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Extrapulmonary tuberculosis: tuberculous meningitis new developments

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Abstract. – Tuberculosis (TB) can involve any organ system in the body. Extrapulmonary involvement can occur in isolation or along with a pulmonary focus as in the case of patients with disseminated tuberculosis. Tuberculosis meningitis (TBM) is the most severe form of extrapulmonary tuberculosis. TBM a medical emergency, is still a major cause of serious illness in many parts of the world. TBM remains difficult to diagnose, and it is usually due to hematogenous dissemination of the tubercle bacillus. The exact incidence and prevalence are not known. The clinical spectrum is broad and may be non-specific making early diagnosis difficult. Improved outcome requires early recognition and treatment of these conditions. Clinical features included fever for more than 7 days, headache, or neck stiffness. While TBM is a disease of childhood, tuberculomas and spinal tuberculosis are invariably an adult manifestation. In HIV infection, TB is often atypical in presentation, frequently causing extrapulmonary disease, and patients have a high incidence of TBM. Clinical response to antituberculous therapy in all forms of neurotuberculosis is excellent if the diagnosis is made early before irreversible neurological deficit is established. Diagnosis is based on the characteristic clinical picture, neuroimaging abnormalities, cerebrospinal fluid changes and the response to anti-tuberculosis drugs. Diagnosis is best made with lumbar puncture and examination of the cerebrospinal fluid (CSF). Suspect TBM if there is a CSF leucocytosis (predominantly lymphocytes), the CSF protein is raised, and the CSF plasma glucose is <50%. Rapid techniques based on nucleic acid amplification such as PCR are more sensitive and specific as they attempt to detect specific DNA sequences of the organism. The hallmark pathological processes are meningeal inflammation, basal exudates, vasculitis and hydrocephalus. Treatment delay is strongly associated with death and empirical anti-tuberculosis therapy should be started promptly in all patients in whom the diagnosis of TBM is suspected. Corticosteroids reduce the number of deaths. Development of an effective vaccine against tuberculosis hinges on an improved understanding of the human immune response to Mycobacterium tuberculosis (Mtb). The emergence of drug resistant tuberculosis poses a serious threat to the control of this pathogen, and the development of drugs that are active against the resistant strains is vital. Further research into the epidemiology, immune mechanisms, diagnosis, treatment, and prevention of TBM is urgently needed.

Key Words:

Mycobacterium tuberculosis, Bacillus Calmette-Guérin, Extrapulmonary tuberculosis, Tuberculous meningitis, Human immunodeficiency virus, Dexamethasone, Antituberculosis agents, Neurological sequelae.

Introduction

Tuberculosis (TB) is a major cause of morbidity and mortality. It is a major global health problem with 9 million new cases and almost 2 million deaths per year, of which the majority (55%) are in Asia[1]. Extrapulmonary tuberculosis usually presents more of a diagnostic problem than pulmonary tuberculosis. In part this relates to its being less common and, therefore, less familiar to most clinicians. Extrapulmonary tuberculosis has become more common since the advent of human immunodeficiency virus (HIV) infection. In addition, extrapulmonary tuberculosis involves relatively inaccessible sites and, because of the nature of the sites involved, fewer bacilli can cause much greater damage. In 2007, over 13 000 tuberculosis (TB) cases were reported in the USA[2]. Nearly 20% of patients with TB develop extrapulmonary manifestations[3]. While pulmonary disease is the most common manifestation of TB, the involvement of the central nervous system (CNS) is associated with the most severe form of disease, namely tuberculous meningitis (TBM). TBM is one of the most common forms of central nervous system infections,

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especially in many developing countries where tuberculosis remains highly endemic, and it is a severe form of extrapulmonary tuberculosis.

TBM occurs when *Mycobacterium tuberculosis* (Mtb), the bacterium responsible for tuberculosis, invades the membranes and fluid surrounding the brain and spinal cord. TBM is related to the prevalence of TB in the community, and it is still the most common type of chronic CNS infection in developing countries. Bacterial and host's genetic factors play a crucial role in its pathogenesis. TBM usually results from the haematogenous spread of primary or post-primary pulmonary infection, or from the rupture of a subependymal tubercle into the subarachnoid space. TBM differs from that caused by most other common bacteria in that the course is more prolonged, the mortality rate is higher, it is always secondary to tuberculosis elsewhere in the body. The infection usually begins in the lungs, and then travels through the bloodstream to the meninges where small abscesses are formed.

Many investigations have confirmed that genetic factors are involved in the disease, and these include adoption studies, twin studies, genome-wide linkage and population-based case-control association studies[4]. Initially, differences in susceptibility to TBM were recognised through numerous observations, such as the wide range of responses seen after exposure to Mtb.

The clinical features of TBM mimic those of other chronic meningoencephalitides, causing considerable diagnostic difficulty[5]. The disease is difficult to recognize, and a high index of suspicion is necessary to establish the diagnosis. In low-incidence geographic areas, clinicians should suspect tuberculous meningitis in members of immigrant groups from high-incidence areas, as well as in patients who abuse alcohol or drugs and those with immunosuppression from any cause. Early diagnosis is important for the success of the treatment[6]. If detected early, tuberculous meningitis usually responds to standard chemotherapy supplemented with corticosteroids. Meningitis is the most common form of CNS disease and is discussed in depth here.

This review focuses on the various aspects of TBM, and special emphasis is given to recent developments in the early detection, phatogenesis and management of this dreaded disease.

Etiology

Mtb, the causative agent of tuberculosis (TB), is a facultative intracellular parasite of macrophages. It is a gram-positive, aerobic, non-spore-forming, non-motile, pleomorphic rod that is distantly related to the Actinomycetes. Mtb is the most common species to cause TB in humans. It is an obligate pathogen and cannot multiply outside host cells. It can survive for long periods under adverse conditions. Human to human transmission is most common. Mtb is an acid-fast bacterium, and it is very small, only 2 to 4 micrometers in length. It is known as acid fast because of staining characteristics.

The characteristic staining quality of these organisms is their ability to resist decolorization of carbol fuchsin by acid-alcohol in the Ziehl-Neelsen process due to the high lipid content of the cell wall. The Ziehl-Neelsen (ZN) stain and its modifications historically have been essential tools in the identification of mycobacteria (Figure 1).

Lowenstein-Jensen medium is most popular and widely available culture medium to grow Mtb. Like other microbes, it can mutate and affect characteristics such as drug sensitivity, which is the focus of this review. In order for Mtb to establish infection it must first gain entry into resident alveolar macrophages following inhalation of infectious aerosols.

The main cellular reservoirs of these organisms in the host are tissue macrophages, although the organisms also exist extracellularly. Macrophages

Figure 1. Mycobacterium tuberculosis stains with Ziehl-Neelsen stain (one of the most widely used acid-fast staining method).

patrolling the distal airways avidly engulf inhaled bacteria using a variety of phagocytic receptors. In order for *Mtb* to establish infection it must first gain entry into resident alveolar macrophages following inhalation of infectious aerosols. A number of different phagocytic receptors have been implicated in *Mtb* entry to macrophages, with complement receptor and mannose receptor likely the predominant pathways. Intracellular parasitism is accomplished by a variety of mechanisms that include altered trafficking of bacteria during endocytosis, interference with host Ca_2 signaling pathways, and induction of maturational arrest of the phagosome. There are numerous macrophage receptors capable of binding Mtb that can participate in its internalization, including CD11b, CD14, the macrophage mannose receptor, scavenger receptor A, Toll-like receptor 4, and CD44[7].

Epidemiology

TB is an ancient infection that has plagued humans throughout recorded and archeologic history. TB is a social disease with medical implications, and it is remains one of the deadliest diseases in the world. The natural history and clinical manifestations of tuberculosis in children are different as compared with that of adults. The exact incidence and prevalence of TBM in the most parts of the world are not exactly known. In developed countries, despite an overall decrease in numbers of tuberculosis cases, the proportion of extra-pulmonary tuberculosis and tuberculous meningitis cases has increased. Central nervous system TB accounts for about 5% of all extrapulmonary TB and TBM is the most serious complication[8]. TBM occurs in approximately 7-12% of patients with pulmonary tuberculosis, and is associated with high mortality and morbidity[9]. There were an estimated 13.7 million prevalent cases of tuberculosis in 2007 (206 per 100 000 population). During this period, estimated 9.27 million new cases of tuberculosis were registered worldwide. Of these, an estimated 1.37 million (14%) were human immunodeficiency virus-positive (10). It is estimated that 200 million people will display symptoms and that 35 million will die of TB between 2000 and 2020 if control and preventive measures are not strengthened (World Health Organization Annual Report, 2000). Estimates of the prevalence of latent tuberculosis infection (LTBI) are considerably less precise, but it is commonly suggested that up to one-third of the world's population may be affected and at risk of subsequent reactivation. LTBI, which is defined as a positive tuberculin skin test (TST) and no evidence of TB disease, has to be detected and treated to prevent extension and dissemination of TB, especially in children.

