ADENYLATE KINASE VALUES IN CEREBROSPINAL FLUID AS A MARKER TO PREDICT NEUROLOGICAL OUTCOME IN CHILDREN WITH MENINGITIS

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I declare that this thesis is my own work. It is being submitted for the Masters Diploma in Technology (Medical Technology) to the Cape Technikon, Cape Town. It has not previously been submitted for any diploma, degree or examination at any other institution. This work was carried out in the Department of Paediatrics and Child Health, Medical School, Tygerberg Hospital, University of Stellenbosch. The opinions and conclusions drawn are not necessarily those of the Cape Technikon.

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5/5/97
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Date

I DEDICATE THIS WORK TO MY DAUGHTERS

LISA-UGA AND LARA CARLINI

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LIST OF ABBREVIATIONS

ADP	Adenosine-di-phosphate
AK	Adenylate kinase
AMP	Adenosine-5-monophosphate
ATP	Adenosine-5-triphosphate
BM	Bacterial meningitis
CSF	Cerebrospinal fluid
CT	Computerised tomography
DTT	Dithiothreit
EC	Extracellular
g	Gram
g/l	Gram per liter
IC	Intracellular
ICP	Intra cranial pressure
inn	Innumerable
IQ	Intelligence quotient
LDH	Lactate dehydrogenase
LP	Lumbar puncture
M	Molar
mg	Milligram
ml	Milliliter
mmol	Millimol
μl	Microliter
MgSO4	Magnesium sulphate
NAD	Nicotinamide-adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide disodium salt
PEP	Phospho-eno-pyruvate
PK	Pyrovate kinase
RBC	Red blood cells
TBM	Tuberculous meningitis
TEA	Triethanolamine-hydrochloride
TIA	Transitory ischaemic attack
U/l	Units per liter
W	Week

OPSOMMING

Meningitis in kinders is 'n algemene en ernstige siekte. Bakteriële en tuberkuleuse meningitis lei dikwels tot neurologiese komplikasies. 'n Sensitiewe merker om breinskade te voorspel in kinders met meningitis, sal van groot belang wees. Frithz F *et al*, 1982 het voorgestel dat verhoogde adenilaat kinase waardes wel as merker vir breinskade kan dien.

Adenilaat kinase (AK) is 'n ensiem wat in breinweefsel teenwoordig is. Lae konsentrasies is teenwoordig in normale serebrospinale vog (SSV) (< 1 E/L). Verhoogde konsentrasies is gevind in gevalle van isgemiese breinskade (Frithz F *et al*, 1982), maligne breintumore (Ronquist G *et al*, 1977) en bakteriële meningitis. Omdat adenilaat kinase 'n lae molekulêre massa het (22,000 Daltons) in vergelyking met ander kinases (40,000 Daltons), is AK een van die eerste ensieme wat in die SSV aangetref word na breinskade en kan dit dus as 'n betroubare merker van breinselskade beskou word.

Die doel van die studie was om adenilaat kinase waardes in die SSV van kinders met bakteriële- en tuberkuleuse meningitis kwantitatief te bepaal en vas te stel of hierdie waardes neurologiese uitkoms in die kinders kon voorspel.

Agt en tagtig kinders met tuberkulose meningitis (TBM) en drie en dertig kinders met bakteriële meningitis is vir hierdie studie gebruik. Sestig kinders met moontlike meningitis, maar later gediagnoseer met urienweg infeksies, gastro enteritis, brongitis, koorskonvulsies of ander nie-neurologiese infeksies, is as kontroles gebruik.

Die resultate het getoon dat verhoogde AK waardes by kinders met bakteriële- en tuberkuleuse meningitis (TBM) voorkom. Daar was 'n statisties betekenisvolle verskil tussen stadium III en II TBM AK waardes gedurende die eerste week na diagnose ($p=0,03$). Daar was ook 'n statisties betekenisvolle korrelasie tussen SSV AK waardes en laktaat konsentrasies ($p=0,001$) wat op hipoksiese breinmetabolisme gedui het.

Alhoewel AK waardes nie altyd direk gekorreleer het met die kinders se kliniese prognose nie, is daar definitiewe bewyse dat verhoogde AK waardes in die SSV as merker van breinskade gebruik kan word.

SUMMARY

Meningitis in children is a common and serious disease. Bacterial and tuberculous meningitis often lead to neurological complications. A sensitive marker to predict brain damage in children with meningitis could be of great importance. Frithz F *et al*, 1982 suggested that increased adenylate kinase values could indeed be used as a marker for brain damage.

Adenylate kinase (AK) is an enzyme present in brain tissue. Low concentrations are present in normal cerebrospinal fluid (CSF) (< 1 U/l). Increased concentrations were found in cases of ischemic brain damage (Frithz *et al*, 1982), malignant brain tumours (Ronquist G *et al*, 1977) and bacterial meningitis. As AK has a low molecular weight (22,00 Daltons), in comparison to other kinases (40,000 Daltons) it is one of the first enzymes that can be detected in the CSF after brain damage and it can thus be used as a reliable marker for brain cell damage.

The aim of this study was to quantify the AK values in CSF of children with bacterial and tuberculous meningitis and to evaluate their use to predict the neurological outcome in children with bacterial and tuberculous meningitis.

Eighty eight children with tuberculous meningitis (TBM) and thirty three children with bacterial meningitis were included in the study. Sixty children with suspected meningitis but who were later diagnosed with urinary tract infections, gasto-enteritis, bronchitis, febrile convulsions or other non-neurological infections were used as controls.

The results showed raised AK values in the CSF of children with bacterial- and TB meningitis. There was a statistically significant difference of AK values between stage III and II TBM AK values in patients at week 1 after diagnosis ($p=0,03$). There was also a statistically significant correlation between CSF AK values and lactate concentrations ($p=0,001$) which reflected hypoxic brain metabolism.

Although AK values did not always correlate directly with the patients' clinical outcome, there is proof that increased AK values in CSF can be used to predict neurological outcome.

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CHAPTER 1

INTRODUCTION

1. MENINGITIS

1.1 General

Meningitis is the term which refers to the condition when the meninges covering the brain are infected. The term meningitis is said to have been used for the first time by the French army surgeon Francois Herpin in 1803. At post mortem examination of injuries to the skull he noted purulent material between the meninges and called this "new" condition, meningitis. In 1768 the term "acute hydrocephalus" was used by Robert Whytt, Professor of Medicine at Edinburgh University who gave a classical description of the features of tuberculous meningitis (TBM) based on post mortem investigations of 10 cases. He noted "dropsy in the brain" with the accumulation of watery fluid in the ventricles, not knowing that he in fact described the consequence of the disease rather than the disease itself.

The greatest single contribution in the diagnosis of meningitis was made by Heinrich Iraneus Quincke who, when attempting to reduce the raised intracranial pressure present in some cases of hydrocephalus, introduced the lumbar puncture in 1891. In 1896 Otto Heubner was able to recover an organism (*N. meningitidis*) from a living patient using Quincke's method. (Berlin Kliniesche Wochenschrift 1891:38)

Cerebrospinal Fluid (CSF)

In man about 130-140 ml of the cavity enclosing the brain and spinal cord is occupied by CSF. It has a very low specific gravity (1,005) and is secreted continuously into the ventricles, chiefly from the ventricular choroid plexuses.

CSF is normally crystal clear and any deviation from this is considered to be pathological (Macdonald Critchley, 1972). Less than 5 cells x 10^6/l, usually all lymphocytes, are found in normal CSF in children older than 3 months of age. The protein concentration is between 0,1 - 0,4g/l and the glucose 2,2 - 4,5mmol/l in normal CSF.

In cases of meningitis the CSF values are changed and a characteristic picture seen in the different types of meningitis as illustrated in Table 1.

TABLE I

CSF pressure, cell counts, protein and glucose under normal conditions and the characteristic findings in meningitis caused by different organisms

Condition	Pressure mm H_2O	Lymphocytes 10^6/l	Polymorph nuclear leucocytes 10^6/l	Protein g/l	Glucose mmol/l
Normal	<10	<5	0	0,1-0,4	2,2-4,5
Acute bacterial meningitis	usually elevated	>5 - ≥500	1 - ≥500	1 - 5	<2,2
Partially treated bacterial meningitis	usually elevated	>5 - ≥500	1 - ≥500	1+	depressed or normal
Tuberculous *meningitis*	usually elevated	0 - 500*	0 - ≥500	1 - 5	<2,2
Viral or meningoencephalitis	normal or slightly elevated	50 - 200	normally absent	normal	normal

***may occasionally be higher**

1.2 **Bacterial meningitis**

1.2.1 *Pathogens*

In children with bacterial meningitis a specific organism, for example *Haemophilus influenzae*, *Neisseria meningitidis*, *βeta haemolytic streptococcus*, *Escherichia coli*, may be cultured from the CSF. The CSF in these children usually shows an increased polymorphonuclear neutrophil count, the protein concentration is usually raised and glucose concentration decreased.

1.2.2 *Diagnosis*

The diagnosis of meningitis may be confirmed by evaluation of the CSF cell count and the CSF protein and glucose concentrations following lumbar puncture. The causative organism may be identified on microscopy of the CSF after Gram's stain and on culture from the CSF.

1.2.3 *Outcome*

In older children increased intracranial pressure is the rule. In many cases meningitis is associated with inappropriate secretion of anti-diuretic hormone and if the patient is given normal or excessive amounts of water, a further increase in intracranial pressure may result.

Seizures occur in about 30% of children with bacterial meningitis. Stupor, coma and focal neurological signs may occur. Transient or permanent paralysis of the cranial nerves may be noted and deafness or disturbances in vestibular function are relatively common. Involvement of the optic nerve, with blindness is rare. Paralysis of the 6th cranial nerve (a non-specific sign of raised intra cranial pressure), usually transient, is noted frequently early in the course of the disease.

Collection of fluid in the subdural space has been demonstrated in up to 50% of infants suffering from *H.influenzae* meningitis during the acute illness. These effusions appear to be more frequent in the very young, resulting in enlargement of the head circumference.

Major neurological complications of meningitis include deafness (10%), and consequently speech impairment (15%), mental retardation (10%) motor abnormalities (3-7%) and convulsions (2-8%).

1.2.4 *Therapy*

Appropriate antibiotic therapy based on the sensitivity of organisms is given to the patient. The aim of the therapy is to eliminate the causative organism as rapidly as possible and prevent long-term complications.

1.3 Tuberculous meningitis

1.3.1 *Pathogenesis*

TBM in children is caused by M.tuberculosis bacilli and is always secondary to a primary lesion in another organ, usually the lung. It might also be caused by direct spread from a neighbouring focus such as tuberculous otitis media or spondilitis (Smith HV, 1964).

On presentation children with TBM are classified into 3 stages: (described by the British Medical Research Council in 1948) stage I (general, non-specific symptomatology), stage II (appearance of definite neurological signs) and stage III (coma). This staging system is a reasonable reflection of severity and therefore predicts outcome and complications of TBM.

Stage I

It may be difficult to make the correct diagnosis because of the subtle and non-specific nature of the symptomatology. The child seems disinterested in playing, has periods of idly staring into space, shows loss of appetite and may be febrile. The school going child may show rather abrupt mood changes, declining school performance, lethargy and apathy.

