

OVERVIEW

The never-ending story of the fight against tuberculosis: from Koch's bacillus to global control programs

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Summary

*Tuberculosis (TB) is one of the oldest diseases known to affect humanity, and is still a major public health problem. It is caused by the bacillus *Mycobacterium tuberculosis* (MT), isolated in 1882 by Robert Koch. Until the 1950s, X rays were used as a cheap method of diagnostic screening together with the tuberculin skin sensitivity test. In the diagnosis and treatment of TB, an important role was also played by surgery. The late Nineteenth century saw the introduction of the tuberculosis*

sanatorium, which proved to be one of the first useful measures against TB. Subsequently, Albert Calmette and Camille Guérin used a non-virulent MT strain to produce a live attenuated vaccine. In the 1980s and 1990s, the incidence of tuberculosis surged as a major opportunistic infection in people with HIV infection and AIDS; for this reason, a combined strategy based on improving drug treatment, diagnostic instruments and prevention was needed.

Introduction and historical approach

Tuberculosis (TB) is one of the oldest diseases known to affect humanity [1]. It is caused by the bacillus *Mycobacterium tuberculosis* (MT): the bacilli usually multiply in the lungs and are spread by air-borne droplets from infected persons [2]. The disease reached epidemic proportions in Europe and North America during the Eighteenth and Nineteenth centuries [3] and today, with around 10.4 million new cases each year, almost one third of the world's population are carriers of the TB bacillus and at risk of developing active disease. India, Indonesia, China, Pakistan and the Philippines account for 56% of new tuberculosis cases [4], while the Democratic People's Republic of Korea, Lesotho, Mozambique, the Philippines and South Africa have the highest incidence rate, with over 500 new cases per 100,000 inhabitants [5]. In the past, MT displayed a predilection for young people and was called the "thief of youth"; in most cases, it proved fatal, with a death proportion in the pre-chemotherapy era: roughly 65% at 5 years, especially when it was associated to malnutrition, overcrowding and poor living and working conditions [6]. Over the last two centuries, the disease felled an estimated one billion people worldwide, and today, despite effective chemotherapy, it is estimated to be responsible for 1.7 million deaths, including the 380,000 among people with HIV infection normally classified under AIDS deaths by ICD convention, being the second greatest cause of mortality among infectious diseases in the world after human immunodeficiency virus (HIV) [1, 5]. Villemin was the first to prove that human tuberculosis was a contagious

disease transmitted by an infectious agent, *Mycobacterium tuberculosis* [7]. This microbe was discovered and isolated in 1882 by the famous scientist Robert Koch, who was awarded the Nobel Prize for Medicine in 1905. After Villemin's definitive nosographic classification, the ravages of tuberculosis spurred many scientists in the following decades to seek an effective way of curbing the disease, and different and sometimes conflicting public health strategies were implemented [8].

Isolation and staining of the Koch bacillus

Robert Koch observed that the alcohol-methylene blue staining developed by Karl Weigert in 1875 did not easily stain in tubercular lesions [9, 10]. He therefore implemented an innovative method of observing tubercular lesions by means of methylene-blue and a solidified medium of coagulated bovine serum heated to 40°C [11]. Using this staining technique, Koch reported that animal tissues and bacteria stained brown, whereas tubercular bacteria were easily detected on microscopy, as they appeared bright blue [12]. This innovation enabled Koch to prove that the various manifestations of tuberculosis, such as scrofula, pulmonary, extra-pulmonary and meningeal tubercular diseases, had a common cause, and also allowed him to reproduce the disease in experimentally inoculated guinea pigs [6, 11, 13-18]. Inspired by Koch's work, Paul Ehrlich further modified the staining protocol and began working on a better method of staining, using aniline water, fuschin and gentian-violet: the blue background counterstain showed

up the bacilli as red spots [18]. In 1882, Franz Ziehl introduced carbol fuschin instead of aniline, and Friedrich Neelson substituted sulphuric acid for nitric acid, which stained the bacillus a brighter red; this was known as the Ziehl-Neelson (ZN) stain or acid-alcohol-fast bacillus (Aafb), since, once stained, the MT bacillus lipidic wall resists decoloring by acid and alcohol; for this reason, this technique is also used nowadays in order to isolate the MT bacillus [15]. In the following decades, microscopy continued to be the gold standard isolation method, and enabled a diagnosis to be made in a short time with high specificity. Moreover, it was cheap and simple to use [19]. In order to improve sensitivity, other methods of concentration, such as centrifugation, N-acetyl cysteine-sodium hydroxide, bleach, ammonium sulfate or chitin, were tested and used in countries where the disease displayed a high prevalence. In addition, the classical ZN staining was replaced by Kinovon staining and immunofluorescence [19]. In more recent years, other cold staining methods were used to diagnose tuberculosis, such as Gabbet's method and a modified two-reagent cold staining method, which were less time consuming and easier to perform in the field [20]. Currently, the fluorescent stain auramine is used to better detect positive smears. In the past, its use was limited by the need to replace the light source in fluorescent microscopes every 200-300 hours. Subsequently, the innovative use of light-emitting diodes (LED), which last for tens of thousands of hours, proved to be a valid and more economical alternative to fluorescent microscopes, and today the World Health Organization (WHO) recommends replacing conventional ZN microscopy for the diagnosis of pulmonary tuberculosis even in resource-poor countries [21, 22].

