Short Communication

Time discrepancy for tuberculosis-negative microscopy and culture - the diagnostic gap remains: systematic analysis from a large tertiary care tuberculosis-clinic, Germany 2013-2017

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ABSTRACT

Objectives: Patients with open pulmonary tuberculosis (opTB) are subject to strict isolation rules. Sputum smear microscopy is used to determine infectivity, but sensitivity is lower than for culture. This study aimed to investigate the clinical relevance of this mismatch in contemporary settings.
Methods: Differential results between microscopy and culture were determined at the time of microscopic sputum conversion, from all patients with opTB between 01/2013 and 12/2017. In addition, data on HIV, multi/extensive drug-resistant TB status, time to smear- and cultural-negativity conversion were analyzed; and a Kaplan-Meier curve was developed.
Results: Of 118 patients with opTB, 58 had demographic data available for microbiological and clinical follow-up analysis; among these, 26 (44.8%) had still at least one positive culture result. Median time from opTB-treatment initiation to full microscopic sputum- or culture conversion, was 16.5 days (range 2-105), and 20 days (1-105), respectively (median difference: +3.5 days). Sixteen days after de-isolation, >90% had converted culturally. HIV- or multi/extensive drug-resistant TB status did not impact conversion time.
Conclusion: When patients with opTB were de-isolated after 3 negative sputum smear microscopy tests, a substantial part still revealed cultural growth of *Mycobacterium tuberculosis* complex, but it remains unclear, whether smear-negative and culturally-positive individuals on therapy are really infective. Thus, the clinical relevance of this finding warrants further investigation.

Introduction

Tuberculosis (TB) is still a major cause of morbidity and mortality worldwide [1] and therefore, the public healthcare viewpoint of TB infection control and prevention suggests isolating patients with "open" pulmonary TB (opTB) [2]. Traditionally, whether patients are categorized as "open" or not, depends on microscopic detection of acid-fast bacilli obtained from respiratory samples. In this regard, the patient's status can promptly be provided by clinical routine microscopy results. It could be shown, that the quantity of acid-fast bacilli in sputum smears correlates with the time to culture positivity for *M. tuberculosis* before and during TB treatment [3]. In contrast, it takes up to 8-12 weeks, until definitively negative cultural results are available. Because culture is more sensitive than microscopy [4], a diagnostic gap can result, if subjects match criteria for microscopic sterility, but still can be open by culture at low pathogen density and thus, theoretically, may be infectious to close contact persons. Scientific knowledge on this clinically relevant time gap between microscopic- and cultural-negativity is well-known for decades [5], however, diagnostic investigations have hardly been undertaken while patients have been treated in modern diagnostic and therapeutic time era [6]. The purpose of this retrospective analysis was to identify the rate of posi-

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Table 1
Demographics and characteristics of analysis cohort patients (n=58).

Parameter		Analysis cohort (n=58)		
		N (%)	Median (range)	Mean (+/-SD)
Age [years]:			30.92 (9.3;78.1)	36.3 (+/- 17.1)
Gender:	Female	19 (32.8)		
Origin:	European	24 (41.4)		
	African	16 (27.6)		
	other	7 (12.1)		
	unknown	11 (19)		
HIV test result [n]:	Positive	6 (10.3)		
	Negative	52 (89.7)		
MDR/XDR-TB [n]:	Positive	9 (15.5)		
	Negative	49 (84.5)		
Specimen type at first diagnosis TB:				
	sputum	30 (52)		
	BAL	7 (12)		
	provoked sputum	11 (19)		
	Bronchial secretion	10 (17)		
Time [days] until first smear-negative microscopy result after first diagnosis with opTB			0.5 (0;98)	10.6 (+/- 19.6)
Time [days] until completely smear-negative (stop isolation = 3 consecutive smear-negative/microscopy after first diagnosis with opTB)			16.5 (2;105)	26.2 (+/- 28.4)
Time [days] until first culture-negative result (after first diagnosis with opTB)			13.5 (0;88)	21.1 (+/- 23)
Time [days] until completely culture-negative (non-infectious = 3 x consecutive culture-negative after first diagnosis with opTB)			20 (1;105)	32.5 (+/- 30.2)
Main lab results at first diagnosis TB:				
Leukocytes [/nL]			8.1 (1.4; 15)	8.14 (+/- 2.9)
(Reference value: 4-10 /nl)				
Hemoglobine [g/dl]			11.4 (7.2; 16)	11.5 (+/- 1.92)
(Reference value: Men 14-18g/dL; Women: 12-16g/dL)				

