Personalized Medicine for Chronic Respiratory Infectious Diseases: Tuberculosis, Nontuberculous Mycobacterial Pulmonary Diseases, and Chronic Pulmonary Aspergillosis

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Key Words

Personalized medicine · Chronic respiratory infectious diseases · Tuberculosis · Nontuberculous mycobacterial diseases · Chronic pulmonary aspergillosis

Abstract

Chronic respiratory infectious diseases are causing high rates of morbidity and mortality worldwide. Tuberculosis, a major cause of chronic pulmonary infection, is currently responsible for approximately 1.5 million deaths per year. Although important advances in the fight against tuberculosis have been made, the progress towards eradication of this disease is being challenged by the dramatic increase in multidrug-resistant bacilli. Nontuberculous mycobacteria causing pulmonary disease and chronic pulmonary asper-

gillosis are emerging infectious diseases. In contrast to other infectious diseases, chronic respiratory infections share the trait of having highly variable treatment outcomes despite longstanding antimicrobial therapy. Recent scientific progress indicates that medicine is presently at a transition stage from programmatic to personalized management. We explain current state-of-the-art management concepts of chronic pulmonary infectious diseases as well as the underlying methods for therapeutic decisions and their implications for personalized medicine. Furthermore, we describe promising biomarkers and techniques with the potential to serve future individual treatment concepts in this field of difficult-to-treat patients. These include candidate markers to improve individual risk assessment for disease development, the design of tailor-made drug therapy regimens, and individualized biomarker-guided therapy dura-

tion to achieve relapse-free cure. In addition, the use of therapeutic drug monitoring to reach optimal drug dosing with the smallest rate of adverse events as well as candidate agents for future host-directed therapies are described. Taken together, personalized medicine will provide opportunities to substantially improve the management and treatment outcome of difficult-to-treat patients with chronic respiratory infections.

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Introduction

Chronic respiratory infections including tuberculosis (TB), nontuberculous mycobacterial pulmonary disease (NTM-PD), and chronic pulmonary aspergillosis (CPA) are a leading cause of morbidity and mortality worldwide. According to the World Health Organization (WHO) Global Tuberculosis Report 2015, TB affected about 9.6 million persons globally, including ~480,000 cases of multidrug-resistant TB (MDR-TB, defined as resistance against rifampicin and isoniazid). The annual mortality rate was estimated at about 1.5 million [1].

In contrast to pulmonary TB, direct human-to-human transmission of nontuberculous mycobacteria (NTM) seems to be unlikely, although single cases have been reported [2–4]. Incidence rates of NTM-PD in Europe range from 0.2 to 2.9/100,000 persons. The main risk factors are preexisting respiratory disorders such as cystic fibrosis, chronic obstructive pulmonary disease, asthma, and bronchiectasis. However, NTM-PD also occurs in immunocompetent patients without underlying respiratory disorders [5, 6].

The prevalence of CPA is estimated at about 3 million people, with 1.2 million CPA cases occurring in patients with prior TB worldwide [7]; however, CPA complicates also other chronic respiratory disorders, such as sarcoidosis and allergic bronchopulmonary aspergillosis [8, 9].

The diagnosis of chronic pulmonary infections is often challenging, and their therapy is complex and requires a long duration of antimicrobial treatment, which often leads to a high frequency of adverse events, suboptimal adherence to treatment, and poor outcomes (table 1) [10–12]. Therefore, improving individual risk assessment for disease development, the design of tailor-made therapies, therapeutic drug monitoring (TDM), and the use of biomarkers to guide and individualize the duration of antimicrobial therapy are of great importance for the quality of life of patients and to ensure treatment success, as well as for health economic reasons [13–15].

Current Standardized Management Approach

Tuberculosis

Antimicrobial chemotherapy for TB was first introduced in 1946 in a randomized controlled trial on streptomycin monotherapy [16]. The development of high rates of drug resistance after treatment with the drug and similar long-term mortality rates in both trial arms did not support this monotherapy approach. Combination therapies were developed which improved treatment outcomes, even though successful drug regimens had to be given for at least 12 months. In 1970 the first combination of streptomycin and isoniazid with rifampicin or pyrazinamide significantly reduced relapse rates, which led to the development of the current short-course treatment lasting 6 months [17]. The latest WHO guidelines recommend treatment of drug-sensitive pulmonary TB with rifampicin, isoniazid, pyrazinamide, and ethambutol for 2 months (intensive phase), followed by a conservation phase of 4 months of rifampicin and isoniazid, for a total duration of treatment of 6 months [18].

Animal studies led to the hypothesis that the antimycobacterial activity of fluoroquinolones (FQs) might allow a further shortening of treatment to 4 months [19]. Unfortunately, 3 phase III trials, all published in 2014, showed the inferiority of the FQ-containing short-course regimens, leading to increased relapse rates during follow-up compared to standard regimens [20–22].