X-rays and the tuberculosis skin test: two effective diagnostic methods

Wilhelm Konrad von Röntgen was awarded the Nobel Prize for his contribution to the control of tuberculosis through his studies and his 1895 discovery of X-rays, which were used as an effective TB diagnostic technique. By using a Crooke's cathode, he succeeded in producing a shadow of a metal object on a photographic plate. This enabled him to visualize internal organs and detect TB lesions in the lungs, from Ghon foci to apical cavitation. This technique was used as a cheap method of diagnostic screening until the 1950s, together with the tuberculin skin sensitivity test. Nowadays X-rays are still used as a screening tool. In 1890, Koch obtained from tubercular bacilli a concentrated filtrate of liquid cultures, called "tuberculin" or "Koch's lymph" [23]. He believed it was an effective method of curing animals of tuberculosis, and announced his intention to test it in humans. Tuberculin was later nicknamed "la kockine", in French "coquine", which means "mischievous" as in "playful", but also as in "causing mischief" [7]. Koch had prepared the substance by means of crops in glycerin broth in six weeks, killed by heating at 100°C, filtered and evaporated to one

tenth. Up to 0.25 ml of these crops could be injected into humans [24, 25]. It was a clear, brownish liquid, which was easy to store. However, it could be used in humans only in a very diluted form because it caused very violent reactions, such as inflammation at the site of inoculation, intense tuberculous reaction, high fever, expansion of tubercular lesions, more or less extensive necrosis and the onset of nephritis. This liquid was active only if injected subcutaneously, not when ingested. In healthy individuals, the subcutaneous injection of tuberculin did not cause any sort of reaction, while if injected into a subject already affected by the infection, it elicited an intense reaction, including fever, chills and vomiting, as happened to Koch himself [26, 27]. Tuberculin retained a place in the treatment of some forms of tuberculosis until the Second World War; later, it found application in the diagnosis of tuberculosis: the famous "tuberculin reaction" test, which used the same principle involved in the diagnosis of other diseases, such as glanders (Mallein Est) and brucellosis (brucella test) [24]. In 1907, a Viennese pediatrician and immunologist, Clemens Freiherr von Pirquet (1874-1929), used cutaneous tuberculin scratch tests, later called the Pirquet test, to diagnose in children "latent tuberculosis", a term which he himself introduced into medicine. The reaction to tuberculin, which he called "allergy", unmasked contagion even in individuals who showed no sign of the disease or who had overcome the clinically manifested disease [28]. In 1910, a French physician, Charles Mantoux (1877-1947), improved Pirquet's method, by using a cannulated needle and syringe to inject tuberculin between the layers of the skin. This permitted more accurate control of the dose of tuberculin used. Today, the gold standard test used to diagnose TB is tuberculin purified protein derivative (PPD). Developed in the 1930s by two American biochemists, Florence B. Seibert and Esmond R. Long, this is a more sensitive test than tuberculin [6, 28]. In 1952, Carroll Palmer and Leroy Bates published the results of a large study of reactions to one test unit of PPD-S in more than 3,000 hospitalized tuberculosis patients [29]. Fewer than one percent failed to react; skin reactions were normally about 15 mm in size. Some years later, other studies conducted in hospitalized tuberculosis patients and in school children in various parts of the world demonstrated that, in countries with a high prevalence of tuberculosis, a significant percentage of healthy school children displayed skin reactions that were similar in size to those seen in tuberculous patients [30]. This finding confirmed von Pirquet's theory of latent tuberculous infections, as did LB Edwards and CE Palmer's experiments, in which nonspecific tuberculin reactivity was caused by the cross-reaction to the antigen of environmental mycobacteria in guinea pigs [31]. Thus, tuberculin skin test studies contributed to our understanding of tuberculosis and other non tuberculous mycobacterial infections and of the immune response to human and animal TB [32].