As the disease progresses, irritability becomes worse and may alternate with apathy. Fifty percent of the children will experience episodes of vomiting, or complain of

headaches. Children under 2 years of age may have seizures. Stage I lasts from 1-3 weeks.

Stage II

It is marked by the appearance of neurological signs which result from an exudate that forms over the cerebral convexities. Irritation of the meninges produces nuchal rigidity. As time progresses, a thick gelatinous infiltrate and an exudate develop at the base of the brain. Raised intracranial pressure and a tuberculous vasculitis lead to cranial nerve and brain stem involvement consisting of strabismus, ptosis, sluggish pupils and visual disturbances may develop. Cerebral oedema may occur and the patients become confused, disorientated, with slurred speech, grimacing or tremors of the extremities. With time focal neurological signs (hemiparesis) or cranial nerve palsies may develop often as result of infarctions caused by the tuberculous vasculitis.

Stage III

This stage manifests by loss of consciousness, opisthotonus decerebrate rigidity complete hemiplegia and may be accompanied by papilledema (Figure 1). The most common neurological sequelae are developmental retardation, cranial nerve palsies, optic atrophy, deafness, paralysis, continuing stupor or coma, convulsions, pituitary disturbances and hydrocephalus.

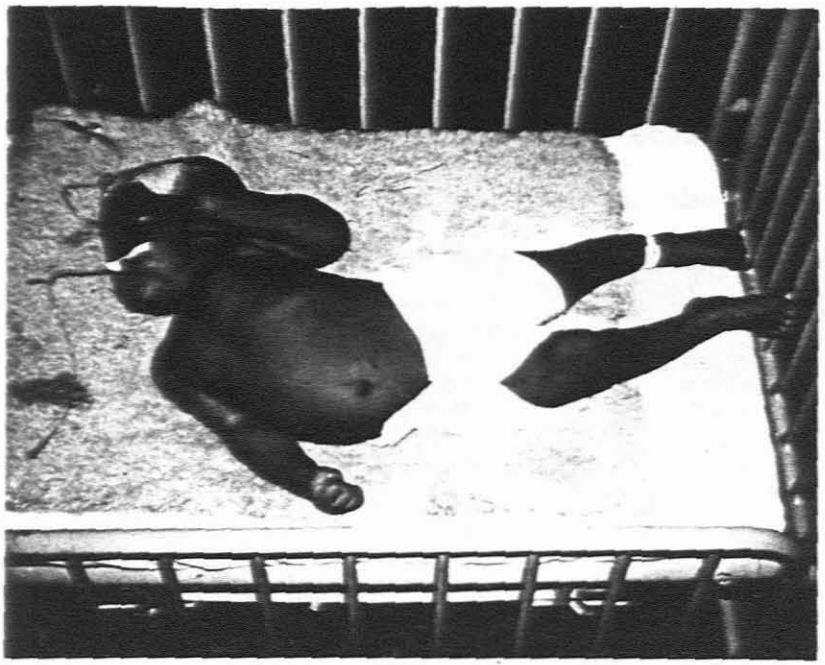


Figure 1

An infant with stage III TBM showing severe arching of the back (opisthotonus) and clenching of the fists caused by severe brain damage. The child also requires feeding by a naso-gastric tube.

1.3.2 *Diagnosis*

Diagnosis is confirmed by changes in CSF, a positive tuberculin test (preferably a Mantoux test) and demonstration of tubercle bacilli in the CSF by microscopy following Ziehl Nielson stain or by attempting to grow M.tuberculosis from the CSF making use of Lowenstein-Jensen medium or the semi-automated Bactec system. Computerised tomography (CT) to demonstrate hydrocephalus and a basal exudate supports the diagnosis except early in the illness.

The CT scan is a noninvasive technique for demonstration of intracranial structures. The method detects small variations in tissue density by computerized assembly of information from multiple tomographic sections through the head. Brain tissue is clearly distinguishable from cerebrospinal fluid-filled spaces. This technique is therefore well suited for the demonstration of ventricular size, and the presence of infarctions (Figure 2), displacements of ventricular system by mass lesions and subdural collections of fluid. Oedematous brain, as in the area of an infarct or contusion, has a lower density than

normal brain, cerebral haemorrhages or calcifications. Some solid tumours are detectable as areas of high density (Nelson, 1983). With the use of a contrast medium vasculitis of the arteries supplying the brain, which is typical of TBM, may also be visualised (Figure 3).

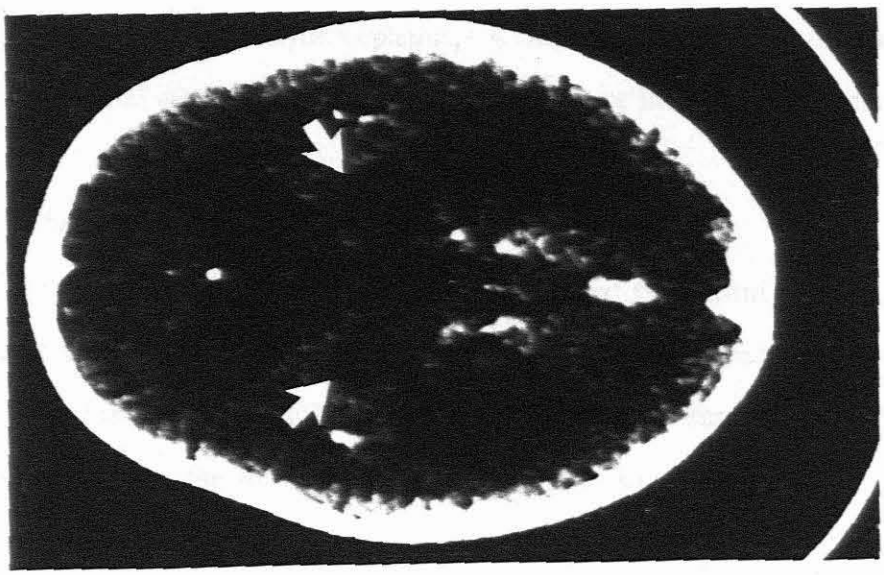


Figure 2

Computerised tomographic scan (CT scan). Widely dilated ventricles and infarction of the basal ganglia in a young child with stage III TBM. (arrows)

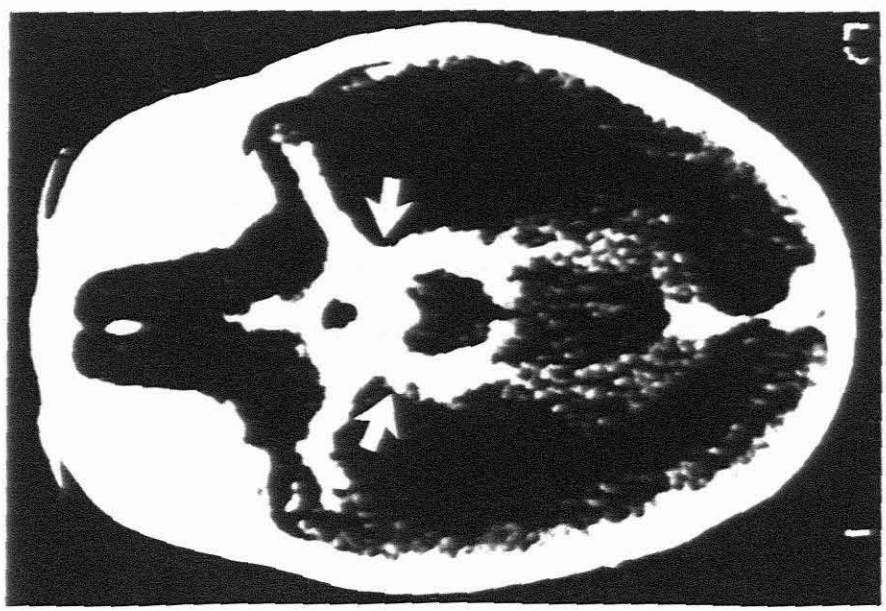


Figure 3

Hydrocephalus and marked basal enhancement on contrasted CT scan indicating severe vasculitis. (arrows)

1.3.3 *Complications*

Major, long-term, complications include death which may affect as many as 20% of children in Stage II and 50% in Stage III, cranial nerve palsies, lowered IQ, deafness, tuberculomas, obstructive hydrocephalus, which may be communicating or non-communicating and which is best assessed by CT scan or intracranial pressure monitoring.

1.3.4 *Therapy*

The major therapeutic aim in treating TBM is to avoid permanent neurological damage. Isoniazid, rifampicin and pyazinamide (and in some cases ethionamide) are the most logical choice for therapy in children. Although the use of steroids is controversial, it is still recommended in the initial phase of the disease, especially in those with potential neurological complications (Holdiness MR, 1990).

1.4 Assessment of progression of disease

Because of the devastating nature of TBM, newer methods of management are constantly being evaluated. Assessment of new methods of managing TBM requires the objective reliable measurement of brain damage, so that the value of new methods of treating TBM can be accurately determined.

The measurement of adenylate kinase (AK) in the CSF has been proposed as an accurate method to evaluate brain cell damage. In the Department of Paediatrics and Child Health of Tygerberg Hospital, AK activity in the CSF of children with TBM and BM has been measured and correlated with neurological outcome.

1.5 Consent

The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Stellenbosch. Where a LP was indicated on clinical grounds for suspected meningitis the procedure was explained to the parent(s). Informed written consent was obtained from the parents or guardians of children with TBM for the children's inclusion in the study and for the ICP measurements during which CSF was obtained.

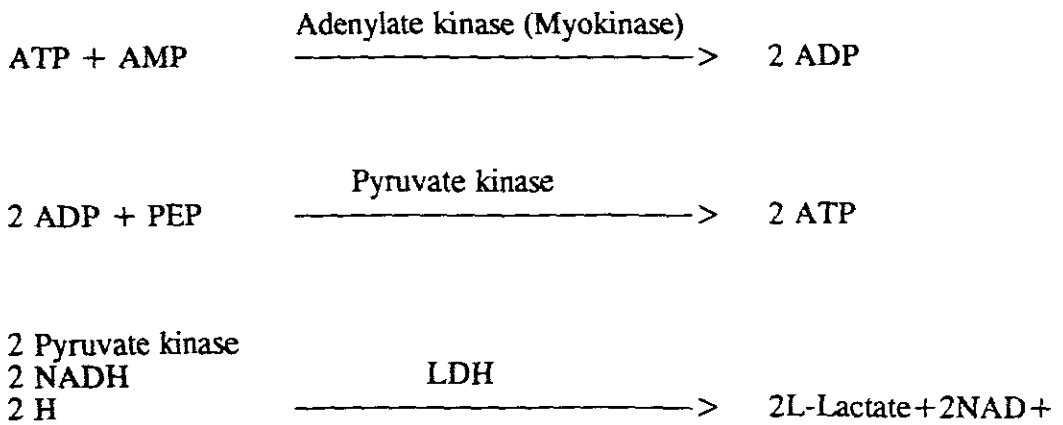
2. ANENYLATE KINASE

2.1. General

Adenylate kinase (AK) (ATP:AMP phosphotransferase EC 2743) or myokinase, is a globular, low molecular protein (Bollensen E & Prange HW, 1989). This enzyme catalyses the dismutation reaction between ATP, AMP and ADP (Bernstein LH *et al*, 1973).

