The present discussion confines to the diagnostic aspects related to 17 KDa antigen, antibody assays in CSF as a means to reinforce the diagnosis of TBM in children arrived at by conventional parameters.

Immunodiagnostic methods have been used by a number of investigators and high levels of sensitivity and specificity have been claimed (CHANDRAMUKI, 1985; VAITYA & WAGLE, 1990). Antigen 5/38 KDa (these are reputed to be the same antigen), 14 KDa restricted largely to M. tuberculosis strains were used. Chandramuki et al showed 100% specificity but sensitivity of 61% with 14 KDa antigen. On the other hand Vaidya and Wagle used a polyclonal specific antibody to detect 38 KDa antigen in CSF and found a specificity of 100% and sensitivity of 82%. However other studies using antigen 5 did not show similar levels of sensitivity and specificity (RADHAKRISHNAN & MATHAI, 1990; SARALA & RAJA, 1991).

Since sensitivity and specificity levels of methods which have used apparently specific antigens of M. tuberculosis did not concurrently exceed 90%, it is not likely that other methods which have used non-specific antigens could have given more satisfactory levels (KADIVAL, 1987; GOGATE, 1993; SRIVASTAVA, 1994). It is thus not surprising that the controlled study (Table 6 in Literature) of International Energy Agency, Vienna failed to identify any
method of antibody or antigen detection exceeding these levels (Vide Appendix -I Personal communication KADIVAL).

In the present study 17 KDa protein identified on SDS-PAGE and isolated (Fig.1) was found to be a \textit{M. tuberculosis} specific antigen reacting with high specificity against sera from human tuberculosis patients but not with healthy controls. The specificity and sensitivity of this protein was later confirmed (85\% and 81\% respectively) by Jagannath et al (ICSU REPORT, 1990). The amino acid sequence of this reference was confirmed by VERBON ET AL (1992) and LEE & BRENNAN (1992). The primary amino acid sequence of this protein has no homology with those of other mycobacterial stress proteins and peptides containing sequences DF DGR, KATYDK were characterised (VERBON ET AL, 1992). Thus the only \textit{M. tuberculosis} specific antigens shown to date are 17 KDa (variously known as 16 KDa and 14 KDa) and 38 KDa of Ivanyi.

The specificity of an immunodiagnostic method is dependant upon the antigen used for antibody detection and antibody used for antigen detection. It is surprising that methods using specific antigen of \textit{M. tuberculosis} (Antigen 5/38 KDa, 14 KDa) also did not yield a satisfactory method. It is also not clear whether the controlled International Atomic Energy Agency study used any of these specific antigen (Personal Communication KADIVAL, Appendix). However, it must be recognised that no attempt was made to simultaneously detect a specific \textit{M. tuberculosis} antigen and its
corresponding antibody in CSF so far by other investigators. The present study has used *M. tuberculosis* specific 17 KDa antigen and a polyclonal antibody reactive with 17 KDa antigen to simultaneously detect corresponding antibody and antigen in CSF samples.

Since antibody is formed in response to infecting organism, it seems reasonable to expect a high degree of specificity from antibody detection tests. KALISH ET AL (1983) detected IgG antibody to tuberculin purified protein derivative in CSF of three patients with TBM by using ELISA techniques. No antibody was found in CSF from 33 patients with a variety of other neurological disorders. Using an RIA, SAMUEL & COLLEAGUES (1983) found IgG antibody in the CSF of 15 of 18 patients with bacteriologically confirmed TBM. Antibody was not found in CSF from control subjects. HERNANDEZ and his coworkers (1984) demonstrated either IgG or IgM antibody in the CSF of all of 20 patients with TBM and in none of 70 control subjects with other forms of meningitis or with non-infectious neurological diseases. However CHANDRAMUKI and her colleagues (1985) found low levels of antibody to mycobacterial antigen in the CSF of 18% of control subjects and 37% of patients with pyogenic meningitis from an area with high prevalence of tuberculosis. Antibody in the CSF was not found in control subjects from an area of low prevalence of tuberculosis.

