Seoul, Republic of Korea
Product	CAD4TB	InferRead DR Chest	JLD-02K	Lunit IN-SIGHT CXR	qXR	AXIR
Version	7	1.0	1.0	3.1.0.0	3.0	1.1.2.2
Intended Age Group (years)	4+	16+	10+	6+	6+	16+
Chest X-ray image format input	DICOM, PNG, JPEG	DICOM, PNG, JPEG	DICOM, PNG, JPEG	DICOM	DICOM, PNG, JPEG	DICOM
Chest X-ray type input	PA	AP/PA	AP/PA	AP/PA	AP/PA	PA
Output	Abnormality score for TB, Heat map, Binary classification "TB" or "not TB"					
Product Development Method	Supervised deep learning (CNN, RNN) plus manual feature engineering	Supervised deep learning (CNN, RNN)	Supervised deep learning (CNN, DBNs)	Supervised deep learning (CNN)	Deep learning to analyse chest X-ray scans.	Supervised deep learning (CNN)

In conclusion, CXR is a useful diagnostic tool for pulmonary TB, particularly in resource-limited settings where other diagnostic techniques may not be available or affordable. CXR can provide valuable information on the presence and extent of TB-related lung abnormalities and can be used to detect TB in high-risk individuals. However, CXR has some limitations, such as the possibility of false-positive results when the interpreting physician confuses other pulmonary pathologies. AI-based CAD systems have the potential to revolutionize TB diagnosis by reducing the limitations of CXR alone, thereby providing accurate, efficient, and consistent diagnostic results.

6.5 NEXT-GENERATION SEQUENCING IN THE DIAGNOSIS OF INFECTIOUS LUNG DISEASES

Next-generation sequencing (NGS) is a high-throughput sequencing technique that has revolutionized the field of genomics and transcriptomics. This technology involves the fragmentation of DNA or RNA into small pieces that are simultaneously amplified and sequenced into thousands or even millions of fragments. The next-generation sequencing process generates a large amount of data that are processed by bioinformatics algorithms to reconstruct the complete genome or transcriptome [37, 567].

NGS has become a fundamental tool in the study of genetic diversity and evolution of microorganisms, allowing the identification of mutations and genetic variations associated with diseases and the elucidation of complex metabolic pathways and gene regulatory networks [512, 585].

The ability to sequence large amounts of genetic material in a short period of time is one of the main advantages of next-generation sequencing compared to traditional sequencing techniques. Furthermore, NGS has enabled an exponential increase in the identification of genetic causes in rare diseases and heterogeneous disorders. NGS is used in research and clinical settings, accelerating diagnosis, and reducing costs. Despite the enthusiasm, there are limitations in coverage and accuracy and challenges in the interpretation of variants and ethical issues. There is a need to define quality and control standards in NGS to further improve its application for the benefit of patients [375].

In the study of bacterial genomics, next-generation sequencing has allowed the characterization of the genetic diversity of *Mycobacterium tuberculosis* populations, which has facilitated the identification of strains associated with resistance to anti-tuberculosis drugs. In addition, NGS has improved the detection and diagnosis of tuberculosis by identifying mutations and genetic variations associated with disease, characterizing the genetic diversity of populations of microorganisms, and elucidating complex metabolic pathways and gene regulatory networks [458, 541, 649].

6.5.1 MOST WIDELY USED SEQUENCING TECHNOLOGIES

NGS technologies have become valuable tools in the diagnosis of infectious lung diseases. Bioinformatics processes in NGS significantly influence disease management and patient care. Lack of standardization leads to variability in bioinformatics procedures, generating inaccurate results that affect patient care. Therefore, in recent years, several guidelines have been proposed to homogenize processes and standardize knowledge of bioinformatics data management [307, 530]. Among the most widely used technologies are Illumina, Ion Torrent, PacBio, and Oxford Nanopore. All these technologies are used in the diagnosis of infectious lung diseases to identify respiratory pathogens, detect mutations associated with antibiotic resistance and characterize the genetic diversity of pathogens. The main features, advantages and disadvantages, and specific applications of each of these technologies are described in Table 6.3.