The assay for AK activity used ATP and AMP as substrates for the reversible reaction ATP plus AMP = 2ADP. The measurement of the reaction rate was as follows:

Figure 4 : Equation of the reaction rate of adenylate kinase



AK is found in both prokaryotic and eukaryotic cells. Because of the reversible reaction between ADP and ATP+AMP, it contributes to the pool of the adenine nucleotides present in all living cells (Kurokawa, Takenaka & Sumida *et al*, 1990).

AK has an unusually low molecular weight (MW), e.g. 22 000 Daltons in comparison to other kinases with a MW of 40 000 Daltons (Russel PJ, Horenstein JM & Goins L *et al*, 1974).

It has been claimed that the integrity of the cellular plasma membrane might be changed when the intracellular concentration of ATP is lowered. This results in increased leakage of intracellular constituents into the extracellular fluid (Aberg T, Tyden H & Ronquist G *et al*, 1982; Edgren E, Hedstrand U *et al*, 1987)

2.2 Iso-enzymes

Several iso-enzymes of adenylate kinase (also known as myokinase) were first discovered in human erythrocytes and human skeletal muscle, brain, heart muscle and spleen and were shown to occur as 3 polymorphic sets of iso-enzymes in man.

In a study by Khoo JC & Russel PJ (1972) the indication from several tissues of humans and rabbits, was that a minimum of two sets of iso-enzymes were present in an individual. The AK from skeletal muscle, erythrocytes and brain were similar and form one set of iso-enzymes. The AK of liver, kidney, spleen and heart muscle were similar and form another set of iso-enzymes.

Similarities and differences amongst the different human and animal species were investigated including reactivity with sulphydryl reagents, the reactions with an antiserum against rabbit muscle, molecular weights iso-electric points and the distribution patterns of iso-enzymes. At least six different sets of AK iso-enzymes were found in 6 different human tissues. The organ specificity of the human AK iso-enzymes appears to be high and can be defined easily enough for consideration as a monitor of organ dysfunctions (Russel PJ, Horenstein JM, Goins L *et al*, 1974).

The two isoenzymes of AK which were found to be present in either the cytosol or in the mitochondria were designated AK_1 and AK_2 respectively. A third isoenzyme AK_3 was reported to be dominant in micro-organisms eg. E.coli AK_3 and differs from AK_1 and AK_2 in its substrate specificity and primary structure despite the sharing of similar enzymatic properties (Kurokawa Y, Takenaka H, Sumida M *et al*, 1990).

According to the abovementioned authors AK_1 isoform is specially located to regulate the cytosol ATP levels, while the AK_2 isoform is compartmentalized to control the mitochondrial ATP levels, which in turn maintain the adenine nucleotide homeostasis. In the inner mitochondrial membrane the ATP/ADP carrier may be essential to complete its catalytic function. The regulation mechanism(s) for each AK isoform function could play an important role in equilibrating the adenine nucleotides pool in living cells (Kurokawa Y, Takenaka H, Sumida M *et al*, 1990)

Toren A *et al*, 1994 named the three isoenzymes present in vertebrates AK1, AK2 and AK3 respectively. AK1 was the only one to be found in red blood cells and in the brain.

The brain is highly dependent on oxygen for its metabolism to maintain normal levels of ATP. Therefore, any condition leading to reduced blood flow in brain tissue would result in a lowered adenylate charge potential in the brain cells. Such lowering may result in a lowered electrochemical potential in the cells. There is an outflow of K^+ and an inflow of Na^+ and water. The cells become swollen and start to leak. Intracellular enzymes will now be found in the extracellular fluid of the brain. Enzymes with a low MW will leak to a greater degree than those with a high MW and can be quantified in the CSF. Being the smallest of the known phosphoryltransferases, AK is one of the first enzymes to leak into the CSF after brain cell damage (Alm PO, Frithz G & Ronquist G, 1979).

The value of CSF AK activity as a measure of brain damage has been assessed in several potentially neurologically damaging situations (Bollensen E, Prange HW 1989; Stensland E, Sandberg S *et al*, 1981; Enlund M, Ahlstedt B *et al*, 1989, Aberg T, 1995). Few articles have however made mention of CSF AK activity in bacterial meningitis and there are no reports of CSF AK activity in patients with TBM.

Frithz G *et al* (1977) measured CSF AK in healthy normal individuals and in 11 patients who suffered transitory ischaemic attacks (TIA). No AK acitivity was detected in any of the normal CSFs. The patients with TIA displayed a raised AK activity.

Bollensen E & Prange HW (1989) found changes in the CSF AK of 17 patients with brain infarction related to the size and site of the parenchyma necrosis. They also suggested that when using a measurement employing photometric techniques, levels of sensitivity should not be lower than 0,2 to 0,3 U/l. Bollensen E and Prange HW (1989) disagreed with other workers, for example Frithz G, Ericsson PER and Ronquist G (1982) who have shown a rise in AK activity in TIA patients and regarded it as having been misinterpreted.

Ronquist G *et al* (1977) found no AK activity in the CSF of 35 normal healthy control subjects but in 11 patients with malignant brain tumours, AK activity was detected.

It was reported that in 33 of 36 patients having open heart surgery the levels of CSF-AK activity were raised after surgery which correlated with a change in an index of intellectual function (Aberg T, Tyden H, and Ronquist G *et al*, 1982).

Stensland E *et al* (1981) determined the CSF AK activity in the CSF of 127 patients with various neurological conditions. The diagnostic sensitivity of AK activity was found te be very low. It was thought that the reason might be that the samples of CSF were taken from patients irrespective of time between onset of the disease and sampling. They found the time interval to be decisively important and eventually came to the conclusion that CSF-AK determination was of limited value in the diagnosis of neurological disease.

Frithz G *et al* (1982) studied the influence of the procedure of sampling and handling of CSF's. They divided the CSF of 28 patients into two samples. The first sample was handled according to the established routine, namely immediately chilled on ice and centrifuged twice. The second sample was taken according to the ordinary CSF routine

of the hospital, namely maintained at room temperature ±22°C and centrifuged twice on arrival at the laboratory, normally after 1-2 hours. The AK values in the samples handled at room temperature were falsely elevated to about twice the level of the samples immediately placed on ice.

Jonssen I *et al* (1979) reported higher CSF AK activity in 27 patients with meningitis than in those patients with meningism.

Hische EAH *et al* (1984) studied the effect of temperature on the evaluation of AK activity. At 30°C the AK activity was 1,5 times of that at 25°C. By increasing the temperature to 30°C and using a dilution factor for CSF of 1,6 instead of 6,0 the sensitivity of the procedure as used by Ronquist G (1977) could be increased 5 to 6 fold. For a group of 15 normal patients without neurological disorders they measured the AK activity from 0,17 to 1,16 U/l (mean 0,57 U/l). It is this latter method that is applied in our laboratory.

AIM

The aim of this study was to determine the AK values in CSF in various forms of meningitis and to seek a relationship between these values and the neurological outcome in children with TBM and BM. Establishing the presence of such a relationship might make it possible to utilise CSF AK activity to accurately predict neurological outcome.

CHAPTER 2

MATERIALS AND METHODS

2.1 INTRODUCTION

The children studied were (a) children without meningitis (the control group) (b) children with bacterial meningitis (BM) and (c) children with tuberculous meningitis (TBM). The study population included all children who presented with suspected meningitis between January 1990 and September 1994 to the department of Paediatrics and Child Health, Tygerberg Hospital, University of Stellenbosch. The ages of these children ranged from just after birth until 12 years. Children came from all population groups and included boys and girls.

2.2 Exclusions

CSF Samples showing blood contamination were discarded because human erythrocytes contain AK which on haemolysis gives rise to falsely increased values.

2.3 MATERIAL

2.3.1 Patient Groups

(a) Control group

The control group consisted of children on whom a lumbar puncture (LP) was performed when meningitis was suspected on clincal grounds. The CSF of these children contained $< 5x10^6/l$ lymphocytes on microscopical examination and no growth was reported on bacterial culture. The children were subsequently diagnosed as having febrile convulsions, bronchitis, gastro-enteritis, pharyngitis, urinary tract infections, or other non-neurological infections. AK determinations were performed in 60 of the abovementioned cases. The values determined in these patients were used to validate the method in the department of paediatrics and were considered normal values.

(b) Bacterial meningitis group

In children with bacterial meningitis a specific organism, eg. *Haemophilus influenzae, Neisseria meningitidis, βeta haemolytic streptococcus, Escherichia coli,* was cultured from the CSF. The CSF in these children showed an increased nuclear lymphocyte count, the protein concentration was usually raised and glucose concentration decreased. This group included 33 children with bacterial meningitis.

(c) Tuberculous meningitis group

All the children of this group had a history and clinical course compatible with the diagnosis of TBM. Children were clinically divided into Stage I, II or III TBM.

Intracranial pressure was measured by CSF lumbar puncture manometry for one hour shortly after the start of therapy and again during the 2nd, 3rd, and 4th week of therapy. In TBM the protein concentration was raised in the CSF, the glucose concentration decreased and the cell count (mainly lymphocytes) increased. CSF was collected after each pressure study. A computerized tomography (CT) scan was carried out during Week one and Week four. Children were hospitalized for 6 months after TBM was diagnosed.

This study included 88 children with TBM.

2.3.2 Collection of Cerebrospinal fluid

Samples of CSF were obtained by lumbar puncture with a spinal 22 x 1.5 needle from patients in the control and study groups. Cell count, lactate, protein and glucose concentrations were determined on the CSF. An aliquot of ± 1 ml was immediately placed on ice, centrifuged twice at 3000 rpm for five minutes and stored at -70°C. For AK determination, supernatants were tested for red blood cells (RBC) with Lenstrip - 5 Code SU15 labsticks and AK determinations were done in batches within 3 weeks after collection.

2.4. METHODS

2.4.1 Measurement of Cerebrospinal fluid lactate, protein and glucose concentrations

CSF lactate concentrations were determined on a TSI model 23L lactate analyzer. L-Lactate is stoichiometrically converted to hydrogen peroxide and pyruvate by the catalytic action of lactate oxidase in the presence of flavine-adenine-dinucleotide. The peroxide diffuses through a cellulose acetate membrane and is oxidized polarographically. The resulting current is measured by a silver chloride electrode and the lactate concentration is displayed digitally. The co-efficient of variation is 6,1%. The normal value for CSF lactate is 3,85 mmol/l. (range 1,1-2,4mmol/l)

CSF protein concentration was determined turbidometrically following precipitation by 3% sulphosalicylic acid. The degree of turbidity was measured by a RAxT analyzer at 625 nm. The normal value for CSF protein is 0,1 to 0,4g/l. CSF glucose concentrations were determined by the Perid metholodogy. Glucose is oxidized by glucose oxidase to gluconolactone, which in aqueous solution is converted to gluconic acid. In the presence of peroxidase the hydrogen peroxide produced is oxidized, and the resulting green colour is read on an Astra-8 autoanalyzer. The normal value for CSF glucose is 2,2 to 4,5 mmol/l.