While TB drug resistance was a known challenge since the streptomycin trial in 1946, the outbreak of extensively drug-resistant (XDR) TB (defined as MDR plus resistance against at least one second-line injectable and at least one FQ) in South African HIV-positive patients in particular directed the attention towards the disease and led to large investments in research for new anti-TB drugs and regimens [23]. While the development of regimens for drug-susceptible TB was based on a large number of clinical trials, the current guidelines for the treatment of drug-resistant TB are mainly based on a retrospective individual patient data analysis from cohort studies [10]. Until May 2016 the WHO had recommended a regimen consisting of at least 4 active drugs plus pyrazinamide for a treatment duration of at least 20 months [24]. During the initial intensive phase of 8 months' duration, the regimen is based on the use of an injectable drug (kanamycin, amikacin, or capreomycin). The other key components of the regimen are FQs of the later generation, of which levofloxacin and moxifloxacin are the most frequently used. Ethionamide or prothionamide, cycloserine/terizidone, or para-aminosalicylic acid are added to

Table 1. Current standards of care and future perspectives for individualized TB, NTM-PD, and CPA disease management

Options for individualized therapy	Timeline	TB	NTM-PD	CPA
Prevention	Current standards	Identify latent infection by TST and IGRAs Limitation: poor positive predictive value Preventive therapy over 3–9 months Limitations: poor compliance, no clear recommendation for MDR-LTBI	None	Antifungal prophylaxis to prevent invasive aspergillosis for high-risk patients (e.g. neutropenic patients, etc.); effect on CPA unknown
	Future perspectives	Use of gene transcription profiles to identify individuals who will develop disease Short-course regimens for LTBI, drugs with a long half-life, active on MDR-TB strains	Identification and elimination of the bacteria's reservoirs Identification of host susceptibility factors: host gene expression profiles to detect genetic predisposition to NTM-PD/define high-risk groups	Identification of host susceptibility factors: host gene expression profiles to detect genetic predisposition to CPA in risk groups
Diagnosis	Current standards	Microscopy, culture, molecular techniques Limitations: insufficient sensitivity for children and extrapulmonary TB, no real point-of-care tests	Microbiology, radiology, clinic Limitations: differentiation between NTM pulmonary infection and disease	Radiology, serology (culture) according to ESMCID/ERS guidelines [49]
	Future perspectives	More sensitive methods, development of point-of-care tests Gene expression signatures to identify patients with TB	Biomarkers for differentiation between infection and disease (e.g. immune reaction against MAC-specific glycopeptidolipid core antigen, transcriptomics)	Biomarkers for differentiation between colonization and infection
Drug resistance testing	Current standards	Phenotypic DST (solid and liquid media), molecular DST (NAATs) Limitations: cultures have a long time until results are obtained, molecular DST is limited to certain drugs, and some tests can be performed only in reference laboratories WGS for prediction of drug resistance limited to few specialized centers	Phenotypic DST (broth microdilution method, CLSI standards), molecular DST (for <i>M. abscessus</i> ssp. only) Limitations: lacking standardized MICs for several antimicrobial drugs used in clinical practice Molecular DST limited to <i>M. abscessus</i> ssp. and two drugs	Broth microdilution for <i>Aspergillus</i> species according to the EUCAST and CLSI [131]
	Future perspectives	Simplification of platforms for drug resistance testing, increase the use of WGS Use of MICs to guide drug selection and dosage	Better understanding of in vitro MIC results and clinical correlations Development of molecular techniques	Better understanding of in vitro MIC results and clinical correlations Development of molecular techniques
Treatment	Current standards	Standardized drug regimens and weight-based dosage Limitations: interperson variability TDM mainly for first-line drugs and limited to few specialized centers	Standard regimens mainly based on expert opinions and traditions Lack of in vitro/in vivo correlations for most of the regimens	Standard regimens based on cohort studies or case reports Limitation: just two prospective studies, which are not really applicable; most studies do not differentiate between CPA forms; no study directly compared two triazole regimens
	Future perspectives	TDM for all drugs with wide availability Dose selection based on drug levels	Prospective clinical trials to evaluate current standards and new antimicrobial drugs	Prospective clinical trials; TDM for all drugs; dose selection based on drug levels
Monitoring	Current standards	Standardized duration of treatment, monitoring based on microscopy (insensitive) and culture (long time until results)	Mainly based on microbiology, combined with radiologic and clinical response Limitation: no outcome definition	Based on radiology Limitation: no outcome definition
	Future perspectives	Biomarkers and gene expression signatures for treatment response	Define treatment outcomes Biomarkers for treatment response	Disease outcome definition Biomarkers for treatment response

Table 1 (continued)

Options for individualized therapy	Timeline	TB	NTM-PD	CPA
Duration of therapy	Current standards	Standardized	Defined as 12 months after culture conversion Limitation: lacking evidence, long-lasting treatment regimens not necessarily leading to cure	Single aspergilloma: surgical resection; all other forms of CPA, at least 6 months Limitation: lacking evidence
	Future perspectives	Tailored according to extent of disease, resistance patterns, and treatment response (biomarkers, gene expression profiles)	Biomarkers to predict clinical treatment outcome early in the course of therapy Gene expression profiles to define risk groups	Prospective clinical trials Gene expression profiles to define risk groups