NGS technologies are valuable tools in the diagnosis of infectious lung diseases. Each of these technologies has its own advantages and disadvantages, and their specific application depends on the type of analysis required. They are constantly changing and being updated, so these descriptions of their advantages and disadvantages can quickly become obsolete. It is necessary to constantly update their performance and recommended implementations. The choice of the appropriate technology will depend on factors such as the complexity of the genome, the length of sequences required, and the available budget.

6.5.2 SEQUENCING DATA PROCESSING AND ANALYSIS

NGS data analysis is a complex process that requires the use of advanced bioinformatics tools for processing, alignment, and identification of genetic variants. The WHO recently published a technical guide for workflow in NGS of *Mycobacterium tuberculosis*. It is mainly structured in four steps DNA extraction and quality control, DNA library preparation, Sequencing, Data Analysis [458]. Since the first three steps vary depending on access to the sequencing platform, available budget, available sample quality, and the desired quality of the output files, we proceed with general comments on the process of analyzing the raw sequencing data. The analysis process begins with obtaining raw sequencing data, which undergo a series of steps before they can be analyzed.

The first step in sequencing data processing is the removal of low-quality sequences and filtering of adapter and contaminant sequences. This is done using quality control programs such as FastQC or Trim Galore, which can identify and remove low-quality sequences and filter out adapter and contaminant sequences [33, 319]. Once low-quality sequences have been removed and adapter and contaminant sequences have been filtered out, sequences are aligned to a genomic or transcriptomic reference using alignment programs such as Bowtie or BWA [144]. This allows the identification of genetic variants and characterization of the genetic diversity of pathogens.

There are several alignment programs available (Bowtie, BWA, HISAT2, TopHat, and other). Each of these programs has its own features and advantages, and the choice of the appropriate alignment program will depend on the type of sequencing data and the purpose of the analysis.

The next step is the identification of genetic variant using bioinformatics tools such as GATK, FreeBayes or SAMtools, or pipelines as MTBseq, which allow the identification of SNPs (Single Nucleotide Polymorphisms), indels and other types of genetic variants. These tools can also be used to identify mutations associated with antibiotic resistance. This program may differ in their ability to detect different types of variants, such as SNPs, indels, and other types of structural variants. These programs use different approaches for the identification of SNPs, such as the comparison of read sequences with a reference genome or the detection of base changes in the alignment of reads [138, 144, 308, 344, 409, 634].

Indels (insertions and deletions) are variants that involve the insertion or deletion of one or more nucleotides in a DNA sequence. The identification of indels can be more difficult than the identification of SNPs, due to the greater complexity of the

Table 6.3
Next-Generation Sequencing technologies

Platform	Key features	Advantages	Disadvantages	Ref.
Illumina	Sequencing technology based on DNA strand synthesis. It uses DNA cluster amplification to generate large amounts of short, high quality DNA sequences. Sequencing products are read simultaneously on millions of DNA fragments using a fluorescent camera system.	Is a high-throughput sequencing technology with high accuracy and reproducibility. It can generate large amounts of data in a short period of time at relatively low cost. The read length is suitable for genetic variant analysis and mutation detection in pathogens.	The read length is limited and does not allow sequencing of longer genomic regions. In addition, there may be problems in sequence alignment due to the high homology between some genomic regions.	[426]
Ion GeneStudio S5 System and the Ion Torrent Genexus System.	A sequencing technology based on the detection of protons released during DNA strand synthesis. It uses DNA cluster amplification to generate high-quality short DNA sequences. Sequencing products are read sequentially using a pH sensor system.	Fast, accurate and easy-to-use sequencing technology. It allows sequencing of short DNA fragments and can detect genetic variants and mutations in pathogens.	Sequencing quality may be affected by the presence of homopolymers, and the number of read errors may increase in repetitive regions.	[328]
PacBio	Sequencing technology based on the detection of fluorescent light generated during DNA strand synthesis. It uses real-time DNA amplification to generate long DNA sequences with high quality.	High-quality sequencing technology that can generate long DNA sequences with high quality, allowing the resolution of complex genomic regions and the identification of genetic variants. It is also capable of detecting epigenetic modifications.	Expensive sequencing technology that requires a large amount of data to generate reliable results. In addition, the sequencing error rate can be high in regions with high homology.	[4, 497]
Oxford Nanopore	Sequencing technology based on the detection of electrical currents generated by the interaction between DNA and pores in a membrane. It uses nanopores to directly sequence DNA molecules without amplification	Portable, real-time sequencing technology that can generate long DNA sequences. It allows the detection of epigenetic modifications and the identification of genetic variants in real time.	Sequencing error rate can be high, especially in repetitive or high-homology regions. In addition, sequencing quality can be affected by the presence of contaminants and genome complexity.	[385]