The majority of human immunodeficiency virus-infected patients with culture-positive tuberculous meningitis had clinical or radiologic evidence of extra-meningeal tuberculosis as well[11]. The incidence in neonates was 31.5 per 100 000 as compared with 0.7 per 100 000 among older children[12]. In Germany, 26 302 tuberculosis cases were registered during 1996-2000.

The proportion of patients with extrapulmonary tuberculosis (including tuberculous meningitis) was 21.6%, and extrapulmonary tuberculosis was most likely among females, children aged, 15 yrs and persons originating from Africa and Asia. Of all patients with extrapulmonary tuberculosis, 58.7% were born in Germany, 32.1% were from places with a higher risk of extrapulmonary tuberculosis than Germany. Females tended to be more likely to have any form of extrapulmonary tuberculosis than males, except pleural tuberculosis[13]. CNS involvement by the disease is estimated to occur in 5-10% of patients, with tuberculous meningitis (TM) as the most common manifestation. In France, the incidence of TB is stable at around 11 cases per 100 000 population since 1997[14]. The proportion of TBM, the most severe form of TB, reported through the French mandatory notification system is very low, at around 1.5% of all TB cases. The incidence of tuberculous meningitis in France (in the year 2000) was estimated as 1.55 cases per million. The incidence rate was 0.7 cases per million when only culture-positive cases were counted. Among 143 tuberculous meningitis cases reported to two agencies of France the Tuberculosis Mandatory Notification System and the National Reference Centre total number of confirmed tuberculous meningitis cases were 91, and TBM is underestimated in France. Capture-recapture analysis using different sources to better estimate its incidence is of great interest[15]. In United States, at a large inner-city medical center, during an 11.5-year period, 34 patients were found to have positive cerebrospinal fluid cultures for *Mycobacterium tuberculosis*, accounting for 1.5% of culture-confirmed tuberculosis cases. All patients were born in the United States, 31 (91%) were black people and 16 (47%) were human immunodeficiency,virus-infected patients[16]. Poverty; lack of functioning public health infrastructure; lack of funding to support basic research aimed at

developing new drugs, diagnostics, and vaccines; and the co-epidemic of HIV continue to fuel the ongoing epidemic of TB.

Multidrug-Resistant Tuberculosis

Tuberculosis is a leading cause of death from infectious diseases world-wide, and multidrug-resistant (MDR) tuberculosis continues to spread in many parts of the world. Given the emergency of multidrug-resistant bacilli, the approach to antimicrobial therapy in patients with TBM has become problematic. The importance of multiply drug-resistant (MDR) tuberculosis (defined by resistance to both isoniazid and rifampin) has been well documented in the literature. The features of MDR tuberculosis in TBM have been characterized mostly by case reports and small case series.

Drug-resistant TB was first noted to develop soon after the introduction of streptomycin as the first useful antibiotic with activity against Mtb. This problem was alleviated by the co-administration of isoniazid and streptomycin together, although soon after the introduction of isoniazid in the early 1950s resistance to this drug was noted as well. The truly modern era of chemotherapy for TB began in the 1970s with the introduction of rifampin, which allowed the shortening of chemotherapy regimens to 9 and eventually 6 months.

The phenomenon of multidrug-resistant tuberculosis (MDR-TB) emerged as a clinical entity in the early 1990s after a couple of decades of widespread use of rifampin. The growing incidence of drug-resistant strains of tuberculosis bacteria is another complication that has TB experts on the alert. A World Health Organization (WHO)-associated global laboratory surveillance network detected an increase in the global case load of MDR-TB from w 274,000 in 2000 to w 500,000 cases in 2007 (5% of the global case burden of TB)[17,18]. MDR-TB, strains resistant to at least isoniazid (INH), and rifampin, are difficult to treat and require drugs that are expensive, toxic, and less effective[19]. The problem of MDR has also been identified in patients with tuberculous meningitis. Drug resistance is more common in human immunodeficiency virus-positive patients, and the problem of multidrug-resistant tuberculosis has also been identified in patients with tuberculous meningitis. MDR tuberculous meningitis is often associated with poor prognosis[20]. The majority of MDR tuberculous meningitis cases either died or experienced significant morbidity. South African serial surveys of antimicrobial sensitivity found an increase in isoniazid resistance from 6.9% to 12.4% in pediatric TB isolates between 1994-2005, a significant development in a treatment naïve cohort[21]. In regions such as Uzbekistan and Azerbaijan, with a high proportion of treatment failure and MDR-TB transmission, reported isoniazid-resistance is as high as 40%-49%[22]. Fortunately, MDR tuberculous meningitis is still not a serious problem in some of the endemic countries. A total of 366 cases, 301 (82.2%) were sensitive to all the four primary drugs tested, while 65 (17.8%) showed resistance. There were 46 (12.5%) isolates resistant to isoniazid (INH), while 9 (2.4%) demonstrated MDR[23]. These data may suggest that MDR in tuberculous meningitis is not yet a serious problem. However, a periodic review is required to ascertain the global incidence of drug-resistant tuberculous meningitis.

The presence not only of MDR tuberculosis but also what came to be called extensively drug-resistant (XDR) tuberculosis. In fact, Mtb strains resistant not only to the front-line drugs isoniazid and rifampicin, but also to an increasing number of second-line drugs, are becoming more common. These strains, are virtually untreatable using current therapeutics and, without the strengthening of the current TB control measures combined with a drive to introduce new anti-tuberculosis drugs, the situation is only set to worsen. XDR tuberculosis is caused by a strain of Mtb resistant to isoniazid and rifampin (which defines MDR tuberculosis) in addition to any fluoroquinolone and at least one of the three following injectable drugs: capreomycin, kanamycin, and amikacin. A definitive diagnosis of MDR-TB and XDR-TB depends on identification of the presence of Mtb and the drug-susceptibility testing (DST) there of. This diagnosis can be achieved only if laboratory quality assurance programs are implemented. The gold standard for DST is the indirect proportion method on agar medium. This method requires obtaining a pure culture of Mtb followed by inoculation onto solid agar medium containing the critical concentration of a specific anti-TB drug (the drug concentration that differentiates bacillary resistance from susceptibility).

Pathogenesis

Transmission of bacteria Mtb to a healthy person is primarily by airborne droplet nuclei. These droplet nuclei contain from one to three organisms, which are then distributed in the well-ven-

tilated areas of the lung, especially the periphery of the midlung fields, most commonly in the right middle lobe, superior segments of the lower lobes and anterior segments of the upper lobes. After inhalation, the droplet nucleus is carried down the bronchial tree and implants in a respiratory bronchiole or alveolus. The primary focus of infection is usually in the lungs but may be in lymph nodes, bones, nasal sinuses, gastrointestinal tract, or any other organ.

Progression of infection is limited by the acquisition of effective cell-mediated immunity. The main cellular reservoirs of these organisms in the host are tissue macrophages. Lung macrophages provide a critical intracellular niche that is required for *Mtb* to establish infection in the human host. Apoptosis of alveolar macrophages and monocytes has been described as a consequence of Mtb infection. Indeed, protective immunity against Mtb is dependent on the interplay between activated T cells, macrophages and other leucocytes. Infection-induced target cell apoptosis may be a successful strategy to eliminate pathogens and assure host survival. Conversely, apoptosis inhibition could represent an adaptive mechanism for pathogen survival, while it may be beneficial for the host to initiate an effective immune response. This parasitic relationship is made possible by the capacity of *Mtb* to block phagosome maturation following entry into the host macrophage, creating an environment that supports bacillary replication.

If the bacillus is able to survive initial defences, it can multiply within the alveolar macrophage. In the lungs, *Mycobacterium tuberculosis* bacteria multiply in alveolar macrophages, the tubercle bacillus grows slowly, dividing approximately every 25 to 32 h within the macrophage and, therefore, there is no immediate host response to infection. Inhaled mycobacteria proliferate in the alveolar spaces and are then transported via lymphatics to regional hilar lymph nodes, from which they can enter the systemic circulation to spread to other organs. Within 2-4 weeks, through blood circulation, bacilli spread to extrapulmonary sites and produce small granulomas in the meninges and adjacent brain parenchyma.

The development of TB infection in humans depends on the mycobacterial strain and the human host, and is multigenically controlled in both. The ability of the host to respond to the organism may be reduced by certain diseases such as silicosis, diabetes mellitus, and diseases associated with immunosuppression, e.g., HIV infection, as well as by corticosteroids and other immunosuppressive drugs. In these circumstances, the likelihood of developing tuberculosis disease is greater. Whether or not an inhaled tubercle bacillus establishes an infection in the lung depends on both the bacterial virulence and the inherent microbicidal ability of the alveolar macrophage that ingests it[24-25]. Our knowledge about how Mtb enters the host cells is currently limited.