2.4.2 Measurement of adenylate kinase

The method depends on the reaction in which AK catalyses the dismutation reaction of 2 molecules of adenosine diphosphate (ADP) into 1 molecule of adenosine monophosphate (AMP) and 1 molecule of adenosine triphosphate (ATP), (Aberg *T et al*, 1982; Ronquist G *et al*, 1982). Hische EAH *et al* (1984) modified this method in which the enzyme activity within the normal range could be estimated. It is this latter method that the Department of Paediatrics applied in the laboratory.

TABLE 2

2.4.2.1 *Reagents used for AK estimation*

Reagents	Freshly prepared
Dithiothreitol (DTT) 80mmol/l	12,34 mg in 1 ml H_2O
Adenosine-5-triphosphate (ATP) 48mmol/l	29,04 mg in 1 ml H_2O
Adenosine-5-monophosphate (AMP) 56mmol/l	27,95 mg in 1 ml H_2O
Phospho-enolpyruvate (PEP) 15,6mmol/l	7,258 mg in 1 ml H_2O
NADH 8mmol/l	5,67 mg in 1 ml H_2O
Lactate dehydrogenase (LDH) Pyruvate kinase (PK)	1:2,5
Triethanolamine HCl (TEA) 1 M	18,57 g/100ml
Potassium chloride (KCl) 1 M	7,456 g/100ml H_2O
Magnesium sulphate ($MgSO_4$) 200mmol/l	4,93 g/100ml H_2O
Myokinase 2mg/1ml	1:1000

Reagents were obtained from Boehringer Mannheim and freshly prepared before each batch for the determinations of AK. KCl and $MgSO_4$ were obtained from Aldrich.

2.4.2.2 *Buffer preparation*

1. Suspend the TEA in ±70ml distilled H_2O, pH to 7,6 with 20% NaOH.

   Make to 100ml with distilled H_2O.

2. Use 20ml TEA, 17,2ml KCl and 1ml $MgSO_4$, made to 100ml with distilled H_2O as the working buffer.

2.4.2.3 *Procedure*

200 μl buffer, 500 μl CSF and 10 μl DTT was pipetted into a cuvette, mixed and incubated at 30°C for 5 minutes. 20 μl ATP, 20 μl PEP, 20 μl NADH and 10 μl LDH/PK solutions were added - the latter mixed together in a ratio of 1:2,5 respectively. The mixture (blank) was incubated at 30°C for 2 minutes. The decrease of NADH absorbance was measured with a Hitachi U2000 spectrophotometer which automatically printed out absorbance readings every 30 seconds.

Absorbance was recorded at wavelength 340 (blank) for 3 minutes. After addition of 20 μl of AMP it was recorded for another 3 minutes (test). The AK activity was calculated as follow:

2.4.2.4 *Calculation*

AK activity, U/l =
A test - A blank x 8/5 x $\frac{1}{6{,}22 \times 10^{-3}}$

A = change in A340/min

8/5 = dilution factor

$6{,}22 \times 10^{-3}$ E340 of NADH in μmol/l

Myokinase was diluted 0,1 μl in 100ml distilled water and repeated with every batch of CSF samples. This 1:10,000 dilution should contain 72 U/l. This department's standardised method measured 71 $\pm$ 9 U/l.

This myokinase from pig and hog muscle was obtained from Boehringer Mannheim (Lot no: 12426724-300).

Table 3 compares different researchers' methods of AK determination. Since the data was published, spectrophotometers became much more sophisticated. The apparatus we used printed the readings every 30 seconds and gave a mean read-out as well. By increasing the temperature from 25°C to 30°C and using a dilution factor of 1,6 instead of 6, the sensitivity of the procedures could be increased 5 to 6 fold.

TABLE 3

Different researchers' methods for AK determination

	Act Neurol Scand 1989;79:53-58 Bollensen et al	Eur Neurol 1979;18:106-110 Ronquist et al	Clin Chemistry 1984;30(2): Hische et al	Department of Paediatrics
CSF	0,5 ml	0,5 ml	0,5 ml	0,5 ml
TEA	53,3 mM	50 mM	200 mM	200 mM
PK LDH	10 µl 20 µl	0,02 ml 0,02 ml	10 g/L 5 mg/ml	ratio 1:2,5
PEP	0,3 mM	0,3 mM	15,6 mM	15,6 mM
NADH	0,2 mM	0,23 mM	8 mM	8 mM
ATP	1,1 mM	1,3 mM	48 mM	48 mM
AMP	1,3 mM	1,3 mM	56 mM	56 mM
DTT	1,0 mM	1 mM	80 mM	80 mM
KCl	133 mM	43 mM	172 mM	172 mM
MgSO4	1,1 mM	13 mM	2 mM	2 mM
Total volume	3 ml	3 ml	800 µl	800 µl
Temperature	25°C	25°C	30°C	30°C

2.5 Evaluation of neurological outcome in children with bacterial meningitis and Tuberculous meningitis

The bacterial meningitis cases were examined before patients were discharged from hospital, to evaluate them for neurological complications. Subsequently a letter was sent to the local health authority clinic sister closest to the patient's home address, to enquire about the development and well-being of these children. In addition, a specific neurological examination was done on all children in the study who could be contacted, and requested to come for a follow-up examination, free of charge, at Tygerberg Hospital.

A full neurological examination was performed by a doctor and included assessment for the following:

1. Motor handicap, (hemiplegia (paraplegia)
2. Intelligence quotient (IQ)
3. Assessment of eyesight
4. Audiogram for assessment of hearing
5. Assessment for cranial nerve palsies

The above findings were then correlated with the CSF AK activity at the time of diagnosis. In the case of children with TBM, CSF AK activity determined at weekly intervals was also correlated with neurological progression or improvement. Children with TBM were hospitalized for 6 months while on therapy. During the first month they had weekly ICP monitoring and AK measurement. After therapy a full neurological examination as above as well as a CT scan was performed. On CT scan, special attention was paid to the detection of causes of permanent brain damage such as infarcts, hydrocephalus and tuberculomas.

2.6 Statistical analysis

Non-parametric statistics were used to analyze the data. The Wilcoxon rank sum test and Kruskal Wallis test were used for comparison of groups. Values were correlated by using the Spearman rank correlation test. A p value of < 0.05 was regarded as significant.

CHAPTER 3

RESULTS

Normal values for CSF AK activity in children have not been established previously. The range of CSF AK activity was therefore established in the CSF of 60 children on whom lumbar punctures were performed for suspected meningitis, but who were later shown not to have meningitis and who were otherwise normal. The median AK concentration in the chidlren was 0 to 1,27 U/l (mean 0,59 U/l).

Myokinase a commercially available pig/hog muscle suspension from Boehinger Mannheim, with the same activity as AK, was used as a control with each assay. In a dilution of 1:10,000 the method used, measured 71 $\pm$9 U/l against the 72 U/l it should be.

3.1 Cerebrospinal fluid Adenylate kinase activity in Tuberculous meningitis

Eighty eight children with a mean age of 32,6 months (range 3,4-156,1 months) with complicated advanced forms of TBM were studied. Details of the sex distribution and clinical staging are summarised in Table 4.

Table 4

Age and sex distribution of patients with Stage I, II and III TBM.

	STAGES		
	Stage I	**Stage II**	**Stage III**
Males	1	17	22
Females	-	28	20
	--	--	--
	1	45	42

Table 5

Available cerebrospinal fluid cell counts and chemistry in children with TBM during weeks 1, 2, 3 and 4

	Week 1	Week 2	Week 3	Week 4	Normal values
Lymphocytes x 10^6/l					
n	79	61	53	41	0-5
mean	267	74	49	43	
range	0-≥500	0-290	0-232	0-240	
Polymorphonuclear neutrophils x10^6/l					
n	79	61	54	42	0
mean	40	13	8	5	
range	0-≥500	0-100	0-87	0-67	
Protein g/l					
n	78	61	54	42	0,1-
mean	3,1	2,7	2,0	1,3	0,4
range	0,3-41	0,3-22	0,2-12,5	0,3-4,1	
Glucose mmol/l					
n	79	59	52	39	2,2-
mean	1,8	2,5	2,6	2,7	4,5
range	0,1-9,1	1,0-4,4	1,3-4,1	1,5-4,0	
Globulin (qualitative) (Pandy's Test)					
n	77	61	54	42	0
mean	3+	3+	4+	3+	
range	0-4+	4+	1-4+	1-4+	
Lactate mmol/l					
n	61	58	54	41	3,85
mean	6,4	4,5	2,9	2,6	
range	1,4-17,9	0,6-11,2	0,7-7,5	0,5-6,2	

3.1.1 *CSF cell counts and chemistry*

The available results of CSF cell counts and chemistry during the first month of therapy in the 88 children evaluated, are summarised in Table 5. CSF was often not available for analysis when lumbar puncture had to be interrupted for technical reasons, or was not carried out as the child had already received a ventriculo-peritoneal shunt, or had died. In other instances a traumatic tap resulted in blood stained CSF unsuitable for analysis. The mean lymphocyte counts in the CSF of the children with TBM decreased as expected from $267x10^6$/l, and ranged from 0-≥500 X 10^6/l in the children evaluated in Week 1 to 43 $x10^6$/l (range 0-240) in Week 4. The polymorphonuclear neutrophils decreased from $40x10^6$/l (range 0-innumerable) to $5x10^6$/l (range 0-≥500 X 10^6/l) at Week 4. The mean protein level showed a consistent decrease from Week 1 (3,1g/l, range 0,3-41g/l) until Week 4 (1,3g/l), range 0,3-4,1g/l). A decrease was also noted in the CSF lactate levels (mean 6,4 mmol/l) and ranged from 1,4-17,9mmol/l in the children evaluated at Week 1 and 2,6 mmol/l (range 0,5-6,2mmol/l) at Week 4), while the mean CSF glucose values increased from 1,8 mmol/l (range 0,1-9,1mmol/l) to 2,7mmol/l (range 1,5-4,0mmol/l) at Week 4. (Table 5)

3.1.2 *CSF lactate values*

There was just one child with Stage I TBM which made comparisons between Stage I, and Stage II and III unreliable. In children with Stage II and III TBM, the mean CSF lactate levels during Week 1 (6,0mmol/l and 7mmol/l respectively) were significantly lower ($p=0,001$) than those during Week 4 (2,7 and 2,5mmol/l respectively). CSF lactate values in children with Stage II and Stage III TBM did not differ significantly at any other time during illness. (See Table 6)

Table 6

Available mean cerebrospinal fluid lactate levels (mmol/l) and range of values in children with Stage I, II and III TBM during Week 1, 2, 3 and 4 of illness

	W1 *mean* value	range	n	W2 *mean* value	range	n	W3 *mean* value	range	n	W4 *mean* value	range	n
Stage I	1,4	-	1	1,7	-	1	2,5	-	1	1,3	-	1
Stage II	6,0	1,8-17,9	32	3,6	0,6-8,1	29	2,9	1,3-7,5	28	2,7	1,7 6,2	26
Stage III	7,0	1,4-14,1	28	5,6	1,7-11,2	28	2,8	0,7-4,8	25	2,5	0,5 5,3	14

3.1.3 *Intracranial pressure measurements*

Intracranial pressure (ICP) was measured at the time of diagnosis and at weekly intervals thereafter for the first month of therapy. In a minority of patients ICP was again not measured for technical reasons, or because the child had a non-communicating hydrocephalus. Follow-up values were also not available in some children who died, had a ventriculo-peritoneal shunt inserted or again when lumbar puncture had to be interrupted. Table 7 summarises the available ICP measurements in the children with TBM.