In the present study of IgG antibody to 17 KDa antigen was detected by ELISA in CSF of 3 out of 90 control subjects.
These three CSF were from children with *S. pneumoniae* meningitis. In patients with bacterial meningitis, low levels of antibodies to mycobacterial antigen (MY-4) was reported by CHANDRAMUKI (1985). COOVADIA ET AL (1986) found that even with purified mycobacterial antigen 5, IgG antibody was detectable in 4 of 49 CSF from South African children with bacterial meningitis.

In this study, positivity among controls to 17 KDa antibody occurred only among pneumococcal infections. The cause of false positive reaction to antibody tests is more difficult to arrive at since 17 KDa peptide antigen derived from *M. tuberculosis* has been shown to be exclusively specific for that pathogen. Cross-reactions between polysaccharides of mycobacteria and other bacterial genera have been reported (MINDEN, 1972; STANFORD, 1974). There could have been antigenic cross-reaction between *S. pneumoniae* on one hand and 17 KDa antigen of *M. tuberculosis*. It is proposed that this is unlikely since polyclonal antisera to 17 KDa do not react with antigens from *S. pneumoniae* (JAGANNATH, Unpublished evidence) and further these antisera in sandwich ELISA for 17 KDa antigen did not reveal positivity. Except these three false positives, none of the remaining nine pneumococcal and eighteen *H. influenzae* meningitis showed positivity in the assays.

Of these three positives one child had exposure to tuberculosis through a family contact and another infant had strong
tuberculin test positivity. In the third child no further evidence could be obtained as autopsy was refused. LORBER (1960) had reported an eight month old infant who had combined tuberculous and *H.influenza* meningitis. In the absence of culture evidence for *M.tuberculosis* in these three cases, it can only be suggested that these cases could have had concurrent tuberculosis.

CHANDRAMUKI (1985) reported low levels of antibody to MY-4 antigen in CSF among 18% of her normal control CSF and DANIEL (1987) states that detection of antibodies in CSF of persons not suffering from TBM could be due to contact with environmental mycobacteria. In the present study none of the normal CSF controls showed positivity to 17 KDa antigen, antibody assays. This observation related to 17 KDa antigen, antibody strengthens the view that 'natural exposure' antibodies are unlikely in immunologically privileged sites like CNS.

During CSF immunoassays for mycobacterial antigens, earlier investigators found CSF from patients with cryptococcosis (SADA, 1983), Staphylococcal brain abscess (BAL, 1983), *H.influenzae* meningitis (KRAMBOVITIS, 1984), Pyogenic meningitis (CHANDRAMUKI, 1985) giving false positive reactions. One must ask why a small number of patients who clearly do not have TBM have been positive. The cause of false positivity in antigen detection methods could perhaps be traced to the polyclonal nature of
antibody probe used, which recognises many epitopes and it has been known for sometime that mycobacteria share antigens with other micro-organisms. Unlike these previous reports, no false positive reaction was observed in 17 KDa antigen detection among the study controls. Based on the above observations on 17 KDa antigen, antibody detection among controls in the study, it is believed that the system is specific enough to be useful for diagnosis of TBM.

Among the cases selected as TBM in the study _M. tuberculosis_ was isolated on CSF culture in 34%. The concurrent assay test was positive (either of the two ELISAs is positive or both the ELISAs positive) in all of the culture positive cases proving the sensitivity of the test. It may be pointed out that antigen was not detected in all of the CSF which were culture positive (Fig.5). The presence, absence or relative preponderance of antigen or antibody in CSF during TB meningitis process is dependant upon the stage of the disease and host-reponse. Methods which detect either antigen or antibody individually and interpret the result in isolation as positive or negative for tuberculosis may thus be misleading. This observation underlines the importance of assaying antigen and its corresponding antibody concurrently in the CSF.
Since the concurrent assay was positive in all the culture proven group (Na) it can be said that positivity in assay (either of the two ELISAs is positive or both ELISAs positive) is equivalent to a positive culture for AFB. The assay was positive in 75% of suspect TBM (Group Nb) reinforcing the diagnosis in them. The necessary and sufficient evidence to detect TBM or not is to apply culture and concurrent immunoassays. 17 KDa antigen, antibody tests reported in a more definite manner TBM or no TBM in suspect group (Chart II).