Meningitis can result from direct meningeal seeding and proliferation during a tuberculous bacillemia either at the time of initial infection or at the time of breakdown of an old pulmonary focus, or can result from breakdown of an old parameningeal focus with rupture into the subarachnoid space. In persons with intact cell-mediated immunity, collections of activated T cells and macrophages form granulomas that limit multiplication and spread of the organism.

TBM develops most commonly after a two-stage process. Mtb bacilli enter the host by droplet inhalation, the *initial point* of infection being the alveolar macrophages. Localized infection escalates within the lungs, with dissemination to the regional lymph nodes to produce the primary complex. During this stage, a short but significant bacteremia is present that can seed tubercle bacilli to other organs in the body. In persons who develop TBM, bacilli seed to the meninges or brain parenchyma, resulting in the formation of small subpial or subependymal foci of metastatic caseous lesions (Rich focus). *The second stage* in the development of TBM is an increase in size of a Rich focus until it ruptures into the subarachnoid space. The location of the expanding tubercle (i.e., Rich focus) determines the type of CNS involvement. The organisms tend to be localized in the center of the granuloma, which is often necrotic. Tubercles rupturing into the subarachnoid space cause meningitis. In other instances, meningitis may arise in the course of miliary TB or from parameningeal infection.

Our understanding of the pathogenesis of TBM dates from the meticulous studies that Arnold Rich and Howard McCordock conducted at Johns Hopkins Hospital in the 1920s and 1930s. In experiments in animals, they showed that the meninges could not be directly infected by hematogenous spread. Then, in a brilliant series of postmortem examinations, they demonstrated that in nearly every case, there was a subcortical or meningeal small granulomas from which bacilli gained access to the subarachnoid space. They postulated that during primary infec-

tion, Mtb deposited in the brain parenchyma and meninges during hematogenous dissemination. Rich and McCordock, found that the initial event in TBM was rupture of a subpial or subependymal granuloma into the subarachnoid space or ventricles[26]. These small granulomas are known as "Rich focus". Organisms proliferate in the caseous centers, eventually leading to the rupture of the tubercle. The fate of these tubercles and the subsequent course of infection are at least, in part, a function of the immunological capacity of the host. Rich foci remain dormant for years. Rich and McCordock challenged both sensitized and non-sensitized guinea pigs and rabbits intravenously with Mtb and *M. bovis* respectively. Though no animal developed acute exudative meningitis, all animals developed few to several granulomatous lesions in the brain parenchyma and meninges. Based on these data, Rich further postulated that tuberculomas or "Rich foci" develop around the deposited mycobacteria. Much later, the rupture of these foci allows dissemination of mycobacteria into the cerebrospinal fluid, causing diffuse, inflammatory meningitis. Indeed, TBM develops when a caseating Rich focus discharges its contents into the subarachnoid space, and the bacilli enter the central nervous system by traversing the blood-brain barrier (BBB). This barrier is principally composed of tightly apposed human brain microvascular endothelial cells. Mycobacteria as an intracellular pathogen are known to use several survival strategies upon infection of the host cells. The spread of Mtb into the subarachnoid space following rupture of a Rich focus triggers a robust inflammatory T cell response.

Studies of CSF cytokine levels in patients with TBM have found elevated levels of TNF-alfa and IFN-gamma. Virulence of mycobacteria is a multifaceted phenomenon based on the expression of multiple genes involved in various stages of host-pathogen interactions including adhesion, invasion, intracellular replication, and dissemination to other sites. The pathogenesis of tuberculosis can be considered as a series of battles between the host and the tubercle bacillus. Understanding how healthy tuberculin reactors control infection without developing disease provides important insight into the mechanisms of protective immunity against Mtb.

There are numerous macrophage receptors capable of binding Mtb that can participate in its internalization, including CD11b, CD14, the macrophage mannose receptor, scavenger receptor A, Toll-like receptor 4, and CD44. The innate response is a form of natural immunity in which the immune cells have never previously encountered the pathogen, but can nevertheless eliminate it. Innate immunity explains why some persons are naturally more resistant to certain viral or bacterial infections. In contrast, the adaptive immune response depends on the immune system's prior contact with a pathogen or antigens (immunogenic components) of that pathogen[27]. It is estimated that approximately 10% of individuals who acquire tuberculosis infection and are not given preventive therapy will develop active tuberculosis.

Several genetic abnormalities increase the host's susceptibility to MT. Indeed, several studies have suggested that genetic factors may affect the susceptibility of a population to tuberculosis, and it has been found that *P2X7* is linked to an increased risk for tuberculosis in some West African, Southeast Asian, North American, and North European populations. Activation of the $P2X^{28}$ receptor, an ATP-gated Ca^{2+} channel, on human macrophages induces intracellular killing of Mtb. Fernando et al[29] examined the association between polymorphisms in P2X and the risk of TB among patients and control subjects from Southeast Asia. They found that only the 1513A/C polymorphism was present in this ethnic group. The 1513C allele was found to be strongly associated with extrapulmonary, but not pulmonary TB. ATP-mediated killing of mycobacteria was shown to be ablated in macrophages from subjects homozygous for the 1513C allele, and significantly impaired in macrophages from heterozygous subjects. Nino-Moreno et al[30] found no significant association of the $P2X^{7}$-762 gene polymorphism with TB. Investigation revealed that single nucleotide polymorphisms in toll-interleukin-1 receptor domain containing adaptor protein gene were more strongly associated with the risk of TBM[31]. Stein et al. analyzed candidate genes related to TNF-α regulation and found that IL-10, IFN-γ receptor 1, and TNF-α receptor 1 genes were linked and associated to both TB and TNF-α. They showed these associations with regard to progression to active TB disease, but not susceptibility to latent TB infection (LTBI)[32]. TNF is commonly regarded to have an essential role in the immunologic response of Mtb infection. Single nucleotide polymorphisms, located at these genes, are thought to influence cytokine levels and regulate resistance and susceptibility of an individual to tuberculosis[33]. The pathogenic mycobacte-

ria have developed strategies to circumvent the major killing mechanisms employed by macrophages and take advantage of the enclosed environment within its host cell to avoid humoral and cell-mediated immune responses. Mycobacterial genetic variability, including large sequence polymorphism (LSP) and single nucleotide polymorphism allows survival adaptation to environmental challenges. Beijing strain of Mtb (highly prevalent in Asia and in the countries of the former Soviet Union) is strongly associated with TBM. However, the tuberculosis caused by the Euro-American strain (the most prevalent strain in Europe and the Americas) is more likely to be pulmonary rather meningeal[33]. The association between the East Asian/Beijing strain and disease progression and cerebrospinal fluid leukocyte count might influence protective inflammatory responses of the brain[34,35].

The mechanisms by which Mtb manipulates the host immune system are attributed to lipids in the cell wall[36]. A variety of unique lipids, such as lipoarabinomannan (LAM), trehalose dimycolate and phthiocerol dimycocerate, anchor noncovalently with the cell membrane and appear to play an important role in the virulence of Mtb[37]. Studies have shown that LAM acts at several levels and that it can scavenge potentially cytotoxic oxygen free radicals, inhibit protein kinase C activity and block the transcriptional activation of gamma interferon inducible genes in human macrophages such as cell lines, and hence contribute to the persistence of mycobacteria within mononuclear phagocytes[38]. Mtb appears to gain entry into macrophages via cell surface molecules, including those of the integrin CR1 and CR3 complement receptors and the mannose receptors. It has been shown that mannose receptors bind the virulent Erdman and H37Rv strains but not the avirulent MT H37Ra strain. Mtb H37Rv has evolved a number of very effective survival strategies, including: (1) the inhibition of phagosome-lysosome fusion; (2) the inhibition of phagosome acidification; (3) the recruitment and retention of tryptophanaspartate containing coat protein on phagosomes to prevent their delivery to lysosomes; and (4) the expression of members of the host-induced repetitive glycine-rich protein family of proteins[39].

Pathological Consequences of Infection

Many of the symptoms, signs, and sequelae of TB and TBM are the result of an immunologically directed inflammatory reaction to the infection[40]. The TBM has a strong propensity to affect the basal parts of the brain. Mtb causes meningitis and parenchymal caseous granulomas. The inflammatory infiltrate extends into the subpial region, the ventricles, and subependymal parenchyma. The pathophysiology of TBM includes basal exudates, hydrocephalus, granuloma, and infarction. The location of the expanding tubercle (Rich focus) determines the type of CNS involvement. When the caseous content of a tubercle on the meninges leaks into the cerebral spinal fluid (CSF), the released material contains a significant amount of mycobacterial antigen. The neurological complications of TBM are initiated by a hypersensitivity reaction that occurs in the subarachnoid space when tuberculoproteins are released by the rupture of a caseous lesion. In the immunologically competent individual, this produces an intense immune reaction. As a result, severe inflammatory changes occur with accumulation of cells and the onset of hyperemia, edema, capillary damage, exudates, and fibrosis.