EUCAST = European Committee on Antibiotic Susceptibility Testing; IGRA = interferon- γ release assay; NAATs = nucleic acid amplification tests; TST = tuberculin skin test.

the drug regimen, provided there is no in vitro resistance to those drugs. In a trial conducted in Bangladesh, patients were treated with a therapy regimen consisting of gatifloxacin, kanamycin, clofazimine, ethambutol, pyrazinamide, prothionamide, and high-dose isoniazid for only 9 months [25]. Patients treated with this short-course regimen showed a relapse-free cure rate of more than 80%, compared to approximately 60% in patients treated with standard regimens. Based on this concept and on further operational study results, but not on clinical trials, the WHO has recommended a short-course regimen for 10–12 months for patients with pulmonary MDR-TB since May 2016 [26]. The application of this recommendation is limited to patients who have no suspected or proven resistance to the drugs mentioned above (moxifloxacin instead of gatifloxacin). Unfortunately, this is only applicable to a small proportion of patients, i.e. in Europe to less than 10% (manuscript submitted). For patients with resistance to second-line drugs, and in particular to the second-line injectable drugs or FQs, two new drugs are available. Delamanid and bedaquiline are licensed under specific conditions due to lack of evidence for their efficacy in large phase III trials [27, 28]. Other drugs which were originally licensed for non-TB infections, such as linezolid and meropenem/clavulanic acid, also have antimycobacterial activity. Both drugs play an increasing role in the treatment of MDR-TB [29, 30].

Nontuberculous Mycobacterial Pulmonary Disease

The current management of NTM-PD differs substantially from TB management. NTM are environmental mycobacteria which can be isolated from soil and water [31]. They are not obligate pathogens, but some may affect certain vulnerable patient populations, e.g. patients

with chronic respiratory diseases [32]. Isolation of NTM from respiratory samples is not necessarily associated with pulmonary disease; it may reflect colonization or infection of the lungs without inducing disease, or contamination of the respiratory samples. Therefore, the diagnosis and management of NTM-PD is often challenging [33]. The American Thoracic Society (ATS) and the Infectious Diseases Society of America (IDSA) suggested criteria that help to differentiate between patients requiring treatment and those who do not [32]. These include the repeated isolation of mycobacteria from respiratory samples, compatible clinical and radiological presentation, and the exclusion of any alternative diagnosis. Nevertheless, these criteria are not generally applicable to all patients with NTM-PD in the same manner [34]. They may for example differ between patients with nodular-bronchiectatic and those with fibrocavitary disease, with the latter disease often necessitating a more aggressive treatment approach.

Treatment recommendations are mainly based on expert opinions and ‘traditions’ [35]. Macrolides (clarithromycin or azithromycin) are the cornerstone of most NTM drug regimens; they are usually combined with rifamycins and ethambutol [32]. But there are exceptions such as *Mycobacterium kansasii* or *M. simiae*. *M. abscessus* subspecies (ssp.) are particularly difficult to treat, as they are resistant to multiple antimicrobial drugs and require long-term intravenous treatment [36]. Relapse-free cure from *M. abscessus* ssp. PD is almost impossible.

Drug sensitivity testing (DST) can be used to guide the choice of drug regimens [37]. Phenotypic DST is performed using the broth microdilution method according to a standardized protocol from the Clinical and Laboratory Standards Institute (CLSI) [38]. Molecular tools for

detecting resistance to clarithromycin and amikacin are available for *M. abscessus* ssp. only [39]. Still, in vitro/in vivo studies evaluating their clinical efficacy are lacking. Clarithromycin and amikacin are the only drugs for which a correlation between in vitro drug susceptibility and in vivo efficacy in *M. avium* complex pulmonary disease (MAC-PD) has been shown [40, 41].

The evidence for the current treatment recommendation is weak [42]. Only one prospective placebo-controlled clinical trial on MAC-PD is available, which compares the treatment outcome of pulmonary MAC infection with and without an aminoglycoside in addition to the standard treatment regimen [43]. Clinical improvement was more common in that study's streptomycin group, but the difference was not significant.

Surgery should be considered for patients with extensive but localized cavitation, severe nodular-bronchiectatic disease, and/or a poor response to medical therapy [44]. The benefit from and risk of surgery has to be well considered, as complication rates can be high [45]. If surgery is feasible, it should be considered for patients with persistent culture positivity after 6 months of medical treatment [44].

A treatment duration of 12 months is recommended after sputum culture conversion [32]. Patients with comorbidities taking long-term multidrug treatment regimens for NTM-PD often face a high risk of adverse events, drug-drug interactions, and noncompliance, reducing the chances of treatment success and cure [46]. Standardized endpoints for treatment outcomes are missing [47] and the risk of recurrence is high [48]. Therefore outcome objectives may vary from sputum culture conversion to simple improvement of clinical and radiological signs, prioritizing a patient's quality of life.