The role of surgery in treating tuberculosis: pneumothorax

In the diagnosis and treatment of tuberculosis, an important role was also played by surgery and other invasive techniques. Indeed, some anatomic and physiological conditions originally observed in patients with other diseases were successfully exploited to treat tuberculosis. The first such case was described in 1696 by Giorgio Baglivi, who reported that the clinical TB of a patient who suffered pneumothorax as a result of a sword wound improved. Later, in 1771, Edmond Claude Bourru, a librarian at the Faculté de Médecine in Paris, first suggested the benefit of lung collapse. Subsequently, in 1834 in London, FH Ramage reported the first successful therapeutic pneumothorax in one of his patients. In order to cure and prevent the spread of TB infection, two surgeons, Edouard Bernard de Cérenville and Max Schede, used a new method of unilateral rib resection, called thoracoplasty, between 1885 and 1890. This enabled them to reduce thoracic cavity volume and collapse tuberculous cavities. In 1888, Carlo Forlanini, an Italian physician, undertook a new therapeutic intervention; he created the first artificial intrapleural pneumothorax by collapsing the lung and filling the pleural cavity with nitrogen. This technique at first appeared paradoxical, since spontaneous pneumothorax had, up to then, been seen as one of the complications of pulmonary tuberculosis. This therapeutic technique was successfully applied until the 1970s and was abandoned only after the advent of anti mycobacterial drug therapies [33, 34]. Thoracoplasty, which had first been introduced by the Swiss surgeon De Cérenville, was taken up by two Norwegian physicians, L Brauer and PL Fridrich, as a diagnostic technique for tuberculosis; its safety profile was very good as it did not involve the pleural space, and so minimized the risk of tuberculous empyema. Surgical treatments for tuberculosis were developed and used until the 1940s. However, they were followed by some complications, such as infections, fistula and empyema. The efficacy of these interventions was reported in various papers. For instance, in 1939 the Briton Oli Hjaltested published the results of a study conducted on 191 patients between 1925 and 1931 [35], while another paper described the outcome of 557 patients treated with pneumothorax between 1930 and 1939 [36]. After the discovery of effective antibiotic therapy, surgical treatments were progressively abandoned in favor of safer treatment. Nevertheless, there are some countries (e.g., former USSR) that kept using surgery abundantly under the claim that their cases are more advanced and complicated by drug resistance.

Introduction of the tuberculosis sanatorium

The late 19th century saw the introduction of one of the first useful measures to combat tuberculosis, that is to say, improving hygiene conditions. Indeed, fresh air, proper nutrition and exercise had been prescribed for phthisis throughout history by famous scientists, including Hip-

ocrates and Aretaeus of Cappadocia. In France, the government started to structure the fight against tuberculosis. "Selection centers" to identify soldiers with consumption, and "health stations", the precursors of "public sanatoriums", were created in order to treat tuberculosis sufferers, and above all to provide them with information on prevention, before they were sent home. Phthisiotherapy, also known as sanatorium treatment, was first proposed by a German physician, Hermann Brehmer. The sanatorium was a place where patients were treated with fresh air, rest, a rich diet, mountain walks and water [6]. In the middle of the Nineteenth century, the Italian doctor Biagio Castaldi, who suffered from pulmonary tuberculosis himself, published a summary of his clinical observations in the Journal of Medical Sardinian States [37]. He reaffirmed the great efficacy of a healthy lifestyle and a high mountain climate in preventing the disease. Indeed, he observed that these conditions could boost the body's mechanisms of defense and repair, first arresting the progression of the disease and subsequently healing local lesions and the general damage produced. At the end of the Nineteenth century, tuberculosis was widespread, especially among the poorer classes, and sanatoria were designed for these categories, too. In these places, people could work in order to obtain adequate treatment and food. Those who could not afford to stay in sanatoria were instructed to remain isolated from other persons living in the same house [13]. Treatment in sanatoria was undertaken in many countries until the Twentieth century, and several hospitals had therapeutic open-air wards. In most cases, however, only patients with minimal disease benefited; most of those with severe complications died within a short time [14].

The prevention of tuberculosis: TB vaccines and Bacille Calmette-Guérin (BCG) immunization

In the 19th century, vaccination against tuberculosis was advocated in Italy by Edoardo Maragliano, followed by Giovanni and Gaetano Petragliani Salvioli, and in France by Albert Calmette and Camille Guérin. In 1886, the Venetian doctor Vittorio Cavagnis made the first pioneering experiment, in which killed bacilli were used in order to achieve a state of preventive immunity against infection. Cavagnis tentatively vaccinated some animals by means of the inoculation of tuberculous sputum treated with carbolic acid solution [38]. In 1891, Jacques-Joseph Grancher and P-Ledox Lebard experimented with immunization based on the principle of Pasteur's inoculations of crops of progressively increasing virulence. They started by inoculating avian tuberculosis bacilli cultures subjected to different aging processes, but had to admit that these cultures, when inoculated into guinea pigs, did not confer immunity against mammalian tuberculosis [39]. In 1895, Edoardo Maragliano claimed that it would always be preferable to use a vaccine with the bacillus of human type that contains the antigenic complex counterpart. He believed that the bodies