Neurological abnormalities occur with the development of an inflammatory exudate that affects mostly the sylvian fissures, basal cisterns, brainstem, and cerebellum[41]. The exudate contains erythrocytes, neutrophils, and macrophages, followed by lymphocytes in more mature exudates. The inflammatory infiltrate is composed of lymphocytes, mononuclear cells, epithelioid histiocytes, and occasional multinucleated giant cells. The hallmark pathologic features are meningeal inflammation (Figure 2), fibrogelatinous basal exudates, vasculitis of the arteries traversing the exudates and obstruction of flow of cerebrospinal fluid resulting in hydrocephalus. This exudates envelops arteries and cranial nerves, creating a bottleneck in the flow of cerebrospinal fluid at the level of the tentorial opening, which leads to hydrocephalus. Because of

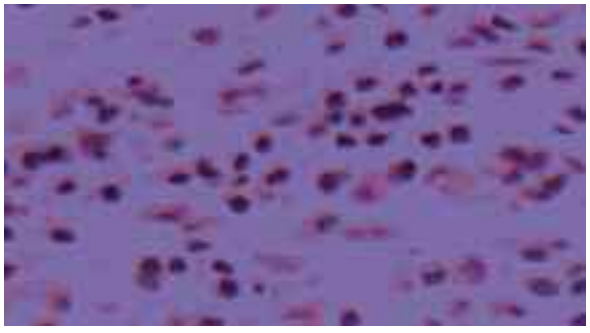

Figure 2. Dura mater with dense fibrosis and lymphoplasmacytic infiltrate.

the predilection of these changes to occur in the basilar portion of the brain, the inflammatory reaction tends to interfere with the function of cranial nerves, to produce hydrocephalus and injure blood vessels in that area[26,42,43].

Basal meningitis accounts for the frequent dysfunction of cranial nerves (CNs) III, VI, and VII, eventually leading to obstructive hydrocephalus from obstruction of basilar cisterns[44]. Formation of hydrocephalus in TBM is due to blockade of the CSF pathway and/or impaired CSF absorption. The reported frequency of hydrocephalus complicating TBM varies among different studies. Hydrocephalus commonly develops, sometimes acutely, and should be treated early by surgical drainage. In the mid-1960s, ventriculoperitoneal (VP) shunts largely supplemented ventriculoatrial (VA) shunts for CSF diversion in persons with hydrocephalus. This was mainly because of the diverse complications of VA shunts, such as pulmonary vascular microemboli and immune-mediated glomerulonephritis, among others. TBM damages nerves, blocks the circulation of CSF and thrombose small blood vessels, and produces multiple cerebral infarcts.

The most serious consequence of TBM is the development of vasculitis in the vessels of the circle of Willis, the vertebrobasilar system, and the perforating branches of the middle cerebral artery, resulting in infarctions in the distribution of these vessels. Cerebral ischemia and infarction occur either as the result of vasculitis caused by direct invasion of the arterial walls by mycobacteria and inflammatory cells or by the compression of the blood vessels at the base of the brain from the inflammatory exudate. The infarctions in TBM can occur in any arterial territory but characteristically affect penetrating vessels supplying basal ganglia. This is attributed to vasculitis, thrombosis, and strangulation of vessels by organizing exudates. In fact, changes in cerebral vessels are characterized by inflammation, spasms, constriction and eventually thrombosis of cerebral vessels. The resultant infarctions lead to hemiplegia or quadriplegia. The most common vessel pathologies involve infiltrative, proliferative and necrotising processes, either in relatively pure form or in combination. TBM vasculitis, as well as subsequent intimal proliferation, was originally considered to be a response to direct implantation of tubercle bacilli from the bloodstream[45]. Vessel pathology appears to be a consequence of its immersion in the local inflammatory exudate. Infiltrative, proliferative and necrotising vessel pathologies have been described, but the relative contributions of each and of luminal thrombosis to brain damage remain unclear[46]. There is some evidence that vasospasm may mediate strokes early in the course of the disease and proliferative intimal disease later strokes. The meningeal veins passing through the inflammatory exudate show varying degree of phlebitis.

Microscopic pathological feature of TBM is formation of epithelioid cell granulomas with Langhans giant cells, lymphocytic infiltrates and caseous necrosis. Granulomas can coalesce to form tuberculomas, causing diverse clinical problems depending on their size and location. Indeed, TBM may results in formation of tuberculoma consisting of caseous necrotic material, epithelioid cell granuloma and mononuclear cell infiltration, and old tuberculomas may become cystic or calcified with minimal inflammatory infiltrate[10].

The term *caseous necrosis* properly refers to the "cheesy" appearance of the necrotic lesion on gross inspection. TB infection of the CNS can sometimes take the form of focal abscesses instead of overt meningitis. The pathogenesis of these infections is similar to TBM; organisms lodge in the CNS at the time of hematogenous dissemination from the site of primary infection. Tuberculomas can be multiple and can involve any lobe of the brain. The size of cerebral tuberculomas is highly variable. In most cases their diameters range from a few millimetres (mm) to three to four centimeters. Solitary tuberculomas are more frequent than multiple lesions. These are tumor-like masses of tuberculous granulation tissue, most often multiple but also occurring singly, that form in the parenchyma of the brain and range from 2 to 12 mm in diameter. in developing countries. However, they constitute from 5 to 30 percent of all intracranial mass lesions. Tuberculomas are the most common brain tumors in India and other areas of the world where tuberculosis is still a common infection. Tuberculomas may appear in the brain or, occasionally, the spinal cord during treatment for tuberculous meningitis or, less commonly, with pulmonary tuberculosis, often at a time when corticosteroids have been reduced (paradoxical reaction).

Clinical Manifestations

TBM occurs in persons of all ages. The lack of specific symptoms and signs in patients with TBM makes early diagnosis difficult. Meningitis is frequent in infants and small children as an early

complication of primary infection, but it may be seen in any age group. Rapid progression tends to occur more often in infants and young children, who may experience symptoms for only several days before the onset of acute hydrocephalus, seizures, and cerebral edema. TBM occurs in approximately 7-12% of patients with pulmonary tuberculosis[47]. The clinical presentation of tuberculous meningitis is either an *acute meningoencephalitis* characterized by coma, raised ICP, seizures, and focal neurological deficits or a slowly progressive illness with persistent and intractable headache followed by confusion, lethargy, and cranial nerve deficits. Clinically, the discharge of tuberculosis bacilli into the cerebrospinal fluid is followed by an insidious prodromal period, marked by the gradual, fluctuating onset of fever, lassitude, weight loss, behavior changes, headache, and vomiting. The disease usually evolves gradually over two to six weeks. However, acute onset has also been described. TBM in older children and adults is frequently obscured by days to weeks of nonspecific symptoms[48,49]. A history of recent tuberculosis contact is common in children (50-90%) as are atypical neurological presentations.

The clinical manifestations of tuberculosis are quite variable and depend on a number of factors. Failure to thrive, loss of weight, irritability, poor appetite, sleep disturbance, vomiting and abdominal pain are often seen in young children[50]. For that reason, in many patients, the disease is difficult to recognize, and a high index of suspicion is necessary to establish the diagnosis. Fever, headache, vomiting, alteration in sensorium and nuchal rigidity are the most frequent presenting manifestations. Seizures, both febrile and nonfebrile, can be the presenting feature in children as can any focal neurological deficit, the commonest being cranial nerve palsies and hemiplegia[51].

Cranial nerve palsies, vision loss, focal neurological deficits and signs of raised intracranial pressure are common in advanced stages. In the elderly, symptoms are even more subtle, often just drowsiness and feeling unwell. Cranial nerve palsies are seen in approximately 25% of cases. The sixth cranial nerve is most commonly affected cranial nerve. Third and fourth cranial nerves are less frequently involved. Cranial nerves are affected either because of entrapment of nerve trunk in thick basilar exudates or because of increased intracranial pressure[52,53].

Visual symptoms include visual impairment or blindness and, occasionally, abrupt onset of painful ophthalmoplegia Complete or partial loss of vision is a major complication of TBM. Various mechanisms postulated for the loss of vision include presence of exudates around the optic chiasma, arteritis, compression of the anterior visual pathways due to hydrocephalus or tuberculoma, and ethambutol toxicity among others. Ocular tuberculosis presents a form of granulomatous uveitis.

Delayed or wrong diagnosis may be detrimental on the ocular structures and the health of the individual[54]. In untreated cases, progressive deterioration in the level of consciousness, pupillary abnormalities and pyramidal signs may develop due to increasing.

Atypical presentations include acute meningitic syndrome simulating pyogenic meningitis, progressive dementia, status epilepticus, psychosis, stroke syndrome, locked-in-state, trigeminal neuralgia, infantile spasm and movement disorders[55-57]. Tuberculous meningitis *in elderly patients* may present as a subacute dementia with memory deficits and personality changes typical of frontal lobe-like disease. In pediatric patients coma, raised intracranial pressure, seizures and focal neurological deficits dominate the clinical manifestations.