Chronic Pulmonary Aspergillosis

So far, the current management approach to CPA was highly heterogeneous due to lack of evidence, missing guidelines, and the inconsistent disease definitions and diagnostic methods used. Recently, an expert group from the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the European Respiratory Society (ERS) proposed clinical guidelines for the diagnosis and management of CPA [49]. According to these guidelines, the diagnosis of CPA requires a combination of radiological and mycological characteristics including: (a) one or more cavities with or without a fungal ball present or nodules on thoracic imaging [preferably by computerized tomography (CT) scanning], (b) direct evidence of *Aspergillus* infection (microscopy or fungal

culture from biopsy of the lung) or an immunological response to *Aspergillus* species, and (c) exclusion of alternative diagnoses – all present for at least 3 months [49].

According to its radiological features, CPA can be classified as (1) chronic cavitary pulmonary aspergillosis, which is the most common manifestation, with the risk to progress to (2) chronic fibrosing pulmonary aspergillosis when untreated [50–52], (3) *Aspergillus* nodule(s), or (4) a single aspergilloma, which are less frequently seen [53–55]. All these manifestations are primarily found in immunocompetent patients. Clinical risk factors primarily are chronic respiratory diseases such as TB, NTM-PD, or other respiratory diseases leading to structural lung damage. Furthermore, (5) subacute invasive pulmonary aspergillosis (formerly called 'chronic necrotizing pulmonary aspergillosis') is usually found in moderately immunocompromised patients [56]. Radiological findings are usually similar to those of chronic cavitary pulmonary aspergillosis, but disease progression is more rapid (<3 months). However, each form of CPA may evolve into another over time depending on the immune status of the host, and may present as an overlap [49].

The diagnosis of CPA can be challenging and requires a combination of characteristics as described above. While the finding of *Aspergillus* hyphae in respiratory samples may support the diagnosis, it does not validly confirm it, since colonization, infection, and even contamination cannot be distinguished from one another. The detection of human *Aspergillus*-specific IgG antibodies is a key feature to diagnose CPA. It discriminates between infection and colonization with a positive predictive value of up to 100% [57]. Several commercial *Aspergillus*-specific IgG assays are available, but they show differences in sensitivity and specificity [58]. This is even more problematic when an in-house assay or *Aspergillus* precipitins are used. The latter is a gel-based immunodiffusion method used for decades to test patients' serum for the presence of precipitating antibodies to *Aspergillus* [59]. Although precipitin testing (beside *Aspergillus*-specific IgG antibodies) is also recommended by the novel ESCMID/ERS guidelines, more recent evidence indicates a poor performance with a sensitivity of 59% and a specificity of 100% [58]. Furthermore, this manual technique is time consuming, provides subjective results, and is difficult to reproduce [58, 60, 61]. Recently, Page et al. [58] compared the diagnostic performance of six *Aspergillus*-specific IgG antibody assays in a large cohort of CPA patients. The IMMULITE (Siemens Healthcare Diagnostics, Tarrytown, N.Y., USA; cutoff 10 mg/l; 96% sensitivity, 98% specificity) and the ImmunoCAP (Phadia AB,

Uppsala, Sweden; cutoff 20 mg/l; 96% sensitivity, 98% specificity) assays performed statistically superiorly to the other assays tested.

Surgical resection of a single aspergilloma is recommended as the treatment of choice. A combination with antifungal treatment is only indicated when the lesion has not been fully resected or multiple *Aspergillus* nodules are present. In case of severe hemoptysis, catheter embolization of bronchial arteries or surgery may be necessary [62, 63]. For all other forms of CPA, long-term antifungal treatment with oral triazoles such as itraconazole or voriconazole for at least 4–6 months is recommended [49]. In some patients, intravenous antifungal treatment (e.g. in patients with intolerance to or treatment failure with oral triazoles) or installation of antifungal agents in an aspergilloma cavity (e.g. when surgical resection is impossible) may be considered [49, 64, 65].

Is Personalized Medicine Established for Treatment of the Disease at Present?

The high incidence of TB, particularly in low-resource settings, determines the widespread use of standardized approaches under WHO guidance [18, 26]. If available, personalized therapy is mostly based on the use of DST to guide the composition of effective regimens. With the exception of rifampicin resistance testing, which can be done in a point-of-care test (Cepheid® GeneXpert; Cepheid, Sunnyvale, Calif., USA), resistance testing is complex [66]. Unfortunately, the treatment duration cannot currently be determined by personalized approaches, as no specific markers for cure have yet been identified. Treatment alterations based on host factors and TDM currently play a minimal role in the management of TB patients [15].