On analysis of CSF/Blood sugar ratio (Fig.10) and CSF cellular response (Fig.11), the assay negative cases among suspect TBM (Nb₂) were not identical to TBM group. These cases were wrongly included in the study as TBM - the wrong diagnosis based on the strength of criteria other than CSF cytology and CSF/Blood sugar ratio. These cases point out the limitations of clinical and indirect supportive criteria routinely applied for diagnosing TBM. CSF cytology appears to be a dominant parameter to differentiate TBM from non-TBM in suspect group in the same manner as 17 KDa antigen, antibody test. The sensitivity (84%) and specificity (97%) levels for 17 KDa antigen, antibody tests point out that concurrent assay can strengthen the diagnosis of TBM one may arrive at by cytology parameter.
STAGE OF DISEASE AND ASSAY POSITIVITY

Various studies are available on CSF antigen, antibody assays in TBM. However, their form of presentation of the data do not allow a critical analysis of assay positivity with respect to clinical staging. In the present study the usefulness of CSF assay results at admission as a pointer to clinical staging of TBM was examined. A characteristic pattern emerged on analysing the total of 76 assay positive cases of TBM in the study. Clinically graded into three stages (MEDICAL RESEARCH COUNCIL, 1948) the stage-specific distribution of antigen antibody positivities was studied (Table 4).

In Stage I, 80% of the cases had antigen only, while in Stage III there were no samples with antigen exclusively. The occurrence of both antigen and antibody in combination is not likely to be observed in Stage I. However, the combination of both antigen and antibody is predominantly seen in Stage III. These observations indicated that the presence of either antigen or antibody is related to the gradual evolution of the meningitis process within the CNS. Once the antigen is released and presented to the immune system, it is the host and tissue response which determines the outcome.

As normal CNS is immunologically 'privileged' and neither mycobacterial antibody nor antigen can be detected in CSF in healthy children, it was apparent that tuberculous meningitis
had an initial stage where mycobacterial antigen(s) are either secreted or occur as a result of mycobacterial degradation. As 17 KDa protein is not a secretory protein, its detection in CSF is because of bacillary breakdown and release only. In Stage I TBM therefore, mycobacterial antigen is detectable. In response to this antigen, the immunological system synthesises antibody which is apparent in later stages of TBM such as Stage II and III. Since newly formed antibody can compete with corresponding antigen, immune-complexes of antigen and antibody as well as free antigen and antibody should occur in Stage II or III. The presence of all three components in CSF of TBM were established this study.

That immune-complexes occurred in CSF of TBM was supported in the study by two observations (i) antigen alone appeared in Stage I and in only 2 of 29 cases in Stage II TBM while none of the Stage III cases had antigen alone. (ii) Immune complexes were fractionated from among the culture positive TBM and they showed individual presence of 17 KDa antigen and 17 KDa antibody (Fig.7).

The presence of Immune-complexes in CSF of TBM cases raises new questions. The Immune-complexes are formed between antigen and antibody and there will be a sequestration of corresponding antigens and antibodies at any given stage of the disease, while some amount of antigen and antibody may remain free. Methods which detect either of them individually will therefore
be insensitive in immunoassays unless methods to dissociate them are carried out. The relative value of an immunoassay system will be determined by the preponderance of a given type of antibody or antigen as the case may be. In the present system 17 KDa antigen and its antibody dominated in Stage II and III cases whereas 17 KDa antigen occurred more frequently in early Stage I TBM. The study therefore indicates that there is a need to develop both antigen and antibody assay for diagnosing CSF of TBM besides dissociating Immune complexes of CSF prior to antigen or antibody assay.

It would have been ideal if all the CSF samples could have been subjected to the dissociation of Immune-complexes prior to antibody and antigen assay. However the objective of the study was to determine the possible factors influencing such assays and it is evident that simultaneous detection of antigen, antibody and Immune-complex will yield better methods of diagnosing CSF of TBM. Rapid methods of diagnosis of TBM like Latex particle agglutination of CSF with plasma membrane antigen (KRAMBOVITIS, 1984) may appear attractive. Heterogeneous ELISAs such as the present 17 KDa system, requiring long incubations may not at present appear feasible for routine use. However when standardised, concurrent assays proposed in the present study will provide a rational background to interpret the results stagewise and reinforce the diagnosis of TBM.
It appears from the literature that no such attempt has been made to simultaneously detect antibody, antigen and immune complexes in CSF irrespective of the antigen or antibody system used. While it goes without saying that \textit{M. tuberculosis} specific antigens and antibodies should be used to achieve necessary levels of sensitivity and specificity, on the basis of the observations in this study it is proposed that the lack of sensitivity observed in other previous studies, where specific antigens were used (Table 4 in literature) could be attributable at least in part to the formation of immune complexes in CSF and the resultant non-availability of free antigens, antibodies.