Convulsions can occur at all stages of the illness. Presenting clinical features of tuberculous meningitis in older children and adults in (Table I). Tuberculoma, presents a more subtle clinical picture than tuberculous meningitis. Intracranial tuberculomas in patients under the age of 20 yr are usually infratentorial, but supratentorial lesions predominate in adults. Solitary tuberculomas are more frequent than multiple lesions. The usual presentation is that of a slowly growing focal lesion, although a few patients have increased intracranial pressure and no focal findings. cerebrospinal fluid is usually normal, and the diagnosis is established by computed tomographic or magnetic resonance scanning and subsequent resection, biopsy, or aspiration of any ring-enhancing lesion.

Diagnosis Criteria

Central nervous system (CNS) TB accounts for about 5% of all extrapulmonary TB and TBM is the most serious complication[58]. Due to its relative rarity and the protean nature of the symptoms, tuberculosis of the CNS remains a formidable diagnostic challenge. TBM accounts for 70 to 80 per cent of cases of neurological tuberculosis[59,60]. The duration of illness before diagnosis is quite variable and relates in part to the presence or absence of other sites of involvement. In most

Table I. The presenting clinical features of tuberculous meningitis in older children and adults as described by recent clinical series.

Frequency/range
Symptom
Headache 50-80%
Fever 60-95%
Vomiting 30-60%
Photophobia 5-10%
Anorexia/weight loss 60-80%
Clinical sign
Neck stiffness 40-80%
Confusion 10-30%
Coma 30e60%
Cranial nerve palsy 30-50%
VI 30-40%
III 5-15%
VII 10-20%
Hemiparesis 10-20%
Paraparesis 5-10%
Seizures: children 50%
Adults 5%
Cerebrospinal fluid
Clear appearance 80-90%
Opening pressure >25 cm H_20 50%
Leucocyte count (103/ml) 5-1000
Neutrophils 10-70%
Lymphocytes 30-90%
Protein (g/L) 0.45-3.0
Lactate (mmol/L) 5.0-10.0
CSF glucose:blood glucose < 0.5 95%
Cerebrospinal protein can be >10 g/l in those with spinal block

series more than 50% of patients with meningitis have abnormalities on chest film, consistent with an old or current tuberculous process, often miliary tuberculosis.

Diagnosis of TBM is presumptive and is based on clinical symptoms, neurological signs, cerebrospinal fluid (CSF) findings, CT scan and MR imaging, the response to anti-tuberculosis drugs. The development of delayed-type hypersensitivity (DTH) in most individuals infected with the tubercle bacillus makes the tuberculin skin test a useful diagnostic tool. The most common method of testing a person for infection by Mtb is the *tuberculin skin test*. The test involves injecting a small amount of liquid filtered from weakened, nonvirulent tubercle bacillus (termed purified protein derivative, or PPD) under the skin of the forearm or administering it with a multipronged device. The former is known as the *Mantoux test* and is preferred because there is no way to be sure that an adequate amount of PPD is being injected in the latter procedure. A positive tuberculin test can provide diagnostic support, but test results may be negative in patients with tuberculous meningitis. The intradermal tuberculin skin test is negative in 50% to 70% of patients with and often becomes positive during the course of therapy. False-positive reactions to tuberculin can be caused by cross sensitization to antigens of nontuberculous mycobacteria (NTM), which generally are more prevalent in the environment as one approaches the equator. The absence of radiographical evidence of pulmonary tuberculosis and/or a negative tuberculin skin test does not exclude the possibility of TBM. A high index of suspicion is necessary for timely diagnosis and prompt initiation of therapy. Confirming the clinical suspicion of TBM has always been problematic.

Death from TBM is strongly associated with delayed diagnosis and treatment, and there is an urgent need to improve diagnostic methods for this disease[61]. Prompt diagnosis is crucial for successful disease management; the case fatality rate for untreated TBM is almost 100%, and delay in treatment often leads to permanent neurological damage[62,63]. The lack of accurate and rapid diagnostic testing for tuberculosis is an important impediment to worldwide tuberculosis control[64]. In most series more than 50% of patients with meningitis have abnormalities on chest film, consistent with an old or current tuberculous process, often miliary tuberculosis. In fact, only about 45% of patients will have chest radiographic evidence of past or present TB and only about half will have a positive tuberculin test[65,66].

A number of strategies have been attempted to improve the laboratory diagnosis of TBM. These include techniques to improve smear and culture positivity, biochemical tests, antigen and antibody tests, immunocytochemical studies, and bacterial DNA detection kits. The newer diagnostic tests and neuroimaging methods are unlikely to be available in many developing countries. Because early diagnosis is the key to a satisfactory outcome, a high level of suspicion is essential. The diagnosis and treatment of TM before the onset of coma is without question the greatest contribution a physician can make to improved outcome[67,68].

Cerebrospinal Fluid Examination

Careful study of the CSF is critical in the diagnosis of all CNS infections. In the presence of meningeal signs on physical examination, lumbar puncture is usually the next step in the diagnostic sequence. Neuroimaging may also show expanding masses and brain shift, which should be iden-

tified before lumbar puncture is performed. Analysis of the CSF is most likely to establish or exclude a diagnosis of meningitis and determine its cause; however, such tests are not often done.

Characteristic CSF changes help in differentiating from other causes of chronic meningitis. CSF is typically under increased pressure and is clear or slightly turbid. Rarely, it may look frankly purulent. Opening pressure (OP) at the time of the initial lumbar puncture (LP) in patients with TBM is elevated in 40-70% of cases, usually in the range 20-40 cm H_2O. CSF should be analyzed for protein and glucose (compared with simultaneous serum total protein and glucose). Total white blood cell and differential counts should also be obtained. A high protein (>50% of the serum protein concentration), lymphocytosis, and low glucose are typical of tuberculous meningitis. In fact, typical CSF changes are mononuclear cell pleocytosis, low glucose levels and elevated protein levels. A minimum of 5 ml should be submitted to the laboratory in a sterile container for mycobacterial culture.

The classic CSF abnormalities in tuberculous meningitis are as follows: (1) an elevated opening lumbar pressure, (2) increased WBC count between 10 and 500 cells/mm^3 with a predominance of lymphocytes, (3) an elevated protein concentration in the range of 100 to 500 mg/dL, (4) a decreased glucose concentration (the median glucose concentration is approximately 40 mg/dL), and (5) a positive culture in 75% of patients requiring 3 to 6 weeks for growth. Indeed, the "gold standard" for diagnosis is demonstration of MT bacilli in the cerebrospinal fluid[10]. The demonstration of acid-fast bacilli (AFB) in the cerebrospinal fluid (CSF) remains the best and the most widely available method, but the sensitivity varies significantly. The diagnostic yield of CSF microscopy and culture for Mtb increases with the volume of CSF submitted; repeat the lumbar puncture if the diagnosis remains uncertain. If tubercle bacilli are seen, the diagnosis is confirmed, but several samples may be required for organisms to be identified, and even then as many as 30-50% of cases are negative. In these cases it may be possible to diagnose TBM by PCR, although the place of this has still not been fully evaluated in TBM.

Many Authors report finding AFB in fewer than 20% of TBM patients[61], but the older literature suggests that much better results can be achieved[69,70]. A prospective study made a bacteriological diagnosis of TBM in 107 of 132 (81%) adults with clinical TBM: acid-fast bacilli were seen in 77 of 132 (58%) and cultured from 94 of 132 (71%)[71]. The CSF should be cultured in all cases of suspected meningitis. Cultures take on even more importance in fungal and TBM, in which the India ink or acid-fast preparations are negative in 50% or more of cases. In fact, culture of the CSF, though the gold standard, is positive in only about 40% of cases and may take up to 6 weeks to return a positive result[72]. The CSF of most patients with TBM contains only 100-102 organisms/ml yet approximately 104 organisms/ml are required for reliable detection with Ziehl-Neelsen and auramine stains[72]. The presence of acid-fast bacilli in the CSF should be diagnostic for the disease (Figure 3). To increase the yield of smear examination at least 6 ml of cerebrospinal fluid is collected and smear is examined for at least 30 min[73], and CSF sediments should always be subjected for smear examination.