For NTM-PD no personalized medicine strategies are established, although its management heavily relies on personal opinions. The criteria for a differentiation between infection and disease are imprecise, making the diagnosis of NTM-PD difficult. Different management approaches depending on the NTM species isolated, the underlying diseases, or different disease presentations (e.g. nodular-bronchiectatic vs. fibrocavitary disease) are discussed but not established [34]. There is a severe lack of knowledge about the correlation of in vitro susceptibility with in vivo efficacy of antimicrobial drugs, making DST-guided therapy difficult [37]. Although patient risk groups are described, specific host susceptibility factors determining the small subpopulation affected with-

in these risk groups are not known, making prevention strategies difficult [32].

For CPA a personalized approach – including, for example, DST-guided therapy, treatment alterations based on (e.g. genetically determined) host factors, and/or TDM to improve disease management and outcome – has not been established so far [49]. Antifungal DST is available, but DST-guided therapy is not used for CPA routinely [67]. Although the novel ESCMID/ERS guidelines for the diagnosis and management of CPA allow for a consistent definition of the disease, a definition of the disease outcome is still missing [49].

Future Perspective: Personalized Medicine to Improve Clinical Management and Outcome (Prevention, Diagnosis, Treatment, and Monitoring)

The diagnosis of latent infection with *M. tuberculosis* (LTBI) that carries the risk for progression to active disease relies on the presence of a positive adaptive immune response to *M. tuberculosis* in the tuberculin skin test or a positive reaction in an interferon- γ release assay in the absence of active TB [68]. The positive predictive values of adaptive immune responses to *M. tuberculosis* are highly dependent on the disease prevalence. In the past years it has been shown that in countries with a low TB prevalence (e.g. Western Europe), the value of the tuberculin skin test and the interferon- γ release assay for the risk evaluation of future TB was very limited (progression rates of <2/100 patient-years) [69, 70].

New strategies for evaluating the risk of progression to disease are essential to reduce the number needed to treat to prevent a case of TB [71] and to lead to better acceptance of preventive chemotherapy in vulnerable groups [72]. Recently, in South African adolescents with LTBI a transcriptomic approach revealed a signature of 16 gene transcripts that was able to differentiate between healthy individuals and those who progressed to active disease [73]. The risk signature predicted TB progression with a sensitivity of 66.1% (95% CI: 63.2–68.9) and a specificity of 80.6% (95% CI: 79.2–82.0) in the 12 months preceding TB. Although the predictive power was reproduced in independent South African and Gambian cohorts with a sensitivity of 53.7% (95% CI: 42.6–64.3) and a specificity of 82.8% (95% CI: 76.7–86), the test has not been validated in individuals from countries with a low TB incidence to date; however, it seems to be a very promising approach which could change the management and definition of LTBI.

Fig. 1. Schematic explanation of TDM. Optimized dose adjustment algorithm in TDM. Blood samples are obtained as dried blood spots (DBS) by finger prick (3–5 spots per sampling time point). Time points are defined in the limited sampling strategy. A multianalyte method is employed to extract the antibiotics from the DBS. The extracts are analyzed using high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS).

Based on the results, an approximate pharmacokinetic profile is calculated. If the drug levels are too high and the patient is likely to experience adverse events, the drug dosage is reduced. Integration of the MIC of the respective pathogen results in a pharmacokinetic/pharmacodynamic (PK/PD) profile. If this profile reveals subtherapeutic drug concentrations, the dosage is increased. LSS = Limited sampling strategy.

The introduction and rollout of molecular techniques has revolutionized the diagnosis of *M. tuberculosis* and led to a more rapid detection of drug resistance and to the initiation of an appropriate therapy for patients with MDR-TB. For example, rapid molecular tests such as the automated, cartridge-based Xpert MTB/RIF (Cepheid) or the line probe assays GenoType MTBDR_{plus} and MTBDR_{sl} (Hain Lifescience, Nehren, Germany) have received support from the WHO for their implementation and are used in many settings across the world [74–76]. By far the most simple and rapid test is the Xpert MTB/RIF, for which the time to result is less than 2 h [77]. The results from the line probe assays are available within just 2 days and, based on the presence of particular drug resistance mutations, can be used to design an individualized treatment regimen for patients with MDR-TB [78]. Another new technology that can be used for resistance testing of *M. tuberculosis* strains as well as for outbreak investigation is whole-genome sequencing (WGS). WGS, in which the entire genome of *M. tuberculosis* is sequenced

and analyzed, has been shown to have a relatively rapid turnaround time and to predict resistance mutations with good sensitivity and specificity [79, 80]; it could be used in the future to guide the selection of anti-TB drugs for patients with MDR-TB before phenotypic drug susceptibility results are available. Although it has been shown that WGS can be performed from direct sputum samples [81], in most cases, cultures of *M. tuberculosis* strains are used. WGS can also be used for outbreak investigations, and thus may lead to a better understanding of transmission pathways and to the introduction of measures for preventing further transmission [77, 82]. The cost of WGS – prohibitive in the past – can be less than £70 per bacterial genome [83], which is insignificant when compared to the costs of MDR-TB therapy [13]; however, the use of molecular tests is limited in some settings due to their relatively high cost, technical complexity, and the requirement of an adequate laboratory infrastructure.