Table 7
Available mean intracranial pressure measurements (mmHg) with the range of values found in children with Stage I, II and III TBM during Week 1, 2, 3 and 4 of illness

	W1 mean value	range	n	W2 mean value	range	n	W3 mean value	range	n	W4 mean value	range	n
Stage I	25	-	1	15	-	1	12	-	1	14	-	1
Stage II	26	5,7-54,8	40	15	6-35	31	13	4,6-45,0	27	12	8,6-19,1	27
Stage III	25	5,1-61,4	39	17	5,1-60	29	13	4,7-27	25	14	2,8 35	17

Stage I

In Week 1 the single patient at Stage 1 TBM had a ICP of 25mmHg, 15mmHg in Week 2, 12mmHg in Week 3 and 14mmHg in Week 4.

Stage II

In the first week the 40 patients in whom ICP was measured had a mean ICP of 26 mmHg (range 5,7-54,68mmHg). In the second week the mean pressure was 15mmHg (range 6-35mmHg) for 31 patients. In the third week the mean ICP was 13mmHg (range 4,6-45,0mmHg) for 27 patients. In the fourth week 27 patients had a mean IC pressure of 12 (range 8,6-19,1mmHg).

Stage III

The 39 children in whom ICP was measured during Week 1, had a mean pressure of 25mmHg and their pressures ranged from 5,1 -61,4 mmHg. In Week 2 twenty nine patients had a mean pressure of 17 mmHg and ranged from 5,1-6,0mmHg. In Week 3 twenty five patients had a mean pressure of 13 mmHg and ranged from 4,7-2,7mmHg and in Week 4, 17 patients had a mean pressure of 14mmHg ranging from 2,8-35mmHg.

3.1.4 *CFS adenylate kinase levels*

3.1.4.1 *All patients*

Table 8 summarises the available CSF adenylate kinase levels in children with different stages of TBM during Week 1, 2, 3 and 4 of illness.

Table 8

Available mean cerebrospinal fluid adenylate kinase levels (U/l) and range of values in children with Stage I, II and III TBM during Week 1, 2, 3 and 4 of illness.

	W1 mean value	range	n	W2 mean value	range	n	W3 mean value	range	n	W4 mean value	range	n
Stage I	2,229	-	1	2,024	-	1	1,852	-	1	2,178	-	1
*Stage II	2,949	0-9,218	29	2,425	0-15,829	29	1,999	0,1-9,060	27	1,647	0-5,874	29
*Stage III	5,624	0-18,932	29	2,066	0-8,746	30	1,507	0,171-3,344	24	2,206	0,326 9,260	17

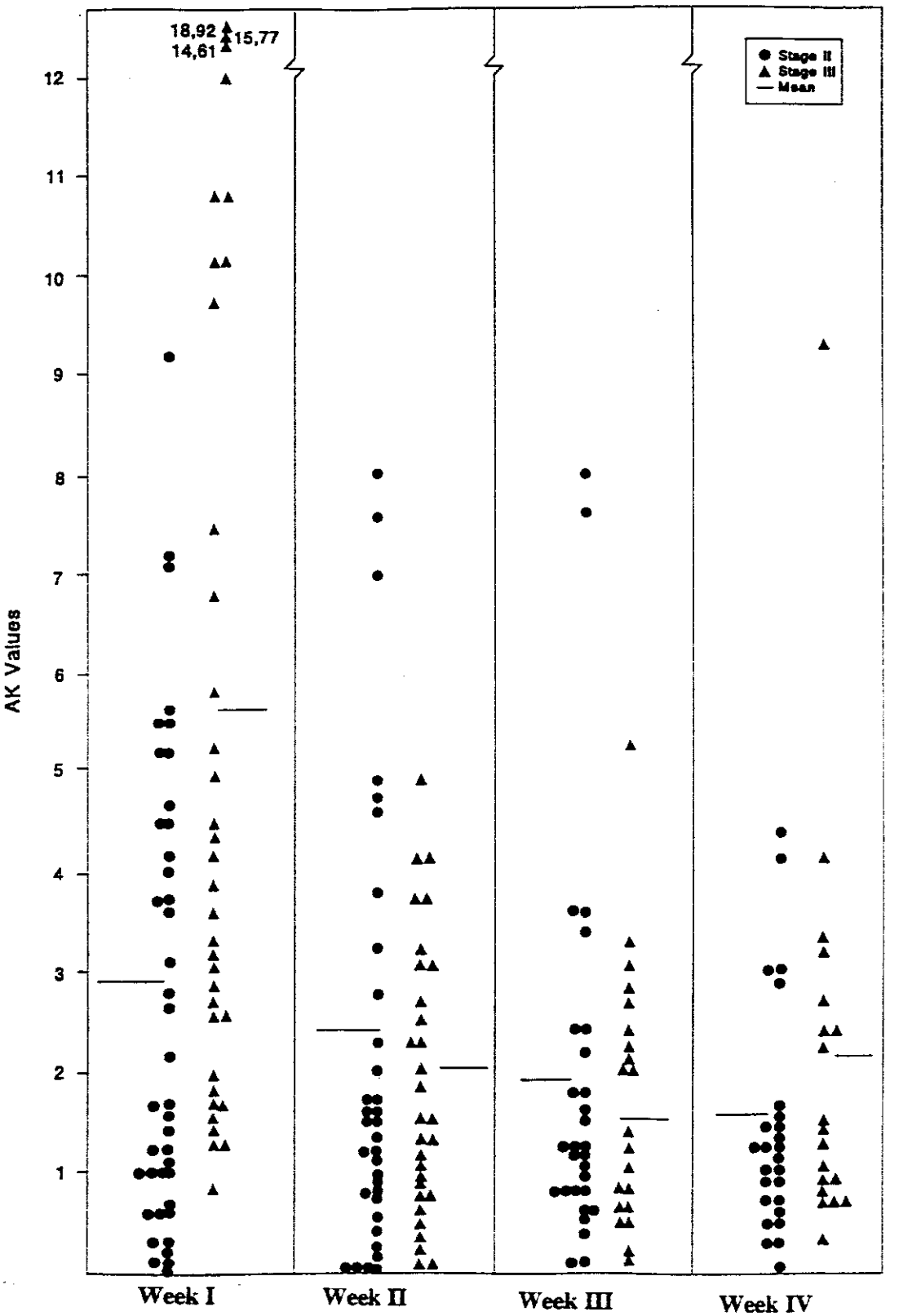
*p 0.03

The CSF AK levels of the Stage III patients at week 1 were significantly higher than those of Stage II patients at Week 1 (p=0.03), but no significant differences were found in CSF AK levels during Week 2 (p=0,87), Week 3 (p=0,55) and Week 4 (p=0,55) when comparing patients with Stage III TBM to those with Stage II TBM.

The one patient with Stage I TBM had an AK value of 2,229 U/l in Week 1, 2,024 U/l in Week 2, 1,852 U/l in Week 3 and 2,178 U/l in Week 4.

At Stage II 29 patients had a mean AK of 2,949 U/l (range 0-9,218) in Week 1. In Week 2 the mean AK value was 2,425 U/l (range 0-15,829), in Week 3 1,999 U/l (range 0,1-9,060) and in Week 4 1,647 U/l (range 0-5,874). At Stage III 29 patients had a mean AK of 5,624 U/l in Week 1 (range 0-18,932) for 30 in Week 2, 2,066 U/l (range 0-8,746). In Week 3, 1,507 U/l (range 0,171-3,344) and Week 4 2,206 U/l (range 0,326-9,260).

Fig. 5 Adenylate Kinase values at Weeks 1 to 4 in Stage II and III TBM
Only during Week 1 of treatment did CSF AK values differ in Stage II (mean value 2,95 U/l) and Stage III (mean value 5,62 U/l) TBM children (p=0,03)



Each symbol (●) represents the AK values in a single patient with Stage II TBM
Each symbol (▲) represents the AK values in a single patient with Stage III TBM

Due to the fact that the AK values at Week 1 of Stage III patients were higher than corresponding values in Stage II patients, as well as the fact that clinically Stage III patients have a worse prognosis than Stage II patients, further analysis was performed on Stage II and III separately.

3.1.4.2. *Stage II TBM patients*

The CSF AK values at Week 1 in patients with Stage II TBM who developed specific complications, were compared to those of patients who did not develop these complications.

Death

The AK values (Week 1) of the 2 patients (7%) who died were 4,63 U/l and 1,37 U/l while the median level of the patients who lived, was 1,81 U/l ($p = 0.89$). Although 2 patients died, they were not excluded from further analysis of CSF AK values as an indicator for the development of complications, because all the fatalities occurred after the development of complications.

Development of granulomata

Three patients (10%) developed granulomas during their clinical course, and their Week 1 AK values were 1,26 U/l, 5,65 U/l and 2,74 U/l, while those who did not develop granulomas had a median AK value of 4,03 U/l during Week 1 ($p=0,35$).

Cranial nerve palsies

The 7 patients (24%) who had cranial nerve palsies at presentation, or who developed cranial nerve palsies during their clinical course, had a median AK value at Week 1 of 2,83 U/l compared to median AK value of 4,63 U/l in the 22 patients (76%) who did not have cranial nerve palsies ($p=0,63$).

Hemiplegia or Paraplegia

Twelve patients (41%) presented with, or developed hemiplegia or paraplegia. The Week 1 median AK level was 2,83 U/l which did not differ from the median value of 1,54 U/l in patients who did not have hemi- or paraplegia ($p=0,43$).

Deficient hearing

The 2 children (7%) who had deficient hearing at the end of therapy had Week 1 AK levels of 5,65 and 0,6 U/l, while those who had normal hearing at the end of therapy, had a median AK value of 1,81 U/l at Week 1 (p=0,86). Only 9 patients (31%) with Stage II TBM had IQ's greater than 90 at the end of therapy. Their CSF AK levels at week 1 (Median = 1,54 U/l) did not differ from the AK levels at Week 1 (Median = 1,62 U/l) of those children who had IQ's less than 90 at the end of therapy (p=0,85).

Brain infarction on CT scan

Four children (14%) developed brain infarctions. Their CSF AK levels at Week 1 (median = 1,81 U/l) did not differ from the CSF AK levels (median = 2,74 U/l) of the Stage II children who did not develop infarctions on CT scan (p=1).

Impairment of vision

One child (3%) with a Week 1 CSF AK value of 5,65 U/l had impaired vision at the end of therapy. The median AK value at Week 1 of the children with normal vision was 1,81 U/l.

Hydrocephalus

Every patient with Stage II TBM developed hydrocephalus and therefore no comparisons were possible.