Nucleic acid-amplification tests, such as polymerase-chain-reaction (PCR) assays, have been evaluated for their effectiveness in detecting the presence of bacterial DNA in cerebrospinal fluid. The CSF polymerase chain reaction (PCR) assay represents a significant advance in the diagnosis of TBM. The results of PCR studies in the CSF have shown a 94-100% specificity but sensitivities ranging from 75% to 100%[74,75]. Variations in the PCR methodologies have been attempted to improve the sensitivity and specificity of the test. A recent PCR advance is the use of real-time amplification and product detection by fluorescence using FRET

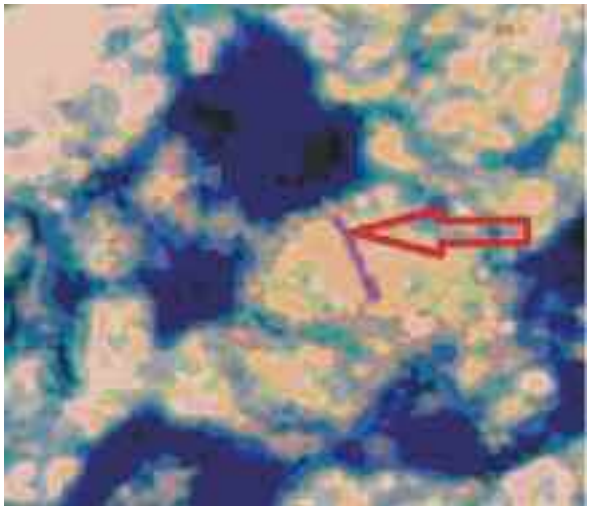

Figure 3. Single acid-fast bacilli (*arrow*) in the CSF of an adult with tuberculous meningitis (Ziehl–Neelsen stain; counter stain methylene blue).

(fluorescence resonance energy transfer) probes or SYBR green[76,77]. A modification of this test is the quantitative nested realtime PCR[78]. Real-time PCR is the fastest system and holds promise. However, to date most studies have used sputa. A more recently developed technique is the use of mycobacteriophage amplification, which detects viable organisms in specimens. The technique is simple, does not require expensive equipment, and results are obtained within 24-48 hours[79,80]. Authors suggest that the "filtrates" and not "sediments" are likely to reliably provide a polymerase chain reaction-based diagnosis[81]. CSF "filtrates" contain a substantial amount of Mtb DNA and (ii) "filtrates" and not "sediments" are likely to reliably provide a PCR-based diagnosis in "suspected" TBM patients. Enzyme-linked immunospot assay (ELISpot assay) is a novel assay for the rapid detection of Mycobacterium tuberculosis-specific T-lymphocytes[82]. Presence of acid-fast bacilli in CSF smear or culture and positive polymerase chain reaction for mycobacteria or immunoglobulin M enzyme-linked immunosorbent assay in CSF were considered as definitive evidences of TBM. "Definite" TBM is defined as the isolation of Mtb from one or more CSF cultures, and/or positive TB-PCR.

Neuroimaging

TBM is the commonest cause of subacute and chronic meningitis in the developing countries. Cranial tomography (CT) and magnetic resonance imaging (MRI) are the main imaging techniques used in its localization and characterization[83,84]. CT and MRI have revolutionized the diagnosis and management of TBM, but these studies may be normal early in the course of illness. CT and MRI of the brain show the pathological changes of TBM and provide diagnostic information at presentation and when complications occur. Brain CT or MRI studies of TBM patients may show hydrocephalus, parenchymal enhancement, contrast enhancement of basal cisterns, cerebral infarct, focal or diffuse brain edema, abscess or tuberculoma. Commonly identified neuroradiological features of TBM include basal meningeal enhancement, hydrocephalus (Figures 4 and 5), and infarctions in the supratentorial brain parenchyma and brain stem. CT is pathologic in the great majority of patients with TBM and is helpful in assessing the complications associated with the disease. In the literature, the use of CT in diagnosing TBM and its complications is well established. The characteristic computed tomographic changes include basal enhancement, presence of exudates, hydrocephalus and periventricular infarcts. The presence of high density within the basal cisterns on non-contrast CT scans is a very specific sign for TBM in children[85]. The role of CT is defined for the acute setting in detecting hydrocephalus for surgical management.

MRI is superior to CT in the evaluation of patients with suspected meningitis. The detection of abnormal meningeal enhancement on MRI is indicative of meningitis in a specific clinical setting. MRI showing evidence of exudates, hydrocephalus, infarction, and tuberculoma either in isolation or in various combination. On CT or MRI of the brain, tuberculomas usually appear as discrete lesions with a significant amount of surrounding edema.

Contrast medium enhancement is often impressive and may result in a ringlike lesion. A gadolinium-enhanced study can detect meningeal enhancement early in course of illness. Basal pial areas, particularly the interpeduncular fossa, were noted as the most preferred site of focal

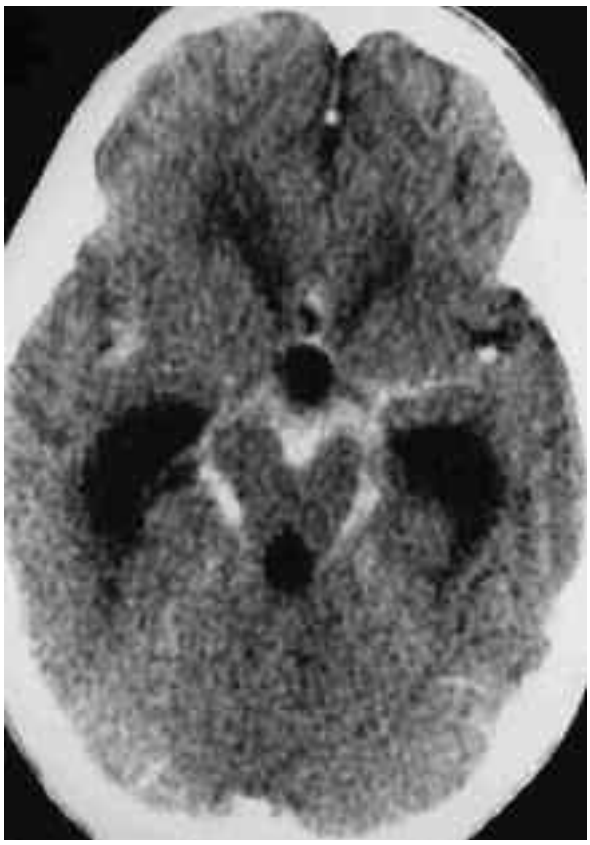

Figure 4. A computerized tomographic (CT) image of the Head showing thick basal exudates, ventricular dilatation is present.

Figure 5. Contrast enhanced computed tomography showing thick basal exudates, meningeal enhancemen.

meningeal enhancement[86]. The majority of infarctions in chronic meningitis are located in the basal ganglia, internal capsule, and thalamus and are rare in the major vascular territories and brain stem. The diagnosis of ischemic lacunes is based on MRI showing evidence of lacunes in basal ganglia which were defined as T2 hyperintense, T1 hypointense, and hyperintense on diffusion-weighted imaging (DWI) with a diameter of less than 1.5 cm[87]. Meningeal enhancement of contrast enhanced CT scans or MRI is observed in approximately half of the cases, and communicating hydrocephalus is a frequent sequel[88,89]. magnetization transfer (MT) MRI is an important technique for the detection and characterization of infectious meningitis of different aetiology. Visibility of the meninges on precontrast T1 weighted MT images may be considered to be highly suggestive of TBM.

Developments and Application of Mycobacterial DNA in Urine

Sputum microscopy is not able to easily detect paediatric, extrapulmonary, or HIV-associated tuberculosis, which are now important causes of morbidity and mortality in developing countries. There is a great need to develop alternative rapid-diagnostic methods – appropriate for use in both developing and developed countries – that are more sensitive and specific than sputum microscopy and better able to detect tuberculosis disease anywhere in the body. Evidence is building that urine contains fragments of DNA that are derived from the cell-free nucleic acids in plasma and blood resulting from the breakdown of DNA released from dying human cells and microorganisms. Some of these fragments pass through the kidney and are excreted in urine as transrenal DNA. Alternative strategies to diagnose tuberculosis by use of nucleic acid amplification methods to detect fragments of mycobacterial DNA in urine have been developed over the past decade with varying sensitivities and specificities[90]. Methods using quantitative PCR on urine samples to detect transrenal mycobacterial DNA are under development.

Tuberculous Meningitis in HIV-Infected Patients

Although not a neurological disease *per se*, AIDS is wide-ranging relevance to neurological practice and the multiple infections that can be associated with nervous system manifestations. Tuberculosis remains an important issue due to globalizationand the HIV epidemic. The HIV epidemic has had a major negative effect on TB control efforts. The consequence is an increased incidence of TBM in areas with high or rising HIV prevalence rates. Since 1985 there has been a moderate increase in the incidence of systemic tuberculosis and TBM and this increase has been mainly, although not exclusively, a consequence of the HIV epidemic. Approximately 30% of patients with tuberculosis are HIV seropositive[91,92]. Approximately 10% of all AIDS patients develop pulmonary or extrapulmonary tuberculosis, and CNS tubercular involvement occurs in 10% of these[93,94]. TBM is usually caused by the acid-fast organism *Mycobacterium tuberculosis* and exceptionally by *Mycobacterium bovis* or *Mycobacterium fortuitum*. The emergence of AIDS has led to a marked increase in cases caused by both the classic organism and also by the two atypical mycobacteria. In fact, tuberculosis has re-emerged in the last two decades in developed countries, mainly due to the HIV epidemic and immigration[95,96], and may be the first clinical manifestation of HIV infection[97].

