



Early View

Invited review

Advances in diagnostic tools for respiratory tract infections. From tuberculosis to COVID19: changing paradigms?

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TITLE PAGE

Title: Advances in diagnostic tools for respiratory tract infections. From tuberculosis to COVID19: changing paradigms?

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Abstract

Respiratory tract infections (RTI) are one of the commonest reasons for seeking healthcare, but are amongst the most challenging diseases in terms of clinical decision making. Proper and timely diagnosis is critical in order to optimize management and prevent further emergence of antimicrobial resistance by misuse, or overuse of antibiotics. Diagnostic tools for RTI include those involving syndromic and etiological diagnosis: from clinical and radiological features to laboratory methods targeting both pathogen detection and host biomarkers, as well as their combinations in terms of clinical algorithms. They also include tools for predicting severity and monitoring treatment response. Unprecedented milestones have been achieved in the context of the COVID-19 pandemic, involving the most recent applications of diagnostic technologies both at genotypic and phenotypic level, which have changed paradigms in infectious respiratory diseases in terms of why, how and where diagnostics are performed. The aim of this review is to discuss advances in diagnostic tools that impact clinical decision making, surveillance and follow-up of RTI and tuberculosis. If properly harnessed, recent advances in diagnostic technologies, including omics and digital transformation emerge as an unprecedented opportunity to tackle ongoing and future epidemics while handling antimicrobial resistance from a One Health perspective.

1. General introduction

Respiratory tract infections (RTI) are amongst the most common reasons for seeking healthcare. Despite important advances in the last two decades, clinical management is challenging at several levels. Many RTI are caused by viruses for which antibiotics are not effective and in many cases are self-limiting. Misuse and mismanagement of antibiotics is particularly relevant in RTI and contributes to the emergence of antimicrobial resistance (AMR) while hindering treatment. The aim of the current review is to discuss advances in diagnostic tools that have an impact on clinical decision making and follow-up.

Pneumonia is the leading cause of death among children under 5 years old and the fifth cause of death among adults over 69 [1]. By contrast, for decades, the leading cause of death from a single infectious pathogen is tuberculosis (TB) [2]. In 2020, COVID-19 overtook TB as the infectious disease causing highest mortality; and although global TB control efforts were not on track, even before the advent of the pandemic, TB control strategies have been further impacted by it [3]. According to the World Health Organization (WHO) report 2021 [4] reduced access to TB diagnosis and treatment in 2020 resulted in the first year-on-year increase (of 5.6%) since 2005: estimated numbers account 1.3 million TB deaths among HIV-negative people and an additional 214 000 among HIV-positive people, with the combined total back to the level of 2017.

Existing surveillance programs regularly monitor respiratory syndromes with focus on Influenza viruses [5] and very efficient initiatives provide a coordinated and agile research response to infectious disease outbreaks [6]. Usefulness of monitoring became evident with the reported clusters of patients with pneumonia of unknown cause in late December 2019 that led to the description of the novel human pathogen SARS-CoV-2 [7]. The initial predictions were that 60% of the global population could be infected [8]. During 2020 and 2021, we witnessed a pandemic with devastating effects on health and economies but simultaneously a historical game-changer. The SARS-CoV-2 is unquestionably a One Health disease and has highlighted the concept of syndemics: a synergistic effect of the pandemic overlapping with endemic diseases and contextual determinants of health such as cultural, socio-economic factors, climate and environment [9]. The influence of non-communicable diseases is well known and the impact of smoking and air pollution is finally being highlighted [10]. New vulnerable populations are resulting from population ageing [11] and the increased use of immunosuppressive therapies for a wide range of medical conditions [12].

2. Why do we need diagnostics? The concept of diagnostic tools

The concept of diagnostics refers to: 1) interpreting the clinical profile to predict the presence of the illness (syndromic diagnosis), 2) finding an explanation for the illness (etiology) and 3) predicting the

course of disease and need for follow-up (prognosis) (Figure 1). These three above mentioned aspects have been reflected in this review.

Diagnostic tools must be accurate and need to be properly assessed and monitored, as emphasized by the introduction of the European *in vitro* diagnostic and medical devices regulation [13]. Risks and benefits need to be quantified, based on scientific evidence and fit for use in the population for which they are intended (age, comorbidities, immune status). Finally, they need to be used at the right time and interpreted appropriately to affect care optimally.