of desiccated bacilli were able to induce the production of specifically bactericidal substances in the organism into which they were inoculated [40]. On the basis of his research, he proposed a dual solution for the treatment of tuberculosis: an active specific therapy based on the administration of a tuberculin prepared by himself and a passive serum therapy, based on the administration of serum obtained from vaccinated animals. In 1901, Francisque Crotte proposed an "electrical" remedy for TB. A year later, von Behring, together with Romer and Ruppel, publicized a method called "jennerization" of cattle. They used a culture of tubercular bacilli of human origin, which had been maintained for over six years in the laboratory and then dried under vacuum, the virulence of which had proved extremely low in the guinea pig. Increasing amounts of the culture were injected intravenously into calves aged about six months on two occasions, initially with a 6-week interval, and then a 3-month interval, between injections. Another line of research was also moving forward; this was aimed at immunizing humans by administering live bacteria obtained from patients. Indeed, in France, Cavagnis had been injecting progressively higher doses of a mixture of phenolized water and saliva from tuberculosis patients. In 1905, two French bacteriologists and pasteurians, Albert Calmette and Camille Guérin, began their search for an anti-tuberculosis vaccine at the Pasteur Institute in Lille. On 8 January 1908, a strain of tuberculosis bacillus isolated by Nocard was cultured on pieces of potato cooked in 5% glycerinated beef bile; after 230 successive cultures, Calmette and Guérin observed in 1920 that these bacilli were no longer able to infect guinea pigs or rabbits, even at high doses [41]. The strain was called Billié Calmette-Guérin, from which they were able to produce a non-virulent strain which they formulated into a live attenuated vaccine, later called Bacille Calmette-Guérin or BCG [42]. In 1921, BCG was first administered to humans by two French physicians, Benjamin Weille-Hallé and Raymond Turpin, at the Charité Hospital in Paris. The vaccine was administered orally to an infant born to a mother who had died of tuberculosis shortly after giving birth; the child survived and did not contract the disease. The vaccine soon became popular throughout Europe and, over the next seven years, more than 100,000 children were immunized. Subsequently, the oral formulation of BCG was replaced by a subcutaneous formulation according to the method of Loeffler and Matsuda (1913), in which the bacterial bodies killed by heat were first dried and then heated to 70°C for 15 days. The subcutaneous vaccine proved more active, but soon they noticed that it often resulted in considerable damage, causing the skin to ulcerate and even liquefy; they therefore adopted intradermal administration as the method of choice. A vaccine developed by Koch and named "Tauruman", made by using living human tuberculosis bacilli with attenuated virulence, was marketed in the early years of the 20th century. In 1930, 250 children immunized with a BCG vaccine in Lübeck in Germany were accidentally contaminated by virulent tubercular bacilli; seventy-three subjects died

of tuberculosis in the first year and a further 135 were hospitalized [43]. The Second World War was followed by a resurgence of tuberculosis throughout Europe and Asia, and in 1948 UNICEF undertook a tuberculosis control program of tuberculin testing and BCG vaccination in children. Routine vaccination was discontinued in the 1970s and in the following decades, but is still implemented in many countries with a high prevalence of tuberculosis, in order to prevent childhood tuberculous meningitis and miliary disease, and in healthcare, military personnel and other people at high risk of exposure to tuberculosis.

New prophylactic and therapeutic vaccines

Since the beginning of the 21st century, new prophylactic vaccines have been tested in order to better control the pulmonary form of TB. One strategy could be to boost the current BCG vaccine with new subunit vaccines. Moreover, more effective recombinant BCG or attenuated live vaccines have been tested for use in primary immunization [44]. In the past century, the BCG vaccine was used in various countries and protected many children and adults from TB. However, its effectiveness against pulmonary TB was variable. For this reason, various formulations of new-generation TB vaccines are currently being tested and are in various phases of clinical trials, the aim being to achieve lasting immunization [45]. In 2016, 13 candidate vaccines were tested in clinical trials, including candidates for the prevention of TB infection and candidates for the prevention of TB disease in people with Latent TB Infection [5]. These candidate vaccines are intended not only to provide stronger immunological responses against MT, but also to elicit long-lasting responses, which will require stimulation of memory T- and B-cell responses [46]. Currently, 8 of the 13 vaccines in clinical development are subunit vaccines; 6 of these contain or express either Ag85A or Ag85B proteins. A major challenge to TB vaccine development is the lack of diversity in both the antigens included in TB vaccines, and the immune responses elicited by TB vaccine candidates. Both will need to be expanded to maximize the potential for developing a successful candidate by 2025. [47] Live attenuated vaccines interfere with phagosome biology and host intracellular pathways, including apoptosis and autophagy. In recent studies, mucosal vaccination was found to be superior to parenteral vaccination, and this innovative route of administration is currently under study [48]. In recent years, a new hypothesis of cure was introduced by a new formulation of TB vaccine. The effectiveness of this vaccine is based on the theory that mycobacterial antigens can enhance bacterial killing. A phase II study of *Mycobacterium indicus pranii* (Mw) vaccine administered via the aerosol route is being examined in guinea pig and mouse models. RUTI is a non-live polyantigenic vaccine that may be used as a prophylactic vaccine together with short intensive antibiotic therapy; it is currently being tested in a phase II trial [5, 49].