Even with modern chemotherapy the prognosis of TBM is closely linked to the stage of the disease at the time of diagnosis. Any attempt to improve the prognosis of TBM must thus at present depend upon early diagnosis. In this study the tuberculous antigen (17 KDa) was detected in the CSF of majority of patients with Stage I disease. This observation is important since it means that the diagnosis of TBM by this method can be established at an early stage of the disease. Stage II and III cases had more antibodies than Stage I of TBM. Thus antibody synthesis appears to be directly related to the severity of TBM and could be a useful marker for prognosis in TBM.
17 KDa antigen, antibody system had more sensitivity for antibody detection than for antigen (Fig. 4 and 5). But the simultaneous presence of antigen and antibody in Stage II and III argue that in many instances the diagnostic sensitivity could have been affected by the formation of Immune-complexes, although this needs to be proven on an experimental ground. Immune-complexes were detected in the study from among CSF of proven group. Nevertheless it is obvious that the combination of antigen, antibody detection methods should prove valuable in the diagnosis of TBM at an early stage based on the data presented herein. The need for Immune-complex dissociation as a preliminary step is also evident from the results.

Very little information is available as to what happens in Immune-complexes in mycobacterial diseases. The relation between Immune-complex formation and clinical manifestation is uncertain. However, as mentioned earlier Immune-complex formation influencing at least in part the sensitivity of diagnostic assay procedure deserves emphasis.

The detection of tuberculous CSF antigen and antibody in CSF at a particular time would depend on the balance between antigen and antibody in CSF which in turn would depend on the duration and intensity of infection. ASHTHEKAR (1987) found no direct correlation between circulating Immune-complexes and CSF
antigen, antibody levels. This indicates that the antigen and antibody levels in the blood pool and CSF pool are independent of each other. Alternatively dilution effect in blood is considerably greater than the CSF pool and hence individual values would not be comparable.

The formation of immune complexes is complicated and the highest probability of such complex formation is when the antigen and antibody are in the phase of equivalence. During the phase of antigen excess with lower antibody, only antigen would be detectable and during the phase of antibody excess only antibody could be detectable in the complexes. Immune-complexes fractionated from among CSF in the study contained both 17 KDa antigen and antibody. From a diagnostic view point concurrent estimation of antigen, antibody and Immune-complex is necessary. Detection of antigen or immune complex might point to current infection while antibody positivity the prognostic trend. The extent to which the trend of association between TB antigen and antibody in CSF stagewise would be affected by variations in immune complex formation needs further evaluation.

CONVENTIONAL DIAGNOSTIC PARAMETERS

The clinical diagnosis of TBM is difficult to establish. The initial symptoms of TBM such as vomiting, fever, irritable behaviour seen in this study are well known (ILLINGWORTH, 1960),
nonspecific in the early stages of the disease and are also observed in a number of other neuroinfections. Their persistence and occurrence in a young child in contact with tuberculous adult should lead to the consideration of possibility of TBM.

The duration of illness before referral to hospital was long among the children studied averaging 18 days and a result 60% of children were already in coma at admission. It is traditionally taught that there is a long history of illness in TBM; but in some children in the study, particularly those below 18 months, there was a much shorter history of less than one week. The acceleration of symptoms in them to Stage II and III of the disease might have been caused by intercurrent illness like measles or malnutrition.

There are few classical signs pathognomonic for TBM. VI cranial nerve palsy in a child with altered sensorium and nuchal rigidity were indicative clinical signs. Choroidal tubercles often emphasised (EDITORIAL BMJ, 1971) were seen in only one of the children studied. KILPATRICK (1986) found no child with choroidal tubercles in his series of 100 CSF culture positive TBM from Egypt. Choroidal tubercles are found only when meningeal involvement is part of miliary tuberculosis. The case with choroidal tubercle had evidence of miliary tuberculosis. Miliary tuberculosis with meningeal involvement accounted for 3-17% of TBM cases
in various studies (UDANI, 1974; BENEKAPPA, 1983; KILPATRICK, 1986; DONALD, 1990). Convulsions with motor paralysis, seen in nearly half of the children studied, were an important feature. Generalised convulsions reflect underlying cerebral edema in early stage and vasculitis in later stage. It has been known for a long time that convulsions are not uncommon in TBM (LORBER, 1960).