Never before have we witnessed such social awareness of infectious disease; the importance of non-pharmacological measures, of a test result and of understanding the immunity behind it. COVID-19 provided an unprecedented response from industry during the massive testing and vaccination campaigns that ensued with more than 1,000 new tests, new diagnostic approaches and modification of existing techniques or the development of new ones[14] . While we must continue to monitor the virus closely, it is time to change the dynamics regarding the value of diagnostics of RTI globally. If this is properly addressed, an unprecedented opportunity emerges to face current and future epidemics.

3. Advances in syndromic diagnosis

One of the first challenges in RTI is correcting frequently inconsistent case definitions. Clinical signs and symptoms are similar for many lower respiratory tract infections and several other diseases (e.g. pulmonary embolism, acute heart failure, asthma). Global burden estimations require valid, reliable and timely data [1], so appropriate definitions are essential and important progress has recently been made [15]. Those aspects that have shown advances are highlighted below.

Pneumonia is defined as an acute illness affecting the lungs usually presenting with cough, sputum production, and rapid and difficult breathing with a new or worsening pulmonary infiltrate on a chest radiograph. The diagnostic processes range from simple to relatively complex procedures combining clinical, radiological and laboratory features. Specific investigations should be considered in some endemic settings (e.g. TB, mycoses) [16] and also in cases of pneumonia associated with air pollution [17]. Depending on the clinical setting and accessibility, the plain chest radiograph is not always required for diagnosis, nevertheless it still remains a relevant tool. Lung ultrasound has shown better accuracy than chest X-ray for bacterial pneumonia in emergency departments [18] and an advance during the pandemic has been its increased use for both diagnosis and follow-up [19]. The additional value of computed tomography has been widely recognized for COVID-19 pneumonia [20].

Tuberculosis. One of the main advances in definitions is the change from addressing two disease states (latent TB infection or active TB disease) to evaluating a continuous spectrum of metabolic bacterial activity and antagonistic immunological responses by adding two additional clinical states: incipient (asymptomatic phase of early disease, between latent infection and subclinical TB) and subclinical TB (described as having viable and detectable bacteria, but without TB-related symptoms) [21, 22]. Recent WHO guidelines endorse automated nucleic acid amplification tests for detection of TB and resistance to rifampicin and isoniazid, providing more options for early diagnosis of TB [23].

Bronchiolitis is a disorder commonly caused by viral infections in infants, typically beginning with rhinitis and cough, which may progress to tachypnoea, wheezing, rales, use of accessory muscles, and/or nasal flaring [24]. Many respiratory viruses cause a similar constellation of signs and symptoms. The most common aetiology is respiratory syncytial virus (RSV), which infects 90% of children in the first 2 years of life, with up to 40% experiencing lower RTI during the initial infection [25]. Recent advances include the definition of different endotypes and the identification of relevant risk factors for recurrent wheezing and asthma development [26].

Bronchiectasis is characterized by a permanent and progressive dilation of the airways because of a vicious cycle of inflammation, infection, and repair of the bronchial mucosa, which leads to malfunctioning of the mucociliary system and destruction of the bronchial wall [27]. Awareness bronchiectasis has increased considerably in recent years [28, 29] and advances include a clearer definition of the disease [30] as well of its exacerbation. Now acute deterioration in three or more of the following symptoms for at least 48 hours: cough, sputum volume and/or consistency, sputum purulence, dyspnea and/or exercise tolerance, fatigue and/or malaise, or hemoptysis; lead clinicians to make changes in bronchiectasis treatment [31].

The definition of **Chronic Obstructive Pulmonary Disease (COPD) exacerbation** has also improved by highlighting a spectrum of different exacerbation types that can require different interventions. Identifying exacerbation triggers is of the utmost importance for proper treatment. Best-known are viral and bacterial RTI with a significant proportion of co-infections. However, in one third of cases, they remain unidentified [32].

Recently, a novel treatment approach based on "**treatable traits**" recognizes that airway infection is only one of many treatable traits in a given patient that benefit from specific management; such as airway clearance techniques, prompt treatment of exacerbations or oral or inhaled antibiotics. This

approach makes it possible to differentiate those individuals in whom symptoms or exacerbations are driven by a different treatable trait that will not benefit from antibiotic treatment [33-35].