The discovery of streptomycin and other anti-tuberculosis drugs

During the 21st century, antibacterial chemotherapy was developed, with the discovery of numerous active molecules against the Koch bacillus, such as thiosemicarbazone, acid or para-PAS and hydrazide isonicotinic acid. In the 1930s, tuberculosis was shown to be resistant to sulphonamides and, in the 1940s, to penicillin. The Ukrainian microbiologist Selman Waksman, who was awarded the Nobel prize in 1952, coined the term "antibiotic". He first isolated actinomycin in 1940 and streptothricin in 1942, both of which would later be used effectively in TB therapy, but which were too toxic in their first formulations. Other anti-tuberculosis chemotherapeutic agents were developed in the following years, such as isoniazid, rifampicin, ethambutol and pyrazinamide, and more recently, viomycin and ciprofloxacin, used to treat drug-resistant infections. The strategy of early diagnosis and targeted therapy with chemotherapy is now considered to have the absolute best cost/benefit ratio.

Tuberculosis and AIDS: the challenge of multidrug-resistant tuberculosis

In the 1980s and 1990s, the incidence of tuberculosis surged as a major opportunistic infection in people with HIV infection and AIDS, as a result of their immune system impairment. Today, TB is still a significant cause of both illness and death in developed countries, especially among immunosuppressed individuals; indeed, subjects with HIV have a higher TB-related mortality rate than the general population [50]. Moreover, the risk of developing tuberculosis is estimated to be between twelve-twenty times greater in people living with HIV than in those without HIV infection [5]. In 2015, 15% of tuberculosis patients worldwide (1.2 million people) had HIV co-infection, and in parts of sub-Saharan Africa, the figure was as high as 50-80%. Tuberculosis is the leading cause of death in people with HIV infection and AIDS; one in three AIDS sufferers dies from tuberculosis [51, 52]. While the incidence of HIV-related tuberculosis has declined in developed countries, owing to effective anti-TB and anti-HIV treatment, it remains high in many developing countries.[53] In a study published by Karo B et al. in 2017, HIV-infected patients who contracted TB showed reduced CD4+ cell counts; in these patients, it is important to start adjunctive TB preventive therapy [54].

New strategies for reducing HIV-related TB infections could be: the use of cheaper, more effective and less toxic drugs; early diagnosis based on PCR technologies in the case of MDR-TB infection, as this may be cured with bedaquiline and delamanid, which are effective against both sensitive and resistant strains; and, in the meantime, improving the use of ART in HIV patients. In the near future, an integrated approach will have to be developed, including new and more sensitive diagnostic tests, together with rapid and effective therapy [55].

Towards the elimination of tuberculosis: tuberculosis control strategy

In 1993, the WHO declared TB a global emergency, and in 1995 defined a tuberculosis control strategy – the directly observed treatment, short-course strategy, also known as DOTS – the most important health breakthrough of the decade in terms of the number of lives that could be saved [56, 57]. Thanks to the DOTS strategy, the global incidence of tuberculosis has fallen by 1.5% every year since 2000, and from 2000 to 2015 the mortality rate declined by about 22% [58]. Nevertheless, morbidity and mortality rates worldwide are still high, especially in developing countries; indeed, 60% of TB deaths occur in six countries: India, Indonesia, China, Nigeria, Pakistan and South Africa [50]. In 1998, the complete MT genome sequence was published, which raised expectations of developing better therapies for TB and vaccines to prevent it [59]. In recent decades, however, multidrug-resistant tuberculosis (MDR-TB) has emerged and is now present in most countries. Patients infected with strains resistant to isoniazide and rifampicin are incurable by means of first line therapy. The response to tuberculosis treatment may be affected by multiple factors associated with the host-pathogen interaction, including genetic factors and the nutritional status of the host [60]. Drug resistance surveillance data indicate that, in 2015, approximately 480,000 people contracted MDR-TB worldwide and 97,000 MDR-TB patients were started on treatment. Surveillance of TB drug resistance over the last two decades has informed and guided the response to the MDR-TB epidemic, and recent innovations in molecular diagnostics have prompted a definitive shift to routine surveillance [61]. In 2016, an MDR tuberculosis treatment regimen of less than a year's duration was recommended on a global scale. Five priority actions, from prevention to cure, are required. The issues of health system barriers, diagnostic and treatment challenges and inadequate funding for care and research must be urgently addressed [62]. Developing new drugs, improving diagnostics and introducing new TB vaccines are critical components of a strategy to combat TB in general, and drug-resistant TB in particular [63]. In TB endemic areas, children are particularly vulnerable to drug-resistant TB; for this reason, a major goal is to improve access to currently available vaccines and treatment, in order to curb the continuing spread of drug-resistant TB, especially in younger people. According to the WHO Stop TB program, daily treatment with isoniazid for subjects at high risk of active tuberculosis is an effective preventive measure at both the individual and collective levels. Other preventive measures are recommended in national programs and in the guidelines of the WHO stop TB program, the aim being to provide universal access to TB diagnosis and treatment [64]. The discovery of *Mycobacterium tuberculosis*-specific immunodominant antigens has led to the development of interferon gamma-release assays, which have been shown to have high sensitivity and specificity for TB disease [65]. Despite considerable progress, however, these techniques should be improved