TDM is of increasing interest in the management of TB treatment (fig. 1). Measuring drug concentrations in

patients' blood may contribute to increased efficacy and optimized dosing in difficult-to-treat patients by adjustment of drug dosages according to drug plasma concentrations [84] and may particularly reduce the toxicity of second-line drugs. In particular, TDM avoids subtherapeutic drug concentrations that may result in functional monotherapy. Therefore, it might have the potential to prevent the development of resistance to additional drugs [85].

Drug levels are mostly determined either as peak concentrations ($C_{\max}$) or as areas under the curve (AUCs). It is recommended to use the ratio of these parameters and the minimal inhibitory concentration (MIC) of the respective *M. tuberculosis* strain to address the pharmacodynamic effect [86]. The parameters that are employed (e.g. $C_{\max}$ /MIC or AUC/MIC) to assess the pharmacodynamic effect are drug specific and still subject to discussion. Determination of drug kinetics requires several blood samples. The sampling strategy is dependent on the targeted parameter and the respective drug. $C_{\max}$ can be determined by analyzing two blood samples, but AUC requires a minimum of 10–15 samples involving a considerable amount of time and costs. Therefore, limited sampling strategies that minimize the number of samples but still achieve an AUC approximation are required [87].

Analysis of blood samples is performed either as a single [88] or (especially in regimens containing multiple second-line drugs) a multianalyte approach using high-performance liquid chromatography and tandem mass spectrometry [89]. Due to its complexity this assay is restricted to a few specialized centers. For the use of TDM at sites lacking this specialized infrastructure, the collection and transport of dried blood spots may facilitate sample logistics [15]. Dried blood spots have been developed for five different antimicrobial drugs so far [87].

Reference values for drug concentrations as well as the benefit from TDM, however, remain unclear. Studies that report an association of TDM with treatment outcome and acquired drug resistance are scarce [90–92]. The reported associations were often not based on those reference values but used regression or multivariate analysis [84, 90–92]. Further studies are needed to confirm or revise the current reference values and to assess the benefit from TDM.

A major difference between mycobacterial infections (NTM and TB) and CPA and other infectious diseases is the duration of treatment recommended to achieve relapse-free cure. However, not even consistent definitions of disease outcome (at least for NTM-PD and CPA) are currently available; while treatment for common bacte-

rial pneumonia is recommended to last from 3 to 14 days, treatment for pan-susceptible TB is recommended for 6 months and treatment for MDR-TB for at least 20 months with combination antimicrobial therapy [11]. Newly proposed short-course regimens of 9–12 months are rarely applicable for patients in Europe (manuscript submitted). Treatment for CPA and NTM-PD is generally recommended for 6 and 12 months, respectively, despite large inpatient variability in disease severity and response to therapy [34, 49].

Patients with chronic respiratory diseases would benefit from biomarkers that serve as surrogates to identify treatment success or failure early in the course of treatment [14, 15]. As an example, TB-specific, mainly interferon- γ -driven transcriptional profiles and their changes upon treatment initiation in patients with susceptible TB have been described [93–95]. Transcriptional changes that can be detected as soon as 2 weeks following therapy initiation were characterized in patients with susceptible TB [93]. In addition, the kinetics of cellular markers (i.e. peripheral T-regulatory cells, exhaustion T cells) and inflammatory proteins (i.e. vascular endothelial growth factor, interferon- γ -inducible protein 10) in the course of treatment was also described [96–98]. Biomarkers or compound markers consisting of blood markers, radiological assessment by chest X-ray, CT or positron emission tomography-CT, and clinical scores could eventually help to subsequently individualize the duration of therapy for patients affected by chronic respiratory infections [14, 99, 100].

Prevention strategies for NTM-PD and CPA are unknown. They could be based on identification and better understanding of the bacterial or fungal reservoirs and of host susceptibility factors. WGS of the host genome could detect a genetic predisposition to NTM-PD and CPA acquisition, but no studies have investigated this until now. Differentiation between colonization and real infection as well as contamination of respiratory samples is one of the key challenges in NTM-PD and CPA management. For NTM-PD, IgA directed against an MAC-specific glycopeptidolipid core antigen has been analyzed, showing the potential to differentiate colonization from infection with a high specificity of 89–100%, even if its sensitivity is highly variable at 52–92% [101–103]. Further studies need to be conducted to prove its usefulness. Concerning treatment, in vitro studies aiming at a better understanding of current and future drug regimens for NTM are being conducted [104–108]; still, prospective clinical trials to examine in vitro and in vivo correlations are lacking. A few novel antimicrobial drugs for the treatment of