When potentially pathogenic microorganisms are present over time, a **chronic bronchial infection** occurs. Chronic bacterial infection can be clinically defined as evidence of positive respiratory tract cultures of the same microorganism by standard microbiology, on two or more occasions, at least 3 months apart over 1 year, while in a stable state [30]. Several novel techniques such as automated molecular diagnostic systems might be incorporated into the diagnostic scheme for exacerbations following careful interpretation. Both acute and chronic respiratory infection cause a progression of the disease and a worsening quality of life [29]. This is a very common situation in diseases such as COPD and bronchiectasis, and less frequently it also occurs in asthma. Chronic airway infection has been associated with altered pulmonary immune response and worse clinical outcomes [36, 37].

Spectrum of disease. A major challenge in each of these clinical conditions is correlating syndromic and etiological diagnosis leading to the concept of spectrum of disease. Diagnosis is straightforward if a primary pathogen such as *Mycobacterium tuberculosis*, *Legionella pneumophila* or *Pneumocystis jirovecii* is identified. In other situations, more careful interpretation is needed such as when identifying virus (infection or asymptomatic shedding), environmental fungus or environmental mycobacteria (infection, colonization or contamination). The most frequently isolated bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* are potentially commensal. This is also the case for *Staphylococcus aureus* and *Pseudomonas aeruginosa* and other non-fermenting bacilli (e.g. *Achromobacter xylosoxidans*, *Stenotrophomonas maltophilia*) when underlying disease is present. Non-primary pathogens might gain access and trigger symptoms or disease (eg. by activating virulence factors) or remain quiescent (biofilm, intracellular survival), so better understanding of host pathogen interaction is crucial [38]. Improving microbiological methods and added number of vulnerable patients, has added *Nocardia* spp., fungi and environmental mycobacteria to the spectrum of potential pathogens. Recent advances include guidelines to assess definitions of disease stages for environmental mycobacteria [39-41] and also for pulmonary aspergillosis, depending on the interaction between *Aspergillus* and the host [27].

4. Advances in diagnostic tools identifying the etiological agent

Microbiological diagnostics are critical at three levels: 1) identifying the etiological agent and guiding appropriate therapy, so adjusting spectrum and duration and decreasing misuse and overuse of antibiotics to prevent the emergence of AMR; 2) surveillance of local resistance patterns and

screening to identify patients colonised with resistant pathogens and adoption of infection prevention and control measures to prevent spread; and 3) detecting emerging pathogens. An important concept is that detection might refer to screening (e.g. detecting carriers of resistant pathogen) or to etiological diagnosis. Independent of the sophistication of the methods, it is crucial to generate timely, understandable results that can inform clinical decisions.

A diagnostic test can be used to demonstrate the presence or absence of infection or to detect evidence of a previous one. Generally speaking, it can be categorized into direct diagnosis, including microscopic examination, culture, antigen detection and molecular detection; and indirect diagnosis, covering immunological tests. The pandemic has brought advances in every single technique [42].

Appropriate sample and methodology.

Accurate microbiological diagnosis of RTI requires good quality specimens. Samples must be collected taking medical condition into account, either from the upper (nasal/ throat specimens) or lower respiratory tract (bronchoalveolar lavage fluid, tracheal aspirate). Additionally, improved detection methods are making the use of new types of specimens possible and even saliva and oral mucosa may have a role to play in some situations [43-46]. But this is not true for all microorganisms, it depends on the pathogenesis and whether they are primary pathogens or potentially commensal. Particularly for COVID-19 diagnosis, pre-analytical factors have been particularly relevant, such as the swabbing methods and the use of different matrices and viral transport media [47, 48]. Lastly, reliable transportation in optimal conditions (temperature, time) to the laboratory are key in insuring valid results.