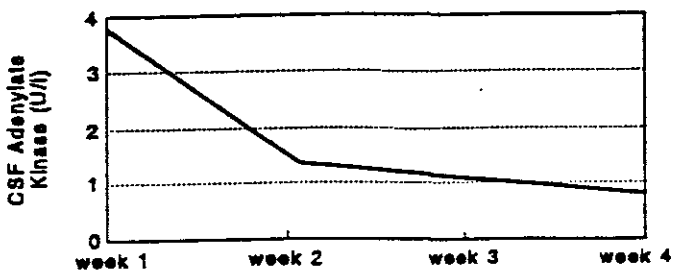
Consecutive AK activity determination in the same patient

There were 17 patients with Stage II TBM who had at least 3 CSF AK determinations and at least 2 CT scans. The successive CSF AK levels of these patients tended to give an indication of clinical course and worsening or improving CT scan results, and some of these results are discussed below.

In 2 patients (Figures 6 and 7) the CSF AK levels decreased rapidly and remained within normal limits. The children improved clinically although on CT scan both still had hydrocephalus at 4 weeks. At the 6 month follow-up both children were neurologically normal and the one child (Figure 6) had a normal CT scan. The second child (Figure 7) still had prominent ventricles on CT scan.

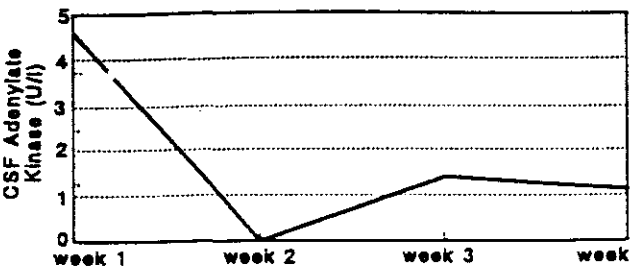
Available serial CSF AK values in children with Stage II TBM at Week 1, 2, 3 and 4

Figure 6



IQ 88, CT scan normal

Figure 7



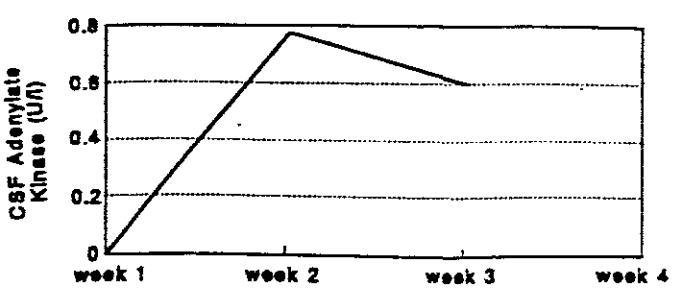
6 months: milestone normal, ventricles still prominent

Graphs illustrating rapid decreases in CSF AK accompanied by a good neurological outcome at 6 months follow-up.

In 2 patients (Figures 8 and 9) the initial very low CSF AK increased over the 4 week period, but still remained within normal reference values for controls. Both these patients had only minimal hydrocephalus on CT scan. Both had low IQ's (68 and 56) but neither had motor, vision or hearing impairment.

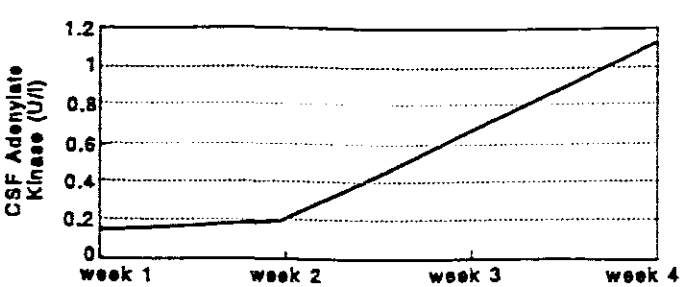
Available serial CSF AK values in children with Stage II TBM at Week 1, 2, 3 and 4

Figure 8



IQ 68, minimal hydrocephalus

Figure 9



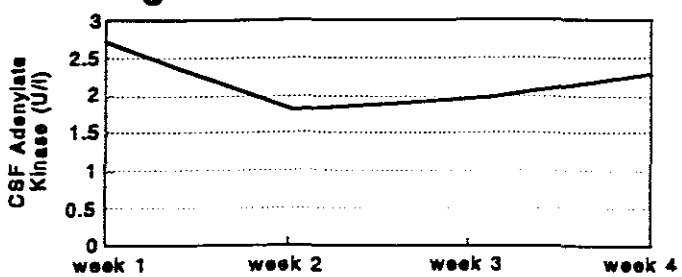
IQ 56, normal CT scan.

Graphs illustrating CSF AK increases during first month of treatment but remained within normal limits. Both patients had only minimal hydrocephalus at 6 months follow-up. Although having no motor deficit, normal vision and hearing, both children had a low IQ. (68 and 56 respectively

In 6 patients (Figures 10-15) an increase in AK value at a specific time coincided with the development of a major complication. In 5 of these patients an initial decrease in the CSF AK value which corresponded to clinical improvement, was followed by an increase in the CSF AK level which also corresponded to the development of complications such as a tuberculous granuloma (Figures 10 and 11), worsening of hydrocephalus (Figure 12), development of infarct (Figures 13 and 14). In one patient (Figure 15) the initial increase in the CSF AK level coincided with a sudden clinical deterioration necessitating a tracheostomy and admission to the intensive care unit.

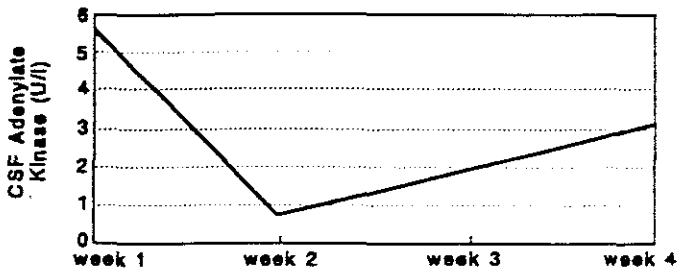
Available serial CSF AK values in children with Stage II TBM at Week 1, 2, 3 and 4

Figure 10



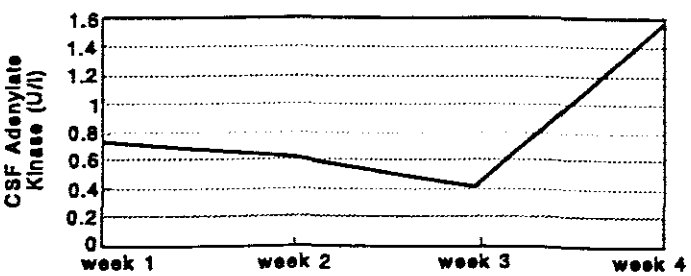
Tuberculous granuloma developed during first month of therapy. At follow up IQ 79, minimal hydrocephalus

Figure 11



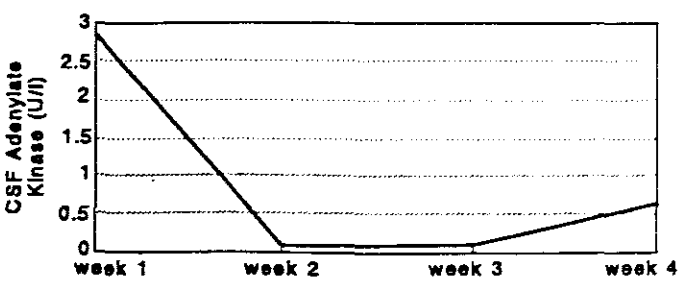
IQ 67, tubeculous granuloma developed during first month of treatment. At follow up optical atrophy and basal enhancement

Figure 12



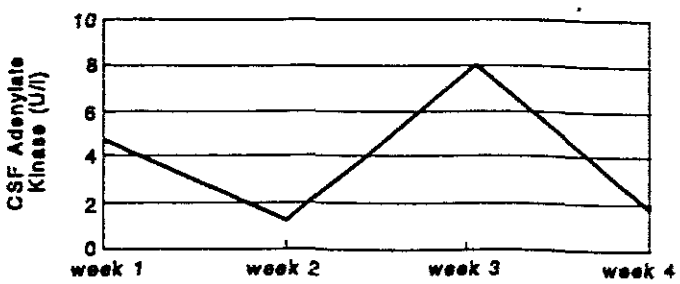
Worsening of hydrocephalus during week 3-4. At follow up IQ 84 with hemiparesis

Figure 13



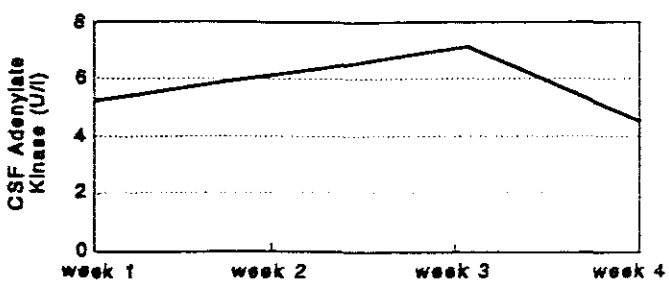
An infarction developed during weeks 3-4. At follow up this child had hemiplegia

Figure 14



An infarction developed during week 2-3. At follow up this child had hemiparesis and an IQ of 81.

Figure 15



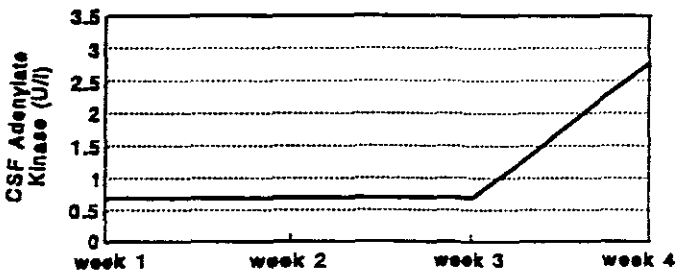
A sudden clinical deterioration during week 2-3. At follow up this child had and IQ of 70 and marked hydrocephalus.

Graphs illustrating increases in CSF AK values, coinciding with development of major complications

In 7 patients (Figures 16-22) an increase or decrease in CSF AK occurred which did not correspond with a coinciding improvement or worsening of clinical condition or CT scan picture at the fourth week. However 2 patients (Figures 17 + 18) had to have ventriculo peritoneal shunts after the fourth week, 1 had minimal hydrocephalus (Figure 16), 2 had infarctions (Figures 20 + 22) and 1 had hydrocephalus (Figure 21) at 6 months follow-up.

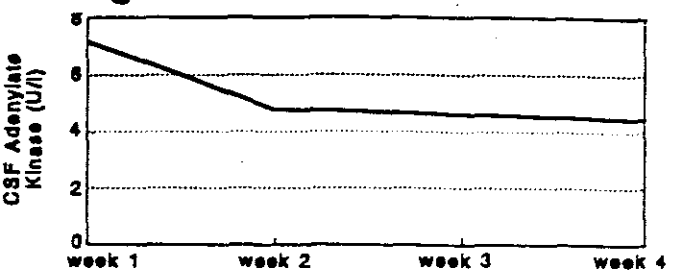
Available serial CSF AK values in children with Stage II TBM at Week 1, 2, 3 and 4

Figure 16



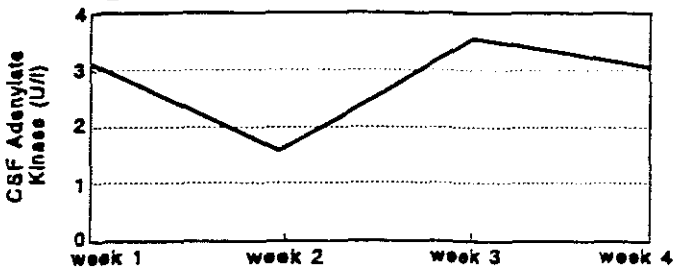
IQ normal, minimal hydrocephalus at 6 months follow-up.