in order to extend their use to low-resource countries and to cases of paucibacillary TB [66, 67].

Conclusions

In Western countries, tuberculosis has greatly diminished in recent decades, and in these areas vaccination remains a selective remedy for vulnerable groups. In the last decade, however, tuberculosis morbidity has increased, rising from three million to ten million new cases annually; this rise is associated with the growth of co-infection with AIDS, which occurs especially in developing countries, particularly in Africa. In addition, the problem of under-notification of cases of TB must be remembered, which will hamper future efforts to reduce the incidence of the disease. In highly endemic countries, the WHO continues to maintain mass vaccination to prevent childhood tuberculosis, though it is acknowledged that this cannot break the “chain” of disease, which is sustained by adults with full-blown pulmonary forms. Since 2012, the WHO has implemented the TB end strategy, which is based on the development of new tools to better detect, prevent and treat tuberculosis. In the coming years, the challenge will be to succeed in translating innovative discoveries into public health targets and socioeconomic interventions in local tuberculosis control programs.

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Authors' contributions

MM and IB conceived the study, performed a search of the literature and they drafted and revised the manuscript. GB revised critically the manuscript. MM, GB and IB read and approved the last version of the manuscript.

References

- [1] Saeed BW. Malignant tuberculosis. *J Ayub Med Coll Abbotabad* 2006;18(3):1-2.
- [2] Nasr Puyan. The fight against tuberculosis, the loathsome killer. *Int.J.Curr.Microbiol App Sci.* 2015;4(1):380-93.
- [3] Keal JL, Davies PD. Tuberculosis: a forgotten plague? *J R Soc Med* 2011;104(5):182-4. doi: 10.1258/jrsm.2011.100384.
- [4] Tanday S. Will we ever eliminate tuberculosis, the voiceless disease? *Lancet Respir Med* 2017;pii:S2213-2600(17)30080-2. doi: 10.1016/S2213-2600(17)30080-2.
- [5] World Health Organization. Global Tuberculosis Report 2016. Available at: http://www.who.int/tb/publications/global_report/en/. Accessed on 16th August 2018.
- [6] Frith J. History of tuberculosis Part 2 - The sanatoria and the discoveries of the tubercle Bacillus. *Journal of military and veterans' health* 2014;22:2.
- [7] Chalke HD. The impact of tuberculosis on history, literature and art. *Medical History* 1962;6:301-18.
- [8] Barberis I, Bragazzi NL, Galluzzo L, Martini M. The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus. *J Prev Med Hyg* 2017;58(1):E9-12.
- [9] Weigert C. Färbung der Bacterien mit Anilinfarben. Breslau: Universität at Breslau; 1875.
- [10] Ziehl F. Zur Färbung des tuberkelbacillus. *Dtsch Med Wochenschr* 1882;8:451.
- [11] Cambau E, Drancourt M. Steps towards the discovery of mycobacterium tuberculosis by Robert Koch, 1882. *Clin Microbiol Infect* 2014;20(3):196-201.
- [12] Marten B. A new theory of consumptions - more especially a phthisis or consumption of the lungs. London: Knaplock T; 1720.
- [13] Daniel TM. The history of tuberculosis. *Resp Med* 2006;100:1862-70. Available at: [http://www.resmedjournal.com/article/S0954-6111\(06\)00401-X/fulltext](http://www.resmedjournal.com/article/S0954-6111(06)00401-X/fulltext).
- [14] Herzog H. History of tuberculosis. *Respiration* 1998;65:5-15. Available at : <http://www.karger.com/Article/PDF/29220>.
- [15] Sakula A. Robert Koch: centenary of the discovery of the tubercle bacillus, 1882. *Thorax* 1982;37:246251. Available at :<http://thorax.bmj.com/content/37/4/246.full.pdf>.
- [16] Daniel TM. Pioneers in medicine and their impact on tuberculosis. Rochester, NY: University of Rochester Press; 2000. pp. 74-6, 179.
- [17] Garrison FH. An introduction to the history of medicine. Philadelphia & London: WB Saunders & Co; 1921. pp. 578-90, 721.
- [18] Boire NA, Riede VAA, Parrish NM, Riedel S. Tuberculosis: from an untreatable disease in antiquity to an untreatable disease in modern times. *J Anc Dis Prev Rem* 2013;1(2):1-11.
- [19] Singhal R, Myneedu VP. Microscopy as a diagnostic tool in pulmonary tuberculosis. *Int J Mycobacteriol* 2015;4(1):1-6.
- [20] Gupta S, Prasad V, Bairy I, Muralidharan S. Comparative evaluation of two cold staining methods with the Ziehl-1950 method for the diagnosis of tuberculosis. *Southeast Asian J Trop Med Public Health* 2009;40(4):765-9.
- [21] Hänscheid T. The future looks bright: low-cost fluorescent microscopes for detection of mycobacterium tuberculosis and coccidia. *Trans R Soc Trop Med Hyg* 2008;102(6):520409.
- [22] Chang EW, Page AL, Bonnet M. Light-emitting diode fluorescence microscopy for tuberculosis diagnosis: a meta-analysis. *Eur Respir J* 2016;47(3):929-37.
- [23] Koch R. *Über bakteriologische forschung. Verhandlungen des X internationalen medizinischen kongresses.* Berlin, Germany: August Hirschwald; 1890.
- [24] Conti AA, Lippi D, Gensini GF. Tuberculosis: a long fight against it and its current resurgence. *Monaldi Archives for Chest Disease - Pulmonary Series* 2004;61(1):71-4.
- [25] Besozzi G, Nardini S, Migliori GB. Tuberculin test and tuberculosis control in a low prevalence country today. *Monaldi Archives for Chest Disease* 1997;52(4):323-420.
- [26] Bazin H. Vaccination: a history. Montrouge: the John Libbey Eurotext; 2011.
- [27] Koch R. Weitere mitteilungen über ein heilmittel gegen tuberculose. *Dtsch Med Wschr* 1891;101-2.
- [28] Nasser Pouyan. The fight against tuberculosis, the loathsome killer. *Int J Curr Microbiol App Sci* 2015;4(1):380-93.