Pathogen Identification. For culture based methods, the arrival of Matrix-Assisted Laser Desorption/Ionization-Time Of Flight (MALDI-TOF) mass spectrometry to clinical labs has been a major development in rapid identification, with the additional potential for detection of antimicrobial resistance and even typing, among other possible/putative/ future uses. In culture independent detection, recently antigen detection has increased its identifiable targets. Additionally, and of particular note, molecular methods have increased the diagnostic yield for virus and atypical bacteria, showing unprecedented development during the COVID-19 pandemic. Molecular techniques are also becoming available at point-of-care (POC) or near-POC as different portable molecular diagnostic instruments are being developed [49]. These syndromic multiplex panels can be used to detect the pathogen DNA or RNA most commonly associated with RTI, including viruses and bacterial atypical pathogens [50-54]. Advantages include decreased time to detection, the possibility of quantification and detection of resistance and virulence genes. Serological diagnosis of atypical pneumonia has been replaced by molecular tests and is now only used in cases such as Q fever.

Antibody detection has seen a comeback, mostly for immunoprevalence and vaccine response studies of COVID-19 (enzyme-linked immunosorbent/chemiluminescence/ fluorescence microparticle and lateral flow immunoassays). The pandemic has also enabled the development of **advanced sensing technologies** based on microfluidics, nanotechnology and material science [55] as well as on targeting structures such as serum extracellular vesicles [56]. The main benefits of these assays is that they are portable, miniaturized, low cost and highly integrated POC devices [57]. In the move to a new paradigm of personalized medicine, POC diagnostic testing has been proposed to improve the quality of antibiotic prescription [58] but accuracy is variable and settings need careful adjustment[59].

Susceptibility testing Advances involving the use of selective chromogenic media shorten the detection time for resistant bacteria. The gold standard is Phenotypic testing because it shows the overall phenotype, although multiple resistance mechanisms can lead to difficult phenotype interpretations. The long time to result has been improved by selective culture media and analytical methods starting from a bacterial pellet, which is particularly useful in systemic infections [60] and also applicable to mass spectrometry or nanosensors [61]. Genotypic tests detect resistant genes in a fast and sensitive way, but genes may be present but not expressed, and the tests only detect known resistance genes. As a pragmatic summary, phenotypic testing can tell what antibiotics to prescribe, while genotypic testing can tell what not to prescribe.

Molecular epidemiology. Available typing methods for respiratory pathogens are laborious and time-consuming, but in recent years whole genome sequencing (WGS) has emerged as the gold standard method for detecting outbreaks and preventing clonal dissemination in medical settings. However, this is not so easily implemented in real-time practice. In this sense, the current COVID-19 pandemic has seen unprecedented generation and global sharing of large numbers of SARS-CoV-2 sequences in a record time with global sharing of variants of concern in common databases, powering influenza surveillance programs into COVID-19 efforts [62]. The next step involves real-time molecular epidemiology [63]. Genomics have expanded rapidly bringing the opportunity to move these approaches from academic to public health decisions and surveillance, as well as strengthening global cooperation with other disease control programs [64]. Phenotypical typing methods are also improving, such as Fourier-Transform InfraRed spectroscopy [65].

Extended culture techniques, automated molecular diagnostic systems and DNA sequencing technologies have revealed unknown airway microbiota, including an abundance of species that are refractory to common diagnostic tools. The interactions that occur between these microbial species can profoundly affect the expression of pathogenicity and virulence [27]. Large numbers of

microorganisms, including bacteria, fungi and viruses, collectively known as the microbiome, coexist in the lungs of healthy subjects and patients with respiratory diseases [66].

Microbiome is defined as the set of the genes and gene products (RNA, proteins, metabolites) produced by resident microbial communities. Research into the chronic lung disease microbiome began in the early 2000s, when ecological techniques of microbial DNA analysis were applied to sputum samples [67, 68]. Since then, the number of studies of the respiratory microbiome has grown exponentially. Whilst traditionally bacterial infection in the airways has been characterized using culture-based methods, 16S ribosomal RNA and other metagenomic approaches provide a powerful method of determining microbial identities and relative abundances [69].

5. The impact of omics technologies on precision approaches

General concepts of omics. There is growing clinical interest in understanding biological mechanisms beyond the molecular level to include biological functionality. In this sense, systems biology aims to explore how the interactions between biological components (genes, proteins, metabolites, etc.) contained in a biological tissue, cell, fluid or a whole organism affect its functionality (biological processes) as a whole, thus making it possible to characterize a biological system in a complete and integrated way [70]. Omics cover the set of high-throughput technologies that provide a global vision of a dynamic biological process through the analysis of genes (genomic), Ribonucleic acid-RNA (transcriptomic), proteins (proteomic) or metabolites (metabolomic).