Legend: min – minimal range; max – maximal range; SD – standard deviation; n – number; HIV – Human-immunodeficiency virus; MDR/XDR – multi/extensive drug resistant tuberculosis; pTB – pulmonary tuberculosis; mg – milligram; dL – decilitre; mL – millilitre; opTB open pulmonary TB

tive cultural findings in subjects with initial opTB disease, at time of sputum smear conversion during controlled TB therapy, and to examine the average duration time of this unconformable finding.

Patients, methods, and study design

A systematic laboratory database query from a large German tertiary care university hospital identified all patients with culturally confirmed *Mycobacterium tuberculosis*-complex-infection, obtained from respiratory specimens between January 2013 and December 2017. Corresponding individuals with available data in electronic treatment charts were reviewed retrospectively for clinical and microbiological parameters, including additional microscopic and cultural findings. Routine laboratory results and demographic data were collected at time of diagnosis and during the follow-up TB treatment course. According to national infection control guidelines [2], patients with opTB have to deliver at least three consecutively negative lower respiratory tract smear samples after a 2-week course of therapy, before discharge from TB isolation, usually treated as inpatients. In addition to national guidelines, all obtained respiratory tract specimens requested for acid-fast bacilli staining underwent both smear microscopy and cultural testing, after a routine local laboratory departmental note, and was repeated in case of any positivity. All individuals were followed as outpatients in the same clinical department after de-isolation. Appointment schedules were individualized, as needed for e.g., drug toxicity checks. At any visit, patients were asked to deliver a sputum sample for smear testing. Treatment was in concordance with contemporary national therapy guidelines principles [5]. The study was approved by the Frankfurt University Medical School Ethics Committee (Vote-No. 2018-195, September 20, 2018).

Continuous variables were displayed as both mean (±SD) and median (ranges), and categorical variables as frequencies and percentages. Kaplan-Meier-curve showed the time until culture conversion, following negative smear microscopy. All calculations were carried out with R for Statistical Computing (The R Foundation, v4.0, Vienna, Austria) and BiAS (BiAS for Windows, v11).

Results

Overall, of the 118 patients with cultural-positive opTB, we identified 58 as the study analysis cohort, eligible for further analysis by simultaneously available culture- and smear follow-up results. The remaining 60 individuals were excluded due to the following reasons (multiple nominations allowed): non-evaluable TB regimens (57%), missing documents (27%), death before conversion (12%), and loss of follow-up (17%). Most patients originated from (central/eastern) Europe (43.2%), followed by (in particular) sub-Saharan Africa (21.2%), Asia (12.7%), and various or unknown (22.9%). HIV-coinfection was reported in 12.7%. A detailed characterization of the remaining analysis cohort is presented in Table 1.

Mean time from TB diagnosis establishment and subsequent anti-TB therapy initiation to microscopic negativity, was on average 26.2 days (SD ± 28.4), compared to 32.5 days (SD ± 30.2) for cultural-negativity, each in three consecutive specimens; the median respective time periods were 16.5 days (range 2-105), and 20 days (1-105), with a difference of +3.5 days (Table 1). Of those who had just reached microscopic sterility (n = 58), there were still 26 (44.8%, 95% confidence interval: 27-62.7%) positive by residual cultural growth of *M. tuberculosis* in at least one specimen obtained from the respiratory tract. Culture negativity rates in the small subgroups of HIV-positive (n = 6) and multi/extensive
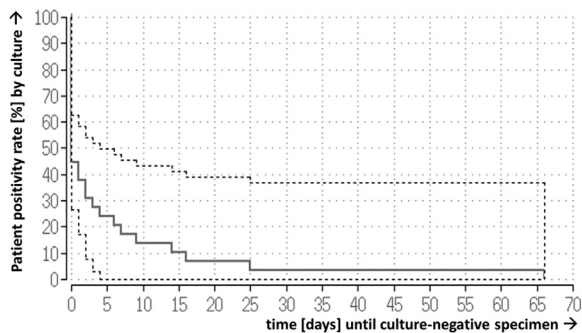
Figure 1. Kaplan-Meier-curve shows the time gap for reaching culture negativity (per individual, from analysis cohort, n = 58), after microscopy conversion to negative for acid-fast bacilli in consecutive three probes (=baseline), obtained from the respiratory tract. Findings are displayed in solid lines (confidence intervals in dashed lines).