NTM-PD are attracting attention. Liposomal amikacin for inhalation is currently under investigation for MAC-PD patients who have failed to respond to standard treatment regimens, and it has shown promising preliminary results [109]. Bedaquiline was given to 10 patients with therapy-refractory *M. abscessus*- and MAC-PD, showing clinical (90%) and microbiological (50%) improvement [110]. Identification of biomarkers guiding the decision when to initiate NTM-PD therapy and allowing the prediction of treatment failure or success early in the course of therapy would be useful, but such biomarkers have not been investigated so far either. For CPA, *Aspergillus*-specific IgG antibody has been shown to differentiate between colonization and infection with a sensitivity of 96% and a specificity of 98% [58]. Antifungal prophylaxis in patients at high risk for CPA may prevent disease development; however, host factors identifying patients at high risk are unknown. Furthermore, personalized disease management including TDM for CPA has not been established so far, even if some studies indicate that routine TDM in patients with invasive aspergillosis may reduce drug discontinuation due to adverse events and improve the treatment response [111, 112]. Despite invasive aspergillosis, CPA requires long-term triazole treatment, which makes TDM even more attractive to improve the individual treatment outcome; however, this would imply a definition of disease outcome, which is currently not established for CPA.

The increased drug resistance of microorganisms and the limited drug treatment options have drawn attention to adjunct host-directed therapy. Vaccination, antiretroviral therapy, and the immunomodulating properties of antimicrobial drugs (e.g. macrolides, clofazimine, pyrazinamide, and co-trimoxazole) belong to this category. In general it is still unknown whether with immunoadjuvants and interfering cytokines ought to tip the balance towards proinflammation or anti-inflammation [113, 114].

A wide range of approaches has been explored, including sunlight exposure, melatonin, supplementation with vitamins A, C, and D, and treatment with corticosteroids [115]. Treatment with corticosteroids was validated by a meta-analysis including 41 trials, including 20 studies conducted before rifampicin was introduced for TB treatment. Corticosteroids were shown to reduce mortality by 17%, consistently across all organ manifestations [116]. The only vitamin that found its way into clinical practice is pyridoxine (vitamin B₆) as a supplement to prevent isoniazid-related neuropathy. The evidence for ascorbic acid (vitamin C) is very poor and based on data from a single group [117]. For vitamin D, several studies have demon-

strated no therapeutic effect on pulmonary TB with the exception of individuals with the *tt* genotype of the *TaqI* vitamin D receptor polymorphism, opening a window to personalized medicine [118].

New anti-inflammatory targets, such as ibuprofen [119], the phosphodiesterase inhibitors cilostazol or sildenafil [120], or the autophagy-inducing drugs verapamil [121] or reserpine [122], aim to restrict organ damage due to aversion of *M. tuberculosis*-induced inflammation. Metformin and statins should augment immune defense [123]. An outstanding example of personalized medicine with promising results is found in a trial evaluating the reinfusion of autologous bone marrow-derived mesenchymal stromal cells into patients with MDR/XDR-TB [124].

Other personalized attempts at searching for underlying immune defects that facilitate chronic mycobacterial or fungal infections in single individuals resulted in various host-directed cytokine-driven therapy approaches aiming to readjust the natural defense mechanisms (table 2). Therapeutic cytokine administration in active TB has been studied for interferon- γ , IL-2, and IL-12. Although a meta-analysis suggested a potential benefit from adjuvant interferon- γ , large randomized trials are needed to further evaluate this option, considering that only 9 trials with aerosolized, intramuscular, and subcutaneous administration routes were analyzed and the microbiological outcomes differed [125]. Clinical data on IL-2 administration are controversial, with beneficial results in MDR-TB but no effect in susceptible TB [114]. It has been suggested that the inflammatory response triggered by *M. tuberculosis* varies with the drug resistance profile; however, further data are needed to implement this compound in a clinical routine. For individual patients with IL-12 receptor- β 1 deficiency, adjuvant treatment with IL-12 has been shown to be beneficial [126].

Patients with chronic respiratory infectious diseases often suffer from multiple comorbidities. A simple disease-focused approach to these patients may fail to encompass the complexities linked to the interplay of their diseases [127]. Transition of care between multiple physicians and between in- and outpatient care may even lead to medical errors [128]. In the outpatient setting, the management of complex cases should be undertaken by experts, and individual solutions and settings tailored to a patient's medical and social situation have to be found by a multidisciplinary team which may be led or supported by case managers or TB link workers who integrate the care for individual patients [129, 130]. Such teams involving physicians, psychologists, physiotherapists, social

Table 2. Examples of approaches to host-directed therapies in chronic respiratory infections