The clinical presentation of TBM in HIV-infected individuals is similar to the clinical presentation in immunocompetent patients; however, HIV-infected patients are more likely to have

an acellular CSF at presentation and have a higher incidence of intracerebral mass lesions. It may be impossible to differenziate TBM from cryptococcal meningitis on clinical and routine CSF findings. In cryptococcal meningitis, headache is often the most dominant and sometimes may be the sole manifestation. In cryptococcal meningitis, meningeal signs may not be demonstrable. Neuroimaging studies are often normal. India ink preparation of CSF is diagnostic.

Despite specific treatment, the mortality of AIDS patients with CNS tuberculosis is high, the most important prognostic factor being the clinical stage of HIV infection at presentation[94]. More importantly, case fatality from TBM is greater in people infected with HIV than in those who are uninfected, although the role of other opportunistic infections upon case fatality is not known and there are no data from people taking antiretroviral drugs.

Drug resistance is more common in human immunodeficiency virus-positive patients. The effect of the HIV epidemic on the burden of tuberculosis in children has been less well characterised than for adults[98]. In several studies, it has been documented that human immunodeficiency virus does not significantly alter the clinical manifestations, laboratory, or radiographic findings or the response to therapy[94-99]. However, some studies, on the contrary, suggest that differences exist between immunodeficiency virus-infected and immunodeficiency virus-negative patients of TBM. TBM is accompanied by TB elsewhere in the body in most of the patients with HIV-TB and the CSF is often acellular; at times, CSF may be completely normal both in cellular and biochemical characteristics[100,101]. In patients with acellular CSF, meningeal signs may not be evident clinically[100]. Apart from these differences, intracerebral mass lesions are more commonly present in HIV-infected patients with TBM[102]. The classic computed tomographic signs of TBM (obstructive hydrocephalus and basal enhancement) were significantly less prominent in HIV-infected patients[102]. In patients with HIV, basal meningeal enhancement and hydrocephalus on CT might be less common and there could be more bacilli. Whilst some maintain that HIV infection does not alter the clinical or radiological manifestations or the outcome of TBM, others report higher mortality, more basal meningeal enhancement and more frequent obstructive hydrocephalus in HIV-positive TBM. Limited autopsy studies comparing HIV-positive and HIV negative TBM describe less inflammation but more bacilli with increasing immunodeficiency[103].

Treatment

Antituberculosis Chemotherapy

If detected early, tuberculous meningitis usually responds to standard chemotherapy supplemented with corticosteroids[104]. Chemotherapy for CNS tuberculosis follows the model of short course chemotherapy for pulmonary tuberculosis. Tuberculosis treatment consists of two phases, *an intensive phase* with a combination of bactericidal drugs to kill the rapidly growing bacilli and a *continuation phase* with fewer drugs to eradicate the slower-growing persistent bacilli[105] (Tables II and III). The optimal drug regimen and duration of each phase are not clearly established. Chemotherapy is given for between 6 and 18 months, dependent upon the guidelines followed and the drugs used. Isoniazid and rifampicin are the key components of the regimen.

Treatment is started with first-line antituberculous drugs which include isoniazid, rifampicin, pyrazinamide, streptomycin and ethambutol. Among the first line drugs, isoniazid is the only

Table II. Recommended treatment regimen for CNS tuberculosis caused by fully susceptible M. tuberculosis[110].

Drug	Drug daily dose		Route	Duration
	Children	*Adults*		
Isoniazid	10-20 mg/kg (max 500 mg)	300 mg	Oral	12 Months
Rifampicin	10-20 mg/kg (max 600 mg)	450 mg (< 50 kg) 600 mg (≥ 50 kg)	Oral	12 Months
Pyrazinamide	30-35 mg/kg (max 2 g)	1.5 g (< 50 kg) 2.0 g (≥ 50 kg)	Oral	2 Months
Ethambutol	15-20 mg/kg (max 1 g)	15 mg/kg	Oral	2 Months

Table III. Antituberculous treatment regimen for tuberculous meningitis (dosage of antituberculous drugs is given in mg/kg for children along with maximum adult dose) [111,112].

Tuberculous meningitis by drug-susceptible organisms (adult, children and human immunodeficiency virus-infected patients)

- *Initiation phase*: 2 months
  Isoniazid (4-6 mg/kg, 300 mg)
  Rifampicin (8-12 mg/kg, 600 mg)
  Pyrazinamide (20-30 mg/kg, 1600 mg)
  Streptomycin (12-18 mg/kg, 1000 mg)
- *Continuation phase*: 4-7 months
  Isoniazid (4-6 mg/kg, 300 mg)
  Rifampicin (8-12 mg/kg, 600 mg)

Multidrug-resistant tuberculous meningitis

- *Initiation phase*: 4 months
  Amikacin or Kanamycin (intravenous or intramuscular 15-30 mg/kg, 1000 mg)
  Ethionamide (15-20 mg/kg, 1000 mg)
  Pyrazinamide (20-30 mg/kg, 1600 mg)
  Ofloxacin (7.5-15 mg/kg, 800 mg)
  Ethambutol or cycloserine (15-25 mg/kg, 1200 mg; 10-20 mg/kg, 1000 mg)
- *Continuation phase*: 12-18 months
  Ethionamide (5-10 mg/kg, 750 mg)
  Ofloxacin (7.5-15 mg/kg, 800 mg)
  Ethambutol or cycloserine (15-25 mg/kg, 1200 mg; 10-20 mg/kg, 1000 m

bactericidal agent that easily crosses the blood-brain barrier (BBB), achieving concentrations in cerebrospinal fluid similar to those in serum. The second-line antituberculous drugs (ethionamide, cycloserine, para-aminosalicylic acid, aminoglycosides, capreomycin and thiacetazone) are kept in reserve.

Treatment for all forms of CNS tuberculosis should consist of 4 drugs (isoniazid, rifampicin, pyrazinamide, ethambutol) for 2 months followed by 2 drugs (isoniazid, rifampicin) for at least 10 months. Isoniazid penetrates the CSF freely and has potent early bactericidal activity[106]. Resistance to isoniazid has been associated with longer times to CSF sterility, which suggests an attenuated bactericidal response. Rifampicin penetrates the CSF less well (maximum concentrations around 30% of plasma), but the high mortality from rifampicin resistant TBM has confirmed its central role in the treatment of CNS disease[107]. Some centres use isonaizid, rifampicin and pyrazinamide for the duration of therapy[108].

Fluoroquinolones (levofloxacin, gatifloxacin and moxifloxacin) are antimicrobial agents which are used for the treatment of drug resistant tuberculosis. American Thoracic Society guideline also recommends a longer duration (9, 12 months) of antituberculous therapy for TBM. The doses of anti-tuberculosis drugs for the treatment of CNS tuberculosis have conventionally followed those used for pulmonary tuberculosis, although this approach has been questioned[109]. The recommended first-line treatment regimen for all forms of CNS tuberculosis is given in (Table II). The diagnosis and treatment of multidrug resistant TM is challenging. A history of previously treated tuberculosis or recent exposure to a known case of multidrug resistant pulmonary disease may identify those at high risk of multidrug resistant TM, but timely confirmation of the diagnosis is problematic.

The benefit of adjunctive corticosteroids in HIV infected patients with TBM is uncertain, although data from the only published controlled trial to include HIV infected patients suggested adjunctive dexamethasone might improve outcome[110].

In majority of patients, antituberculous treatment remains empirical. Hydrocephalus, cerebral infarction, and the expansion of tuberculoma are the commonest reasons for neurological deterioration during the treatment of TBM. Severe hyponatraemia (<120 mmol/l) may cause deepening coma and seizures. There is anecdotal evidence that fludrocortisone replacement therapy and demeclocycline may be useful treatments[110].

Adjunctive Corticosteroids

Adjunctive corticosteroids have long been suggested for the treatment of TBM. Cochrane systematic review and meta-analysis of 7 randomised controlled trials concluded that corticosteroids improved outcome in HIV-negative children and adults with TBM, but the benefit in HIV infected individuals remains uncertain[113]. Corticosteroids are thought to operate via modulation of the production of cytokines and chemokines by macrophages[114,115]. Corticosteroids such as dexamethasone which suppress the production of inflammatory cytokines and chemokines lead to better outcomes and are recommended as adjunctive treatment for patients with TBM Dexamethasone may affect outcome from TBM by reducing hydrocephalus and preventing infarction[116]. Authors demonstrated that dexamethasone decreased cerebrospinal fluid matrix metalloproteinases-9 concentrations early in course of the treatment, and this might be one of the mechanisms by which corticosteroids improve outcome in TBM[117], hypothesized that microglial-derived matrix metalloproteinases (MMPs) have a key role in driving such damage. Dexamethasone suppression

of MMP-1/-3 gene expression provides a novel mechanism explaining the benefit of steroid therapy in these patients.