- [29] Palmer CE, Bates LE. Tuberculin sensitivity of tuberculous patients. *Bull World Health Org* 1952;7:171-88.
- [30] WHO Tuberculosis Research Office. Further studies of geographic variation in naturally acquired tuberculin sensitivity. *Bull World Health Org* 1955;12:63-83.
- [31] Edwards LB, Palmer CE. Identification of the tuberculous-infected by skin tests. *Ann NY Acad Sci* 1968;154:140-8.
- [32] Snider DE Jr. The tuberculin skin test. *Am Rev Respir Dis* 1982;125(3 Pt 2):108-18.
- [33] Donelli G, Di Carlo V. La sanità pubblica italiana negli anni a cavallo della Prima Guerra Mondiale. Armando Editore, Roma, 2016. pp 65.
- [34] Garbarino MC, Cani V, Mazzarello P. A century ago: Carlo Forlanini and the first successful treatment of tuberculosis. *Lancet*. 2018;392(10146):475. Accessed 16th August 2018.
- [35] Hjaltested O, Törning K. Clinical aspects of pneumothorax therapy as illustrated by the results obtained in 191 cases of completed treatment. *Br J Tuberc* 1939;33.
- [36] Mitchell RS. Artificial pneumothorax: a statistical analysis of 557 cases initiated in 1930-1939 and followed in 1949. I. The influence of clinical findings before induction on early and 441 late results. *Am Rev Tuberc* 1951;64.
- [37] *Medical Journal of Sardinian States* 1858.
- [38] Cavagnis V. Contro il virus tubercolare e contro la tubercolosi, tentativi sperimentali. In: *Atti del Reale Istituto Veneto di Scienze*. Venezia: Lettere e Arti; 1886:8.
- [39] Grancher JJ, Ledox-Lebard P. Etudes sur la tuberculose experimentale du lapin. *Arch de méd experim et d'anat pathol* 1891.
- [40] Martini M, Paluan F. Edoardo Maragliano (1849-1940): the unfortunate fate of a real pioneer in the fight against tuberculosis. *Tuberculosis* 2017;106:123.
- [41] Luca S, Mihaescu T. History of BCG Vaccine. *Maedica* 2013;8(1):53-8.
- [42] Martini M, Barberis I, Bragazzi NL, Paluan F. The fight against tuberculosis in the mid nineteenth century: the pivotal contribution of Edoardo Maragliano (1849-1940). *Advances in experimental medicine and biology*. *Adv Exp Med Biol* 2017. doi: 10.1007/5584_2017_125.
- [43] The Lubeck catastrophe. *British Medical Journal* 1931;1:986. Available at: <http://www.bmj.com/content/1/3674/986>. Accessed on 15th October 2017.
- [44] Martín Montañés C, Gicquel B. New tuberculosis vaccines. *Enferm Infecc Microbiol Clin* 2011;29(Suppl 1):57-62.
- [45] Martin C. Tuberculosis vaccines: past, present and future. *Curr Opin Pulm Med* 2006;12(3):186-91.
- [46] Orme IM. Vaccine development for tuberculosis: current progress. *Drugs* 2013;73(10):1015-24.
- [47] Fletcher HA, Schragr L. TB vaccine development and the end TB strategy: importance and current status. *Trans R Soc Trop Med Hyg* 2016;110(4):212-8.
- [48] Gengenbacher M, Nieuwenhuizen NE, Kaufmann S. BCG - old workhorse, new skills. *Curr Opin Immunol* 2017;47:8-16.
- [49] Kaufmann SH, Weiner J, von Reyn CF. Novel approaches to tuberculosis vaccine development. *Int J Infect Dis* 2017;56:263-7.
- [50] Adigun R, Bhimji S. Tuberculosis. StatPearls, Treasure Island (FL): StatPearls Publishing; 2017.