regions containing indels and the variability in their size. Programs such as GATK and VarScan are commonly used for the identification of indels from sequencing data. These programs use different methods for indel identification, such as comparison of read sequences with a reference genome, identification of misaligned read

positions or detection of base read imbalances [306,409].

In addition to SNPs and indels, there are other types of genetic variants that can be detected by next-generation sequencing data analysis programs. For example, structural variants, which include inversions, translocations, and duplications, can be detected by programs such as Delly, Lumpy, Manta, Visor among others [66, 108, 332, 508]. However, adequate detection of sequence copy number changes remains a challenging problem. Recently published machine learning approaches suggested that it is more effective than standard methods at accurately detecting sequence copy number changes in lower quality or coverage next-generation sequencing data, and is equally powerful in high-coverage data, including the identification of novel CNVs in genomes previously analyzed for CNVs using long-read data [244].

6.5.3 APPLICATIONS OF NEXT-GENERATION SEQUENCING IN THE DIAGNOSIS OF INFECTIOUS PULMONARY DISEASES

Identification of differential gene expression and analysis of genetic diversity are two important approaches in *Mycobacterium tuberculosis* research. In Mtb, identification of differential gene expression is often performed using next-generation RNA sequencing (RNA-Seq) technologies. RNA-Seq data can be analyzed techniques, such as differential cell lysis, probe-based ribosomal depletion, and genome-wide metabolic network analysis, scientists can investigate the regulatory networks and gene expression patterns of Mtb and its host during infection to identify genes that are over- or under-expressed compared to control conditions. This approach has been used to investigate the molecular mechanisms underlying *M. tuberculosis* virulence, drug resistance, and host immune response [131, 188, 390, 420, 734].

Analysis of the genetic diversity of *M. tuberculosis* involves the analysis of genetic variation within and between populations of the bacterium. This can be accomplished by analyzing the DNA sequence of multiple strains of *M. tuberculosis*. Next-generation sequencing data are often used to generate complete or partial genomes of *M. tuberculosis* strains. These genomes can be compared to identify mutations and genetic variations that occur in different strains. Genetic diversity analysis is used to understand the epidemiology of tuberculosis, including the spread of drug-resistant strains, the identification of new emerging strains, and the evolutionary history of the bacterium [274, 392, 402, 449, 452, 541, 542, 546, 653].

6.5.4 INTEGRATION OF DEEP LEARNING AND NEXT-GENERATION SEQUENCING FOR TB DIAGNOSIS

NGS enables rapid and accurate identification of Mtb bacteria in sputum samples and other body fluids. While deep learning enables rapid analysis of large genomic datasets to identify patterns of genetic variation and classify Mtb strains. The use of these techniques could allow a faster and more accurate diagnosis of TB, which could help reduce the spread of the disease and improve treatment outcomes. In addition,

it could be a useful tool for monitoring disease progression and identifying potential drug-resistant strains [274].