Chest radiography plays an important role in the diagnosis of tuberculosis in childhood. Normal x-rays were encountered in cases of TBM in the study; but the importance of x-ray as a diagnostic aid when faced with a child having meningitis of unknown origin or as a possible means of preventing TBM cannot be overlooked. The number of children with x-rays compatible with tuberculosis at the time of diagnosis of meningitis in this study is similar to reports by ZARABI (1971), UDANI (1980) and BENEKAPPA (1983). Chest x-ray was helpful only when positive. But a timely chest x-ray in a child, who is failing to gain weight adequately or who is a contact of tuberculous adult, may lead to the prevention of a case of TBM.

In this randomised prospective study heterogeneity was reflected in age and weight distribution between study and control population. It is not surprising, since a group among the controls were pyogenic meningitis — a disease commonly seen at a younger age than TBM. Children below 5 years formed 80% of TBM cases
in the study similar to the experience reported from Bangalore (BENEKAPPA, 1983). Unsuspect household contacts with smear positive TB was responsible for TBM cases seen even in infancy in the study.

Many descriptions of childhood TBM refer to the malnourished condition of the children (PARSONS, 1979) and the observations on Quetlet's Index in the study group (Table 1) again supports this impression. The importance of adequate history and contact tracing cannot be overemphasised. Sixty-four percent of children with TBM in this study had a positive contact. Figures varying between 10-38% have been reported in various studies on childhood TBM (RAMACHANDRAN, 1966; MAGOTRA, 1974; BENEKAPPA, 1983; SHARMA, 1994). Such a history calls for a diligent search and objective clues for tuberculosis. Failure to obtain such a history should not be interpreted as evidence against the diagnosis of TBM.

Forty-five children with TBM in this study had a BCG scar. The protective efficacy of BCG given to the neonate has been estimated to vary from 60-80% (CURTIS, 1984; YOUNG, 1986; TIDJANI, 1986; WAZ-HOCKERT, 1988). A significant protective efficacy of 97% against TBM in children below 5 years was noted in a case control study done at our department (CHITRA, Personal communication, 1993). It should be borne in mind that the
protective effect of BCG may be limited by exposure to a tuberculous adult before tuberculin hypersensitivity has developed. The protective effect of BCG may also be overwhelmed by excessive exposure to smear positive tuberculosis (ROSENTHAL, 1961).

Among the TBM cases studied tuberculin positivity was 62%. Varying figures are quoted for the incidence of tuberculin positivity in TBM. Edith Lincoln in a series of 162 cases from USA during the period 1940-1960 found 87% to give a positive tuberculin test (LINCOLN, 1960), STEINER in 52% (1973) while Illingworth reporting an English series of 184 cases found 58% to be tuberculin positive and Ramachandran working in India found only 36% of 288 children to be tuberculin positive (RAMACHANDRAN, 1970). There is little doubt that negative tuberculin tests will be encountered in TBM. The disease and nutritional status of the child influence its positivity. Tuberculin test, initially negative, became positive in two children in the study a month after therapy and with improvement in general condition. The reported variability in positive admission tuberculin skin tests reflect the severity of illness and type of tuberculin used.

Parental negligence is often blamed for the late stage at which TBM is diagnosed. While this may be so in individual cases, the study found that it is often health personnel who fail to consider the possibility of TBM. The fact that 66 of 76
patients with TBM were seen in Stage II and III of the disease indicates that the diagnosis was not made early at peripheral levels. It is emphasised that mere possibility of TBM is sufficient reason to consider initiation of anti-TB therapy; but establishing the diagnosis, by reliable parameters outlined in the study, is equally necessary to avoid inaccurate reporting of the disease and avoid unnecessary chemotherapy of presumptive cases.