Metagenomics. The first sequencing technique used in respiratory microbiome research was **16S rRNA gene sequencing**. It is based on PCR amplification using primers that target the 16S ribosomal gene in variable regions of bacterial genomes, which can be used for taxonomic classification [71]. It is fast, reasonably low cost, and by converging on a specific region of the bacterial genome requires only a limited sequencing depth and allows the study of microbial communities [72]. However, it targets relatively short gene sequences, which are often shared by different closely related species, making distinguishing them a challenge [73]. Another shortcoming is that 16S sequencing analyses are restricted to the detection of bacteria and archaea since viruses or fungi do not carry 16S rRNA genes. In this sense, **shotgun metagenomic sequencing** is an approach that allows microbiome characterization with a much greater resolution than 16S sequencing. The term 'shotgun' refers to the untargeted sequencing of all DNA present, in contrast to the targeted amplicon-based approaches. By not limiting sequencing to a single region of DNA, metagenomic sequencing can also provide information on the functional characteristics of the taxa present, including their metabolic traits, and their carriage of antibiotic resistance and pathogenicity features. [74]. Furthermore, the inclusive nature of metagenomic sequencing provides information not only on the bacteria present,

but also the fungi and DNA viruses, achieving identification to a subspecies or strain level. The principal limitation of shotgun metagenomic approaches is related to costs, but also to host DNA contamination. Finally, current methods cannot differentiate between live and dead microbes, which may be relevant for several reasons, such as assessing the impact of antibiotic therapy in clinical samples [75]. Novel methodologies to integrate bacterial, viral and fungal communities to allow assessment of the 'interactome' have been recently developed. Rather than focusing on individual taxa, this approach proposes a role for microbial networks in altering clinical outcomes or treatment responses [76].

Transcriptomics involves the analysis of RNA produced by a given genome at a given time and condition, and thus during lower RTI it can provide information regarding pathogen and host dynamics. It can refer to the exploratory analysis of an entire transcriptome, primarily using RNA sequencing, or to a targeted analysis of known RNAs using techniques such as gene expression panels.

Metabolomics is one of the most powerful bioanalytical strategies to obtain a picture of the metabolites in the course of a biological process and is a phenotyping tool [77]. Metabolomics allows the comparison of a chemical fingerprint present in a cellular system or a biofluid under normal conditions with that of altered states produced by disease, pharmacological treatment, dietary intervention or environmental modulation [78].

Clinical impact of omics: There is enormous interest in the potential of the microbiome to improve the understanding and stratification of respiratory diseases and to serve as a biomarker for clinical management. Host-microbiome interactions probably contribute substantially to differences in clinical phenotypes and disease outcomes [79-81]. Recent studies conclude that the microbiome could identify subgroups of patients at higher risk of poor outcome, who could benefit from precision treatment strategies [81]. Important questions still need to be examined, including the role of fungi, viruses and mycobacteria, the interactions with the host, and the usefulness of microbiome profiles for selecting antibiotics and to evaluate therapeutic responses [82]. The gut-lung axis affects disease and treatment. The intestinal microbiome influences the pulmonary microbiome and also lung immune responses by directly seeding the airways with bacteria and distributing bacterial metabolites that act as immune modulators [83].

Regarding **tuberculosis**, advances in systems biology and omics strategies have identified sets of biomarkers with the potential to optimize TB prevention, diagnosis, and treatment. Only a few have been evaluated in clinical trials, so applicability in TB management is still limited [84, 85].

Metabolomics has provided insight into the pathogenesis of TB related to detection of infection and disease progression [77, 86-89]. Urinary metabolomic response has also been characterized during **community-acquired pneumonia**, and different profiles have been identified according to the causative microorganism, atypical bacteria vs pneumococcal or viral [90]; ARDS vs influenza; pneumococcal vs other aetiologies [91]; and pneumococcal vs staphylococcal [92].

6. Advances in diagnostic tools characterizing the host response

The identification of novel biomarkers based on host-pathogen interactions related to the shift from carriage to infection may improve RTI management. There is a need to understand how the pathogens interact with their host to achieve a successful invasion (Figure 2). Inflammatory biomarkers and severity scores can contribute to some levels of stratification [38, 93].