Recently, WHO has published and endorsed the first catalog of resistance-associated genetic variants based on more than 38,000 MTBC isolates to predict clinically relevant resistance phenotypes from genetic data. This mutation catalog provides a common, standardized reference for the interpretation of resistance to all first-line drugs (RIF, INH, ethambutol, and pyrazinamide) and also to second-line group A drugs (levofloxacin , moxifloxacin , bedaquiline, and linezolid), group B (clofazimine), and group C (delamanid, amikacin , streptomycin, ethionamide, and prothionamide [457].

Barely a year later, the Comprehensive Resistance Prediction for Tuberculosis: an International Consortium (CRyPTIC) has published a compendium of data from 12,289 global clinical isolates of *Mycobacterium tuberculosis* , all of which have been subjected to whole genome sequencing and measured for their minimum inhibitory concentrations against 13 antituberculosis drugs processed uniformly in 23 countries in a single assay. This is the largest matched phenotypic and genotypic dataset of Mtb to date. The compendium contains 6,814 isolates resistant to at least one drug, including 2,129 samples that fully meet the clinical definitions of rifampicin-resistant (RR), multidrug-resistant (MDR), pre-extensively drug-resistant (pre-XDR) or extensively drug-resistant (XDR). This combination of an extensive catalog and open availability of an immense amount of data provides an ideal framework for the development of artificial intelligence implementations, especially deep learning [128, 129].

Several investigations have addressed the processing of genome datasets with machine learning techniques [39, 146, 287, 315, 694]. However, most with smaller scale sets than those recently published. With the recent increase in the availability of massive data, a trend to explore deep learning-based solutions can be observed. Moving from the predominance of machine learning classification models (Support Vector Machine, Random Forest, and ensemble models, etc.) to solutions based on deep learning, (convolutional networks, LSTM, GRU, ANN, etc.) [106, 269, 273, 534].

These technologies can also be used to develop new diagnostic approaches, such as early detection of TB. However, the integration of these technologies also presents challenges. Adequate computational infrastructure is needed to process large amounts of sequencing and phenotypic data. In addition, it is important to validate the results in clinical studies to ensure their accuracy and reliability.

6.6 LIMITATIONS

First, deep learning models rely on large amounts of high-quality annotated data for training. However, such data may not always be readily available, particularly in low-resource settings. Additionally, data quality can vary, which may affect the accuracy of the model's diagnosis.

Secondly, deep learning models can be limited by the scope of the data they are trained on. If a model is trained on a specific set of medical images, it may not be able

to accurately diagnose pulmonary infections that present differently or have atypical features.

Third, deep-learning models are often considered “black boxes” because they are highly complex and difficult to interpret. This can make it difficult to understand how the model arrived at its diagnosis, which can be problematic for healthcare professionals who need to justify their diagnoses to patients or other healthcare providers.

Lastly, there may be ethical and legal concerns related to the use of deep learning for medical diagnosis. For example, if a deep learning model produces a false-positive or false-negative diagnosis, this may lead to unnecessary treatments or missed diagnoses, respectively, which can have serious consequences for patients.

6.7 CONCLUSIONS

Timely and accurate diagnosis is essential in treating and preventing the spread of pulmonary infectious diseases like COVID-19, COPD, and TB, which are significant causes of illness and death globally. Deep learning, a machine learning type, has proven useful in medical applications, particularly in analyzing and classifying biosignals and medical images. More recently, there has been growing interest in using deep learning techniques to diagnose pulmonary infections by analyzing cough signals, the ECG, chest radiographs, or computed tomography scans. By training deep learning algorithms on large medical datasets, these models can learn to accurately classify different types of pulmonary infections, leading to more efficient and accurate diagnosis. This improves patient outcomes by enabling early detection of lung infections and enhancing the effectiveness of treatments. Machine learning can also be used to predict drug resistance by summarizing the predictive ability of various factors. This can aid in clinical decision-making and detect single nucleotide polymorphisms as whole genome sequencing data increases.

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