Figure 17



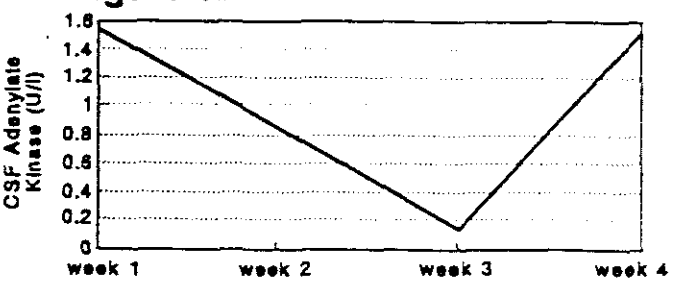
IQ 1 year behind , required a ventriculo peritoneal shunt at end of 1 month of treatment.

Figure 18



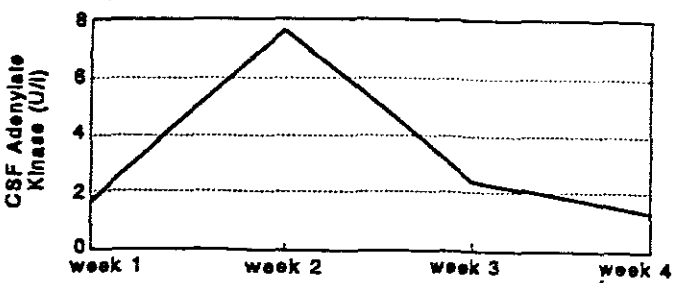
Ventriculo-peritoneal shunt required at end of 1 month of treatment. On 6 month follow up IQ 79.5 and hearing deminished with a partial paresis on NVI and hydrocephalus.

Figure 19



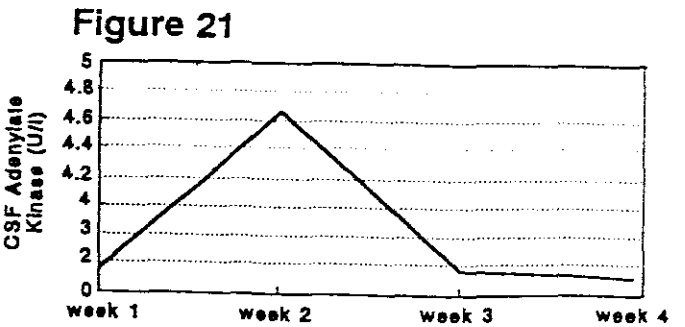
IQ 68, CT scan not normal at 6 months follow up.

Figure 20



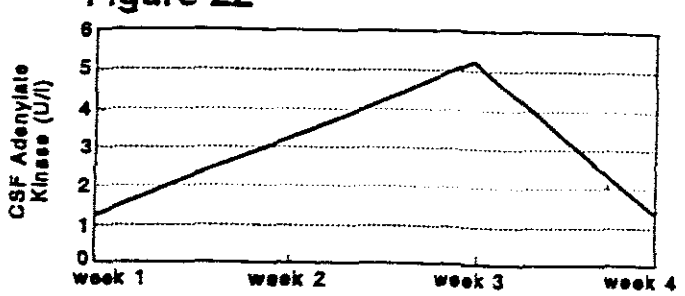
At 6 month follow up this child had a hemiparesis and infarction seen on CT scan.

Figure 21



At 6 months follow up this child had no motor defects but had an IQ of 83 and hydrocephalus.

Figure 22



At 6 months this child had a hemiparesis and an infarction and basal enhancement visible on CT.

Graphs illustrating increases or decreases in CSF AK values in the first month of treatment which did not coincide with improvement or worsening of clinical condition or CT picture. None-the-less the children whose CSF AK values are illustrated in Figure17 and 18 required a ventriculo-peritoneal shunt at the end of the first month of treatment due to failure of their raised intra cranial pressure to resolve satisfactorily. Two children 20 and 22 had infarctions on CT scan at 6 month follow-up which were not present at one month after the start of treatment.

3.1.4.3 *Stage III TBM patients*

Death

Nine (39%) of the 29 patients with Stage III TBM who had AK levels determined during Week 1, died. The AK values at Week 1 of the patients who died (median 3,77 U/l) were no different from the AK levels (median 4,45 U/l) of patients who survived (p=0,78). Although 9 patients died, they were not excluded from further analyses of CSF AK values as an indicator of complications, because all the fatalities occurred after the development of complications.

Tuberculous granuloma

Only 1 patient (3%) with Stage III TBM developed a granuloma. The CSF AK value during Week 1 was 1,45 U/l while the median value for the patients who did not develop granulomas was 4,03 U/l.

Cranial nerve palsies

The AK values (median = 5,71 U/l) during Week 1 of patients with Stage III TBM, who at presentation had, or who developed cranial nerve palsies during their clinical course, were significantly higher than the AK values (median = 2,48 U/l) of those patients with Stage III TBM who did not develop cranial nerve palsies (p=0,015) (Figure 23).

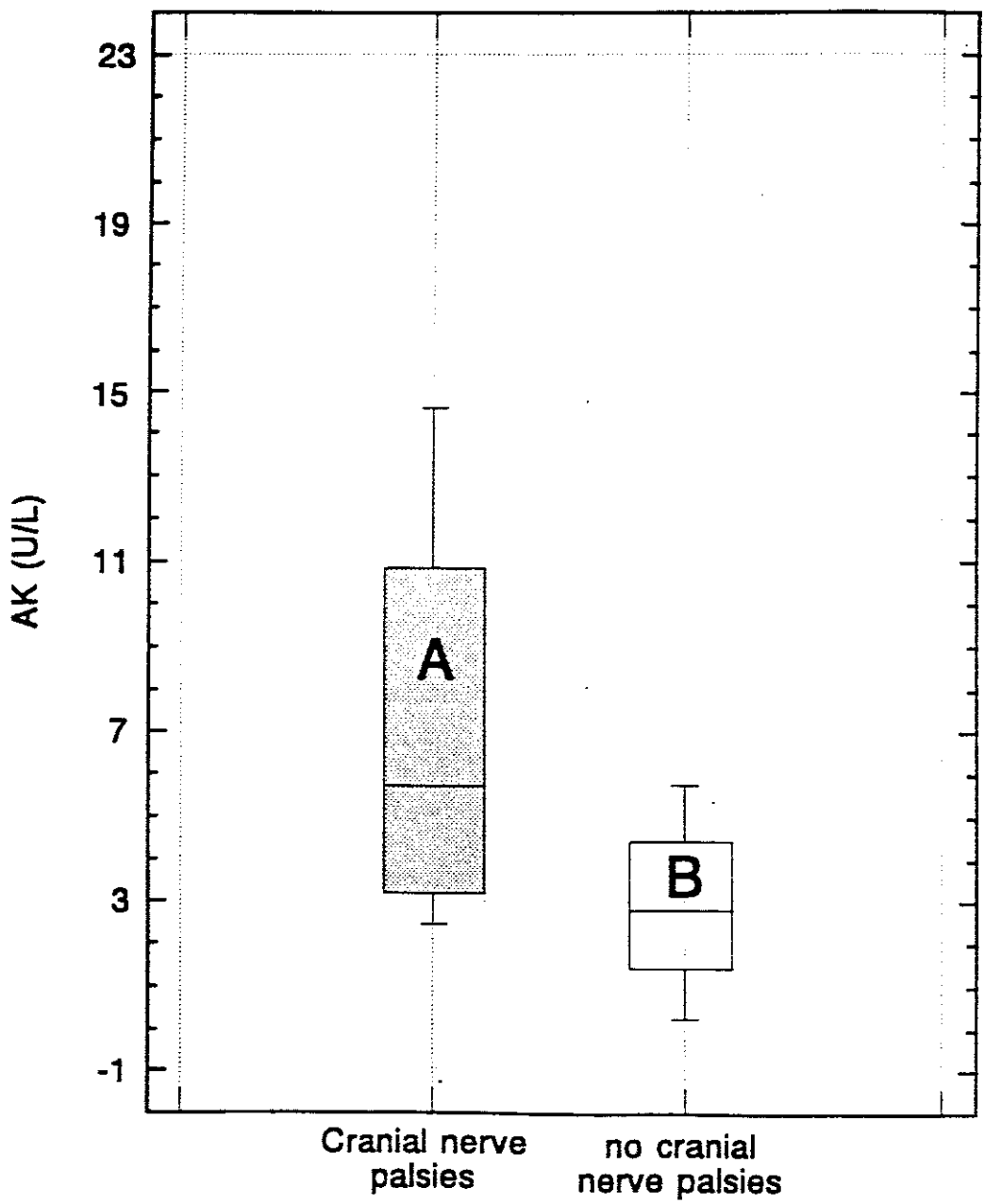
Hemiplegia/paraplegia

Fourteen patients (48%) with Stage III TBM presented with, or developed hemiplegia or paraplegia. Their Week 1 CSF AK values (median = 3,51 U/l) did not differ from those of patients who did not develop hemi- or paraplegia (median = 4,30 U/l) (p=0.28).

Deficient hearing

Eight (28%) of the 29 patients with Stage III TBM had impaired hearing at the end of therapy. Their Week 1 CSF AK values (median = 10,84 U/l) did not differ significantly from CSF AK values (median = 3,51 U/l) of patients with Stage III TBM who had normal audiograms at the end of therapy (p=0,19).

Fig. 23: Multiple Box and Whisker Plot of Stage III TBM children illustrating the CSF AK activity during the first week of treatment in children with (A) and without (B) cranial nerve palsies. CSF AK activity was significantly higher in those who developed cranial nerve palsies. (p=0,015)



IQ

Nineteen (95%) of the 20 surviving patients with Stage III TBM had an IQ of less than 90 at the end of therapy. Only 1 patient (5%) had an IQ of more than 90 and therefore no statistical analysis could be performed.

Impaired vision

There were 6 patients (21%) who developed impaired vision during their illness. Their CSF AK values at Week 1 (median = 9,74 U/l) were not significantly higher than the values of the patients who did not develop impaired vision (median = 3,20 U/l) (p=0,11).

Brain infarctions

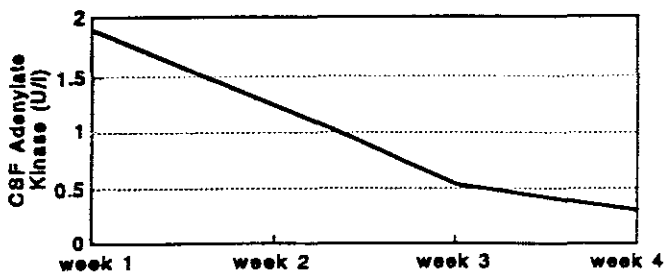
The CSF AK values (median = 4,45 U/l) of the 13 patients (35%) who had a brain infarct on at least one CT scan, did not differ significantly from the values of patients who did not develop brain infarction (median = 3,77 U/l) (p=0,57).