Systemic biomarkers, such as the C-reactive protein (CRP) and procalcitonin (PCT) have been widely analyzed in patients with LRTI although other biomarkers are also being investigated [94, 95]. They are also being implemented at POC. The capacity of biomarkers to distinguish acute bacterial from viral RTI has enabled their use as tools for guiding antimicrobial therapy [96], but in the outpatient setting they might have a limited capacity [97]. Regardless of whether they are used for diagnostic or monitoring purposes, biomarker analysis should be included as an additional criterion to be integrated into decision-making algorithms.

During the COVID-19 pandemic, besides the identification of the causative agent, the detection and monitoring of the host response has provided insights into the pathogenesis of the disease [98, 99]. In fact, immune tools measuring T-cell responses and detecting IFN- γ in vitro (IGRAs) have been used for some years to diagnose TB and nowadays they are under investigation for SARS-CoV-2 [54-59]. Furthermore, second-generation IGRAs which detect other cytokines, such as IP-10, have proved comparable to IFN- γ . Furthermore, IP-10 cytokine has been shown to be very stable when dried on filter paper, which facilitates sample shipment to reference laboratories [100-103]

Since disease outcome depends on dynamic host-pathogen interactions, specific **host genetic signatures**, together with pathogen genomics can be combined in order to identify those individuals with a higher risk of severe disease [104, 105]. Specific **host transcriptomic signatures** have also been identified for this purpose [106-109]. An interesting additional area of host genomics research, is its use during pre-symptomatic stages, which can be important in influenza [110, 111] or TB [112] for predicting disease progression or detecting early forms of the illness. Host determinants for outcome are also related to **acquired risk factors**. Biological therapies targeting cytokines and/or cell

subsets have become essential for the treatment of several immune-mediated diseases and have an impact on RTI, as reviewed elsewhere [12].

Advances in understanding **host immunity** has been unprecedented during the pandemic, providing better knowledge of the immune response for other endemic respiratory viruses. Multiple mechanisms might lead to humoral and cellular responses involved in combatting RTI. While an enormous effort has been put into **vaccine development** and immunogen design, there are still some knowledge gaps in understanding long-term memory and protective immunity. Advances in methods to assess immunogenicity have been very relevant. Vaccines have been traditionally dependent on humoral response activation by means of B-cells producing neutralizing antibodies. However, over the years it has been accepted that cellular immunity mediated by CD4+ and CD8+ T-cells is also critical, evidencing that even a strong antibody response is not sufficient for protection. Recently it has been recognized that local immunity to bacterial or viral RTI can be mediated by a specific cells with a memory phenotype, called tissue-resident memory T-cells (TRMs). These cells are non-circulating, present at the site of infection, and retained in tissues for mediating a protective response in case of re-infection. Studies in mouse models and humans have evidenced that TRMs have a potential role against several respiratory pathogens such as influenza, RSV, *M. tuberculosis* or SARS-CoV-2 virus [113-118]. It is therefore important to develop new approaches and perform immunological studies to characterize TRMs as a diagnostic tool to monitor new vaccines that enhance these cell populations. Finally, the pandemic accelerated novel vaccine formulations including new viral vectored or nucleic acid-based vaccines, as well as the re-emergence of the concept of trained immunity. Live vaccines such as BCG can confer non-specific protection against upper and lower RTI not associated with *M. tuberculosis* through epigenetic re-programming during haematopoiesis of innate cells [119-124].

Finally, better understanding of host-pathogen interactions has allowed the identification of potential host targets and therefore the development of **host-directed therapies**. These methodologies consist of inhibiting host factors indispensable for microorganism replication, intensifying a pathway of the host immune response or decreasing the inflammatory status [125-128]. Metabolic, autophagy and immune response pathways are currently being investigated. Implementation is still a challenge with a need for accurate patient stratification for tailored interventions that prevent major side effects Several randomized clinical trials are ongoing, focussing on TB and COVID-19(<https://clinicaltrials.gov>).