Laboratory parameters aid in supporting the clinical suspicion of TBM. CSF protein rise accompanies the cellular changes. CSF protein levels did not help to distinguish the various groups in the study except healthy controls. CSF proteins has more prognostic value than in predicting the diagnosis. CSF glucose concentration is a more reliable parameter with lesser frequency of fluctuation than proteins or cells in children with TBM (DONALD, 1991). An absolute value of 35 mg% or less was seen in majority of children studied. The CSF/Blood sugar ratio pointed out in the study the assay negative group was not identical to TBM group. Compared to proteins CSF sugar has more diagnostic value.

The question of diagnosis of TBM is not there if CSF can demonstrate AFB in majority of patients. In the present study, though AFB smear of admission CSF was a low yield procedure, its value as a first step in CSF examination must not be forgotten. Success rates in CSF smear positivity varied from 0-20% in
South Africa, India (COOVADIA, 1986; BENEKAPPA, 1983; CHANDRA-MUKI, 1990; TANDON, 1988; JOISHY, 1974) and 10-40% in developed countries (MOLAVI, 1985). Culture positivity in the study was 34%. Culture is achieved with varying success but result cannot influence initial management. Success rates vary from 30-90% depending on the number of samples, time and care taken for examining the CSF (KENNEDY & FALLON, 1979).

Cerebrospinal fluid cellular response - its degree and pattern - appeared a useful parameter based on the observations in this study. Majority of cases of TBM had CSF cell counts ranging 100-500/cu.mm. High pleocytosis of more than 1700 cells, reported by JEREN (1982) was not encountered. Such high numbers were seen in cases of bacterial meningitis only in this study. Absence of cellular response noted by UDANI (1974) was an infrequent observation in the present study. Observations from other reports confirm that normal CSF is not common in TBM (BENEKAPPA, 1983; JEREN, 1982; JACOB, 1993). Sequential CSF cell estimations on follow up lumbar punctures has been suggested by DONALD (1991) for confirmation and monitoring.

The type of cells seen in TBM include lymphocytes in majority of cases and mixed polymorph response in early stages. However, in this study a distinct cell, uniformly found in varying proportions with other cells (10-40%) was the 'monocyte-macrophage
cell'. The distinct morphology - a large cell with indistinct cell margin, abundant cytoplasm and elongated irregular nucleus could be easily recognised. The recognition of the presence of this cell in CSF of TBM is a useful diagnostic clue. The diagnostic usefulness of the mononuclear cell in TBM has been reported by JACOB (1993) who found them in 26 of 28 CSF samples. JEREN (1982), KRAMBOVITIS (1984) observed these cells in their studies on TBM accounting for 36-100% of cells seen on CSF examination. The pathogenetic mechanisms involved in TBM include both cell mediated and humoral response which probably occur at different stages of the illness. The CSF mononuclear cell may reflect these changes. Based on the observations in the study, the importance of looking specifically for mononuclear cells in CSF of TBM as a useful diagnostic marker deserves emphasis.

Despite the advent of more sophisticated diagnostic aids TBM must still be suspected before they can be applied. Despite their limitations, the usefulness and feasibility of conventional diagnostic parameters like tuberculin test, chest x-ray, AFB smear, culture, CSF cytology, proteins and sugar levels cannot be disputed. Besides clinical suspicion, all the
above parameters might not be useful to diagnose TBM at an early stage of the disease. The findings of the present study indicate that concurrent assays for kDa antigen, antibody in CSF help in diagnosis at an early stage of TBM. The assay also picked up 75% of suspect TBM in whom the diagnosis could not have been otherwise established unequivocally.

There is a long history of attempts to establish diagnostic tests for TBM. The method of concurrent assay of CSF for 17 kDa antigen and its antibody by ELISA tests is sensitive and specific enough to be useful and may add considerably to the diagnosis of TBM in children.

The cost considerations and initial standardisation should not be viewed as constraints for establishing this test. ELISA techniques, routinely employed in many hospitals, are not difficult to standardise. Attempts to validate the results of this study from centres which see large number of children with TBM are required. It is suggested that 17 kDa antigen, antibody assay tests on CSF be included as an important measure for
diagnosis of TBM at an early stage and to reinforce the diagnosis in suspect TBM. The need to do so is acute for an important life-threatening infection like TBM in children.