- [51] World Health Organisation. Tuberculosis and HIV. 2014. Available at: <http://www.who.int/hiv/topics/tb/en/> Accessed on 14.10.2017.
- [52] Leutkemeyer A. Tuberculosis and HIV. San Francisco: University of California. Available at: <http://www.hivinsite.ucsf.edu/InSite?page=kb-05-01-06#S3X>. Accessed on 14.10.2017.
- [53] World Health Organization. Tuberculosis: a global emergency. Geneva, Switzerland; 1994.
- [54] Karo B, Krause G, Castell S, Kollan C, Hamouda O, Haas W; ClinSurv HIV Study Group. Immunological recovery in tuberculosis/HIV co-infected patients on antiretroviral therapy: implication for tuberculosis preventive therapy. *BMC Infect Dis* 2017;17(1):517.
- [55] Tiberi S, Carvalho AC, Sulis G, Vaghela D, Rendon A, Mello FC, Rahman A, Matin N, Zumla A, Pontali E. The cursed duet today: tuberculosis and HIV-coinfection. *Presse Med* 2017;46(2 Pt 2):e23-39.
- [56] Dye C, Garnett GP, Sleeman K, Williams BG. Prospects for worldwide tuberculosis control under the WHO DOTS strategy. Directly observed short-course therapy. *Lancet* 1998;352(9144):1886-91.
- [57] Farzianpour F, Kooshad MA. Study of the status of tuberculosis control program based on the implementation of the directly observed treatment short-course strategy (DOTS). *Mater Sociomed* 2016;28(4):249-52.
- [58] Davies, PD. Good news from the WHO (editorial). *IJTLD* 2016;20(7):853.
- [59] Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE 3rd, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S, Hornsby T, Jagels K, Krogh A, McLean J, Moule S, Murphy L, Oliver K, Osborne J, Quail MA, Rajandream MA, Rogers J, Rutter S, Seeger K, Skelton J, Squares R, Squares S, Sulston JE, Taylor K, Whitehead S, Barrell BG. Deciphering the biology of mycobacterium tuberculosis from the complete genome sequence. *Nature* 1998;393:537-44.
- [60] Choi R, Jeong BH, Koh WJ, Lee SY. Recommendations for optimizing tuberculosis treatment: therapeutic drug monitoring, pharmacogenetics, and nutritional status considerations. *Ann Lab Med* 2013;7(2):97-107.
- [61] Outhred AC, Britton PN, Marais BJ. Drug-resistant tuberculosis - primary transmission and management. *Journal of Infection* 2017;74:S128-35.
- [62] WHO. Drug resistant tuberculosis surveillance&response. Supplement global tuberculosis report 2014. Available at: http://www.who.int/tb/publications/global_report/gtbr14_supplement_web_v3.pdf. Accessed on 16th August 2018".
- [63] Manjeliievskaja J, Erck D, Piracha S, Schragr L. Drug-resistant TB: deadly, costly and in need of a vaccine. *Trans R Soc Trop Med Hyg* 2016;110(3):186-91.
- [64] Abouda M, Yangui F, Triki M, Kammoun H, Khouani H, Charfi MR. Tuberculosis prevention. *Rev Pneumol Clin* 2015;71(2-3):159-67.
- [65] Connell TG, Rangaka MX, Curtis N, Wilkinson RJ. QuantiFERON-TB Gold: state of the art for the diagnosis of tuberculosis infection? *Expert Rev Mol Diagn* 2006;6(5):663-77.
- [66] Slim-Saidi L, Mehiri-Zeghal E, Ghariani A, Tritar F. New methods of diagnosis in tuberculosis. *Rev Pneumol Clin* 2015;71(2-3):110-21.
- [67] Davies, PD. Towards the elimination of tuberculosis and a reduction in chest diseases (editorial). *IJTLD* 2015;19(8):875.

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