7. Advances in diagnostic tools to predict severity

Clinical assessment of severity, related to involvement of lung parenchyma in the acute phase and prediction of functional sequelae, is relevant for clinical decisions. In **radiology**, current practices include tedious conventional processes, which rely on specialist technical expertise and are prone to human error. Great progress has been made in deep learning that supports medical radiologists [129-131]. Novel approaches such as **radiomics**, a high-throughput method extracting a tremendous amount of quantitative imaging data using data-characterization algorithms, have shown great potential in characterizing imaging biomarkers [132]. Also radiomics-based machine learning signatures have shown the potential to accurately differentiate ground-glass opacities due to COVID-19 pneumonia from those due to other acute lung diseases [133] or distinction between lung adenocarcinoma and tuberculosis granuloma [134]. Another example would be the Computer Aided Design (CAD) procedure for automatic diagnosis of COVID-19 from chest X-ray images [135].

Several **bronchoscopic technologies** have emerged over recent years, including thin/ultrathin bronchoscopes, radial probe **endobronchial ultrasound** (RP-EBUS), virtual navigation bronchoscopy (VBN), electromagnetic navigation bronchoscopy (ENB), and robotic bronchoscopy [136]. Bronchoscopic transparenchymal nodule access (BTPNA) and transbronchial access tool (TBAT) are novel techniques that combined with navigational bronchoscopic technologies improve access to lung lesions. The introduction of cryobiopsy improved tissue sampling. These innovative techniques allow higher diagnostic yield, also in the context of RTI and TB and other mycobacterial infections during the study of lung nodules and staging of lung cancer [137].

Oxygen saturation monitoring has traditionally been carried out using transmittance pulse oximeters due to their dependability, but they are limited to peripheral regions. Recently, new options being studied include reflectance pulse oximeters that can be used at different body sites (finger, wrist, chest and forehead) and can be scaled down to affordable patches [138].

Regarding prognostic tools, diaphragm **ultrasound** can be utilized to diagnose diaphragm dysfunction, assess severity, and monitor disease progression and could be beneficial both in pneumonia and COVID-19 [16, 139]. Advances have also been made regarding better diagnosis of long-term **structural-functional complications** and a better follow-up of sequelae by standardising prognostic measures such as quality of life and social impact [140-142].

8. Final remarks. Lessons learned.

The pandemic has brought the need for an integrated approach for handling infectious diseases back to the forefront, along with the need for a coordinated effort across multiple disciplines including human, animal and environmental [143]. Unprecedented milestones have been achieved by improving and applying the latest technologies in key areas such as epidemiology, contact tracing, diagnostics and vaccine development.

However, global health inequities in low-income countries related to vaccines and treatments, and also diagnostics still need to be addressed. The pandemic has additional costs related to the previously identified global crisis in AMR and TB [144] and several aspects have been highlighted as opportunities at different levels [145, 146].

POC technologies have allowed community-based testing to be scaled up and used as a public health tool, but they need to be linked to careful evaluation [147] and to demonstrate their diagnostic accuracy in clinical practice [59]. Timely results also involve careful interpretation and this is facilitated by effective interdisciplinary communication starting with understandable reporting of results.

While providing emergency healthcare, clinicians and scientists have carried out clinical research and lessons learned underline the importance of pre-established structures and procedures and the need for improved regulatory consensus and globally connected networks [148]. Cost-effectiveness analyses can be used to assess the value of diagnostics in clinical practice, but they need proper design and reporting [149] in the context of pragmatic clinical trials. Digital transformation of health is ongoing through the incorporation of artificial intelligence to support clinical decision processes [150].

After years of reporting clinical diagnosis and microbiologically confirmed diagnosis in different databases, priorities have been set up in data connectivity by maximizing efforts in FAIR (Findable, Accessible, Interoperable, Recuperable) approaches [151, 152]. Real-world data and real-world evidence is rapidly gaining importance and being formalized in policy frameworks [153].

In summary: Advances in diagnostic tools have been largely accelerated by the pandemic and affect both the design of personalized therapies and public health measures. Transdisciplinary communication is crucial for proper development and implementation of current techniques. Impact of the use of diagnostic tools depends on several contextual factors involving clinical setting, geographical location, connectivity and clinical research resources. It is mandatory to maintain monitoring of pathogenesis of individual RTI for a timely diagnosis and proper interpretation.

Figures

Figure 1. Three pillars in clinical decision making: syndromic diagnosis, etiological diagnosis and prognosis assessment. The most relevant advances regarding methodologies available are presented.

Figure 2. Omics for a better characterization of host, pathogen and host-pathogen interaction factors during RTI

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Why do we need diagnostics?
Clinical decision making

How do we perform diagnostics?