Microorganism	Concept	Stage	Comment
<i>Aspergillus fumigatus</i>	CAR+ T-cell therapy [132]	Rodent model (NOD SCID- γ mice, immunosuppression with cyclophosphamide)	Treatment with dectin-1-specific CAR+ T cells resulted in diminished fungal infection and better outcome in infected mice
<i>Aspergillus nidulans</i>	G-CSF substitution [133]	Human application (case report)	A 16-year-old patient with chronic granulomatous disease and <i>A. nidulans</i> osteomyelitis was successfully treated with liposomal amphotericin B, extensive surgical debridement, itraconazole, and G-CSF
<i>Mycobacterium avium-intracellulare</i>	IL-2 substitution [134]	Human application (case report)	A 39-year-old patient with idiopathic CD4+ lymphopenia and pulmonary infection with <i>M. avium-intracellulare</i> was successfully treated with subcutaneous IL-2 following clarithromycin
<i>Mycobacterium avium-intracellulare</i>	IFN- γ substitution [135]	Human application (case report)	A 20-year-old patient with a homozygous homomorphic I87T mutation in the gene encoding the ligand-binding chain of the IFN- γ receptor and disseminated infection with <i>M. avium-intracellulare</i> was successfully treated with subcutaneous IFN- γ
<i>Mycobacterium tuberculosis</i>	Adjuvant IFN- α inhalation [136]	Human case series	Inhalation of IFN- α reduced the bacterial load in 5 of 7 patients not responding to standard antimycobacterial therapy
<i>Mycobacterium tuberculosis</i>	MSC infusion [124]	Phase I trial in patients with MDR/XDR-TB	In 30 patients, a single adjuvant MSC infusion 4 weeks after antimycobacterial treatment initiation was safe and well tolerated; two grade 3 adverse events were recorded
<i>Mycobacterium tuberculosis</i>	IL-2 substitution [137]	Phase III trial in HIV-seronegative patients with drug-susceptible TB	In 110 Ugandan patients (treated with 225,000 IU IL-2 vs. placebo adjunctive to the first 30 days of standard antimycobacterial therapy) no effect was seen on bacillary clearance or symptoms
<i>Mycobacterium tuberculosis</i>	IFN- γ substitution [125]	Several studies in TB patients	IFN- γ administration varied from subcutaneous or aerosolized (1 trial) and aerosolized (5 trials) to i.m. (3 trials); no treatment discontinuations due to side effects; for i.m. application, sputum conversion improved; symptomatic improvement was reported
<i>Mycobacterium tuberculosis</i>	IL-12 substitution [126]	Human application (case report)	3 months of adjuvant IL-12 to standard antimycobacterial therapy improved the outcome in 1 patient with progressive drug-susceptible TB

CAR = Chimeric antigen receptor; G-CSF = granulocyte colony-stimulating factor; IFN = interferon; i.m. = intramuscular; MSC = mesenchymal stromal cell; NOD SCID- γ mice = severely immunodeficient mice.

care workers, pharmacists, and ambulatory nurses (e.g. for ambulatory intravenous treatment) – as well as other auxiliary staff as required (e.g. such as professional translators for immigrants and refugees) – should work in close collaboration with health authorities and established social support systems such as accommodations for homeless people or methadone replacement programs.

Conclusion

Medicine is presently at a transitional stage between programmatic and personalized management concepts. In the near future, scientific innovations will allow substantial improvements by computer-derived, algorithm-based risk assessment and diagnostics in many disease areas including that of chronic respiratory infectious dis-

eases. They will hopefully lead to precise, tailor-made therapeutic interventions with substantial advances in treatment outcomes.

Recent scientific progress in the area of whole-blood transcriptomic analysis opened the door for improving the prediction of the future development of TB, NTM-PD, and CPA in individuals from risk groups and for the discrimination of active disease from colonization with direct implications for individual treatment decisions. WGS will provide opportunities to identify host genetic factors increasing disease susceptibility and adverse events on therapy. Rapid and automated sequencing of entire genomes of microorganisms provides computed information for the prediction of phenotypic drug resistance by identification of resistance-associated mutations. This technology will enable physicians to select customized treatment regimens shortly after an initial diagnosis and will have a dramatic impact on the management of pa-

Fig. 2. Different elements of personalized medicine for chronic respiratory infections.

tients with MDR/XDR-TB. Prediction of minimal inhibitory drug concentrations by genomic information put into relation to blood level drug measurements will optimize individual treatment outcomes on antimicrobial therapies. With a better understanding of the correlates of immune protection and by identifying distinct immune signatures, host-directed therapies may potentially augment antimicrobial treatments for chronic respiratory infections and at the same time prevent overshooting immune responses causing irreversible tissue destruction. Predictive biomarkers will be used in clinical practice to predict treatment outcomes. They will enable physicians to individualize the duration of therapy needed to achieve relapse-free cure from chronic respiratory infections.

Although this scenario appears futuristic, individual aspects of the personalized medicine concept have already been realized at specialized centers for the benefit

of patients (fig. 2). Putting the elements together and making the concept available and affordable will be a forthcoming challenge.

On average, patients with chronic respiratory infections remain hospitalized much longer than patients with other medical disorders. Personalized medicine also needs to take the psychosocial and physical needs of patients into account by providing individual psychosocial support, physiotherapy, and rehabilitation.

We have no doubt that personalized medicine will improve decision-making for individual patients. However, it should still be patients and physicians who make personal decisions, not computers. While computerized algorithms will improve informed decision-making, they are not intended to serve as a substitute for the physician-patient relationship – only the latter makes medicine truly personal.

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