drug-resistant TB-affected individuals (n = 9), did not differ from other patients with opTB without HIV, or those with susceptible TB isolates, respectively (data not shown). The Kaplan-Meier-curve (Figure 1) for the analysis cohort (n = 58) shows the temporal difference between cultural and microscopic negativity, i.e., the diagnostic gap at time of de-isolation. During follow-up, >90% had culturally cleared TB 16 days later. Only 1 of 58 individuals remained positive by culture beyond day 25, for a total time period of 65 days.

Discussion

In low TB-burden countries, smear-negative and culture-positive sputum patients may account for relevant transmission rates, of up to 17% [7]. Therefore, this large monocentric retrospective German cohort study aimed to investigate the diagnostic gap and the probability of discordant results between smear microscopy- and culture conversion in de-isolated patients with previous opTB. Most patients (>50%) with initial opTB, revealed no growth of *M. tuberculosis* after three consecutive microscopically negative respiratory specimen probes. However, 26 of 58 individuals (44.8%) from the analysis cohort were still culturally-positive, when de-isolation criteria had been fulfilled - and thus could theoretically still infect contact persons. The clinical importance of that "diagnostic lagtime" may define a potential "latent infectious risk". Although, even more relevant than any bacteriological sputum-finding, seems to be the time on effective anti-TB therapy, as discussed within the literature [8,9], and this was confirmed by the second finding in our study: the probability of a positive cultural finding diminished rapidly on continued controlled anti-TB therapy, and growth was only exceptionally observed after >7-14 days following smear-negativity (see Figure 1).

The true infectivity for only culture-positive subjects is hard to determine during effective treatment, because one single viable bacterium in the sample may turn the culture test result qualitatively positive, but hardly will mean infectivity of the person. Beyond the only evidence, better the number of viable bacteria in the sputum sample will correlate with the patient's infectivity, in particular after a prolonged time on controlled therapy. Evidence of acid-fast bacilli in sputum smear or molecular testing, would not necessarily mean TB infectivity, because detection cannot distinguish between viable and nonviable *M. tuberculosis* [10].

This investigation has several limitations, including low case number, and documentation in a retrospective study design, probably resulting in failing statistical significance and broad confidence intervals in Kaplan-Meier curve. This observation may be explained by a broad clinical case spectrum, including cavitary lung and mi-

nor infiltrative TB, with variable bacterial load. Therefore, conclusions should be drawn with caution from the findings. The latter could be overcome by higher case numbers and prospective settings.

In conclusion, we found a substantial part of patients with opTB, still revealed cultural growth of *M. tuberculosis*, when isolation duty was lifted after 3 negative sputum smear microscopy tests. This finding had vanished for >90% of previously opTB subjects 16 days later. Further studies are warranted, to investigate the clinical impact of this finding and its' repeatability, and/or what consequences regarding the prolongation of contact precautions may be drawn.

Author contributions

C. Stephan: conception and design, data acquisition, interpretation, writing the first draft, final validation, submission, project administration, supervision. J. Kessel: data acquisition, writing first draft, review, and approval. E. Göymen: major data analysis, interpretation, critical review, final approval. Ü. Balaban: formal analysis, critical review, and editing, final approval. T. Wolf, N. Wetzstein, C. Küpper-Tetzel, P. Behrens, F. Borgans and M. Hogardt: data acquisition, interpretation, critical review, and final approval. T.A. Wichelhaus: major data acquisition and interpretation, critical review, final approval, and project supervision.

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