Analysis of all of the MMPs demonstrated that conditioned medium from Mycobacterium tuberculosis-infected human monocytes (CoMTb) stimulated greater MMP-1, -3, and -9 gene expression in human microglial cells than direct infection[118]. Foreign antigens including pathogens deposited in the brain parenchyma are not detected efficiently by the immune system in the CNS. These experimental data may explain the clinical observation of delayed "paradoxical" enlargement or development of intracranial tuberculomas, observed several weeks to months in patients receiving anti-tuberculous therapy. There was some evidence that corticosteroid treatment might have a beneficial effect in patients having paradoxical reaction[119].

Prognosis

TBM still causes death, or severe neurologic deficits, despite the advent of newer antituberculous (anti-TB) agents and imaging techniques. TBM, early diagnosis and early treatment are essential for survival (120). The mortality rate associated with treated TBM is 20 to 50% in several series. From this series, those Authors observed that the initial stage of disease at presentation was a major prognostic indicator for mortality. Additionally, patients presenting after 4 weeks of symptoms had 80% mortality, whereas those presenting with less than 2 weeks of symptoms had 40% mortality[121].

Many prognostic factors for TBM have been reported, including age, the stage of the disease, level of consciousness, presence of extra-central nervous system (CNS) TB, isolation of Mtb from cerebrospinal fluid (CSF), biochemical studies of CSF, hydrocephalus, and in farction[122,123]. The prognosis for young infants is generally worse than for older children. Medical Research Council staging is used to assess the severity of tuberculous meningitis (Table III). In this system of staging, *in stage 1* patient is fully conscious and without focal neurological deficit; *in stage 2* patient may be in altered sensorium or has minor focal deficits such as hemiparesis or cranial nerve palsy; and in stage 3 patient is comatose or may have severe focal deficits like multiple cranial nerve palsy, hemiplegia and/or paraplegia[124]. Old age, consciousness changes, TBM *stage III*, and hydrocephalus suggest a poor prognosis for TBM, as does isolation of Mtb from the CSF, or positive CSF TB-PCR. In addition, delay in giving anti-TB therapy has a significant influence on mortality, and early diagnosis and prompt treatment play a key role in survival[125].

Administration of corticosteroids in adult patients is associated with significant decrease in the mortality[126]. Therefore, adjunctive steroid therapy should be administered for at least 2 weeks. However, the optimal dose and duration of steroid therapy need to be investigated further[125].

Prevention

Antibiotic treatment is the standard means of controlling and curing most cases of tuberculosis, but many governments throughout the world seek to prevent outbreaks of the disease by use of the Bacillus Calmette-Guérin (BCG) vaccine developed by researchers Albert Calmette and Camille Guérin at the Pasteur Institute in Lille, France, between 1908 and 1921. In terms of prevention of CNS infection, Mtb is exceptional in that it is the only intracellular bacterium for which a vaccine, BCG, is available that has a protective effect against meningitis. Nevertheless, the salutary effect of BCG vaccination against TBM is found only in children, not in adults, and some Authors suggest that it merely delays the age of onset of meningitis[127].

The mechanism of protection by BCG vaccination is not precisely known[128]. Several large-scale randomised clinical trials from different settings worldwide have suggested a protective efficacy of BCG vaccination against pulmonary tuberculosis that ranges from 0 to 80%[129]. It is universally accepted that protective efficacy of BCG vaccination decreases with age and there is insufficient protection against tuberculosis in adults. In addition, BCG is a live vaccine that can cause serious infections in immunocompromised patients, and cannot be safely given to persons with HIV infection, who are at greatest risk for tuberculosis. However, the greatest effect of BCG vaccination seems to be in preventing severe disseminated disease in young children, including TBM and miliary tuberculosis[130]. There are enough evidences to suggest that a second dose of BCG vaccine does not increase its efficacy.

Future Directions and Public-Health Priorities

Despite great advances in immunology, microbiology, and drug development, TB remains among the great public health challenges. Although global political commitment to reduce the prevalence and severity of TB in endemic areas is increasing, a lot remains to be done. Strong political commitment to comprehensive health

initiatives, together with local community involvement and ongoing advocacy, are required to meet the daunting challenge posed by the global TB epidemic. The lack of accurate and rapid diagnostic testing for tuberculosis is an important impediment to worldwide tuberculosis control.

There is a great need to develop alternative rapid-diagnostic methods appropriate for use in both developing and developed countries that are more sensitive and specific than sputum microscopy and better able to detect tuberculosis disease anywhere in the body. The prevention and management of TBM pose a substantial challenge, especially with the emergence of disease caused by multidrug-resistant pathogens. Existing cases need to be actively found, rapidly diagnosed, and aggressively treated to minimize transmission of disease. MT strains resistant not only to the front-line drugs isoniazid and rifampicin, but also to an increasing number of second-line drugs, are becoming more common. despite a century and a quarter after the discovery of Mtb, remarkably little is known about the pathogenesis of CNS TB. There is need to explore the factors which make a person susceptible to TBM. Development of methods to enhance innate and adaptive defences against MT are an attractive means to provide protection against both MDR and drug-susceptible tuberculosis.

The evidence for a human genetic component in susceptibility to TB is incontrovertible. Recent data utilizing animal models suggests that, in addition to host factors, Mtb genes and their encoded proteins may contribute specifically to bacterial invasion and survival in the CNS. Understanding how these microbial factors affect CNS disease would be essential to developing such preventive strategies. Heritability of disease concordance and immune responses to mycobacterial antigens has been clearly shown, and ranges up to 71%[131]. New approaches have provided early evidence for the importance of gene-gene interactions in regulating resistance to disease, and also the prospect that applying host genetics in the field of vaccinomics could lead to a more targeted approach in designing interventions to aid the human immune system in combating mycobacteria. In fact, not all persons exposed to tuberculosis develop tuberculous meningitis. Further insights into the basic neuropathogenesis of MT through the use of basic science techniques and the development of a relevant animal model are desperately needed to advance our understanding of the disease and uncover potential avenues for intervention.

Understanding the molecular mechanisms of tuberculosis pathogenesis will provide insights into the development of target-specific effective vaccine or drugs candidates for the treatment of the disease. Developing new vaccine strategies or improving upon the existing BCG vaccines currently in use may prove to have the most significant impact on TBM worldwide, with the promise of truly limiting or eliminating manifestations of tuberculosis in the CNS. There are several major hurdles to development of an antituberculosis vaccine. Unfortunately, effective immunity against Mtb and other intracellular pathogens, such as HIV, depends on cell-mediated, rather than humoral, immunity. Because cell-mediated immune responses often involves complex interactions between multiple cell types, development of a vaccine that elicits protective cell-mediated immunity is a tremendous scientific challenge. Inactivated Mycobacterium vaccae (MV) is an investigational vaccine prepared from an environmental mycobacterium that shares numerous antigens with other non-tuberculous mycobacteria (NTM) and with Mtb[132]. Compared with Bacillus BCG, MV has advantages as no need of skin test before injection; short treatment duration, safety, targeting a wide range of people and good compliance[133]. Although a new vaccine to prevent tuberculosis is the ultimate goal, better diagnostics probably represent the next most important step forward, and as such require urgent prioritisation. A reliable diagnostic tool would not only improve individual case management, but also provide a more robust case definition for drug and vaccine trials, and for studies of tuberculosis epidemiology and correlates of protective immunity in childhood.

Better diagnostic tools and new TB drugs are also required to manage patients with clinically active disease, especially in the face of the global emergence of drug resistance. The number of multidrug resistant TB (MDR-TB) cases (resistant to at least isoniazid (INH) and rifampicin (RIF)) was estimated at 489,000 in 2008, with 40,000 cases harbouring extensively drug resistant TB (XDRTB)[134]. XDR-TB strains are currently defined as those resistant to INH, RIF, fluoroquinolone and one of the injectables: amikacin, kanamycin or capreomycin[135]. Drugs which possess the ability to cross the blood-brain barrier (BBB) and achieve therapeutic concentrations in the CSF and brain parenchyma would be specific qualities in drug development for TBM. MDR-TB meningitis is especially challenging to treat due to limited CNS penetration of several 2nd

line anti-TB drugs. Newer drugs effective even against resistant cases with better penetration in cerebrospinal fluid are needed. There are currently at least ten drugs being evaluated in clinical trials. Some belong to chemical classes already employed in first-or second-line treatment regimens and are being explored for more optimized use at higher doses or new drug combinations (rifamycins, fluoroquinolones and oxazolidinones), while others represent potential novel members of the TB drug arsenal, killing MT through previously untried mechanisms of action (nitroimidazoles, diarylquinolines, ethylene diamines and pyrroles)[136,137].

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