There were 17 patients (59%) with Stage III TBM who had at least 3 CSF AK determinations and at least 2 CT scans. The successive CSF AK levels of these patients are illustrated in Figures 24-40.

In 9 patients (31%), (Figure 24-32), the CSF AK levels decreased while the clinical condition and CT scan picture improved or remained unchanged. In 4 (14%) of these patients (Figure 24-27) the initial CSF AK value was <2 U/l and decreased to <1 U/l. Three (10%) of these patients had brain infarctions at presentation and all still had brain infarcts on CT scan at the end of the 6 months therapy. They were severely handicapped with IQ's of 67, 50 and <50. There was 1 patient (3%) (Figure 27) with an initial low CSF AK level, cranial nerve palsy and motor impairment at presentation whose AK levels decreased and who at 6 months only had hydrocephalus, an IQ of 80 but no motor impairment.

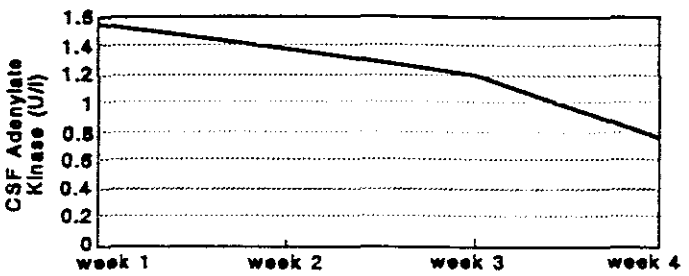
Available serial CSF AK values in children with Stage III TBM at Week 1, 2, 3 and 4

Figure 24



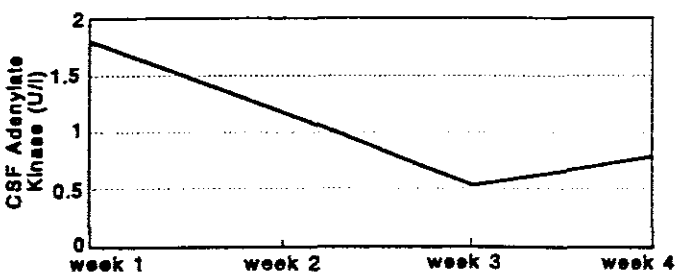
IQ 67, hemiparesis, infarction. AK did not correspond with clinical condition or CT scan. (no w2 AK value)

Figure 25



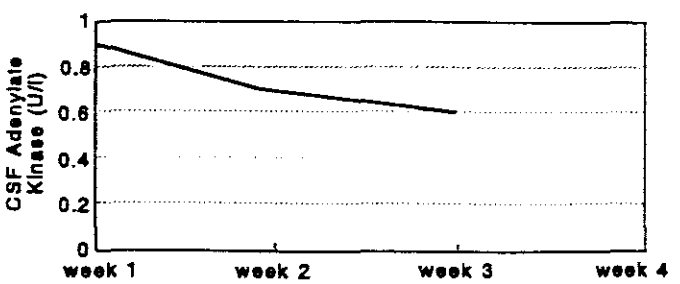
IQ <50, infarction (no w2 AK values)

Figure 26



IQ <50, hemiparesis, infarction (no w2 AK values)

Figure 27



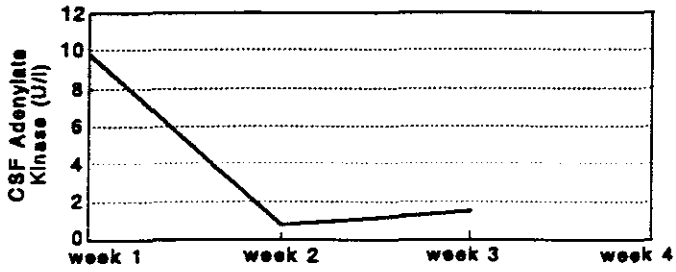
IQ 80, hydrocephalus, milestones normal.. (AK at 6 months was 1,88u/l)

Graphs illustrating CSF AK values decreasing while the clinical condition or CT picture improved or remained the same. Figure 24 and 26 had no Week 2 AK values, and Figure27 had no Week 4 AK value.

The 5 (17%) remaining patients whose CSF AK levels decreased from Week 1 to Week 4 (Figure 28-32) all had AK values above 9,5 U/l at Week 1. One of these, (Figure 28) developed infarct and was in a vegetative state at the end of therapy. There was no Week 4 AK values available. Two patients (Figure 29 + 30) had infarctions at Week 1 and both had infarctions and hemiparesis at the end of therapy. The 2 children who had high initial CSF AK values (Figure 31-32) and no evidence of infarction, both still had hydrocephalus at the end of therapy and IQ's

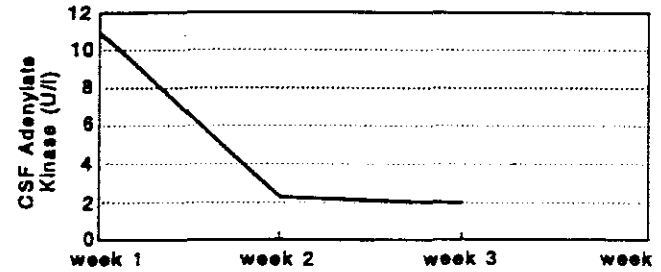
Available serial CSF AK values in children with Stage III TBM at Week 1, 2, 3 and 4

Figure 28



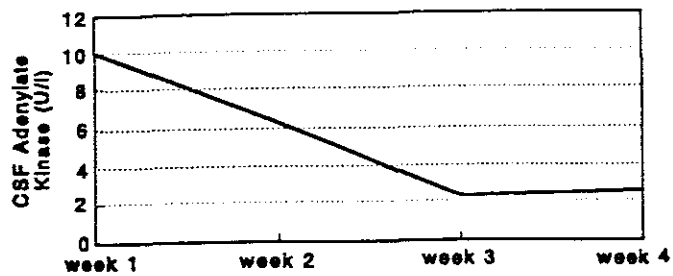
Vegetative, optical atrophy, hydrocephalus, infarction

Figure 29



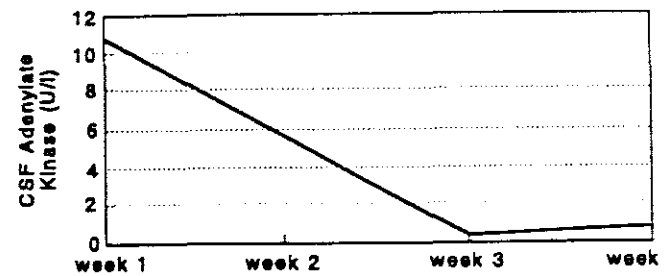
IQ 95, hemiparesis, infarction, vertricles still enlarged. AK did not reach normal value of <1 u/l

Figure 30



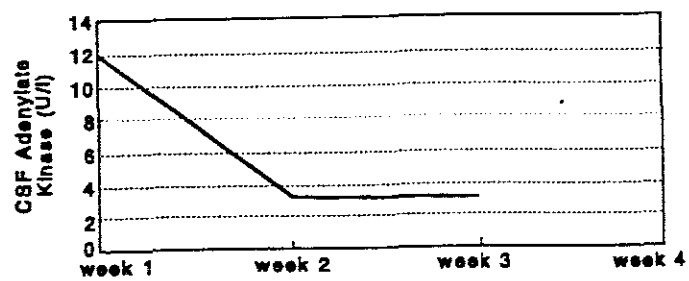
IQ 40.6, hemiparesis, strabismes, hydrocephalus, infarction. AK did not reach normal <1u/l (no w2 AK value)

Figure 31



IQ 80, vision abnormal, milestones normal. (no w2 AK value)

Figure 32



IQ 70, hydrocephalus slightly better, minimal hearing loss. AK decreased but stayed high - patient had to have a ventriculo peritoneal shunt.

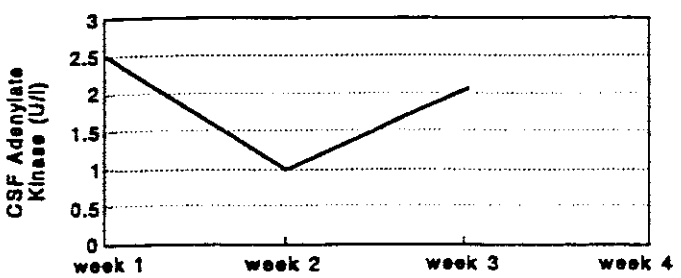
Graphs illustrating CSF AK values decreasing while the clinical condition or CT picture improved or remained the same. However, 2 patients - Figures 30 and 31 - did not have a Week 2 AK value, and 3 patients - Figures 28, 29 and 32 - had no Week 4 AK values. The patient in Graph 32 had to have a ventriculo peritonial shunt.

There were 6 children (Figure 33-38) whose CSF AK showed an increase after an initial decrease, and one (Figure 39) whose AK levels increased consistently. Three of these children (Figure 33, 34 + 39) did not have brain infarcts on initial CT scan but developed brain infarcts. Of the remaining 4 children, 1 died (Figure 35), 2 had infarctions at presentation and were severely handicapped at the end of therapy (Figure 36 + 37), and one who had hydrocephalus initially, still had hydrocephalus at the end of therapy (Figure 38).

There was only one child (Figure 40) who had a low CSF AK level at presentation and whose AK levels decreased persistently despite the fact that he developed a brain granuloma and infarction. However the AK values did not reach the normal < 1U/l and his milestones were normal at 6 months follow-up.

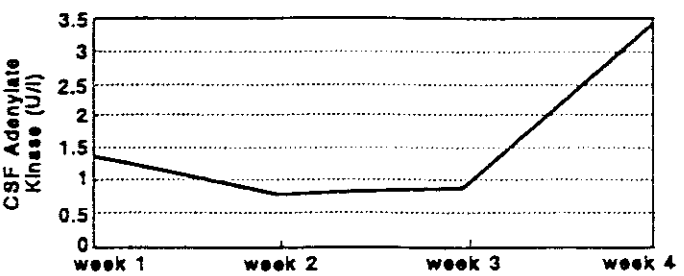
Available serial CSF AK values in children with Stage III TBM at Week 1, 2, 3 and 4

Figure 33



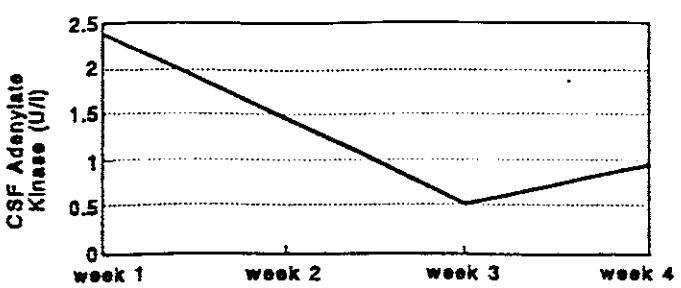
IQ <50, hydrocephalus, strabismes. Patient developed infarction

Figure 34



Hemiplegia, hydrocephalus, patient developed infarction

Figure 35

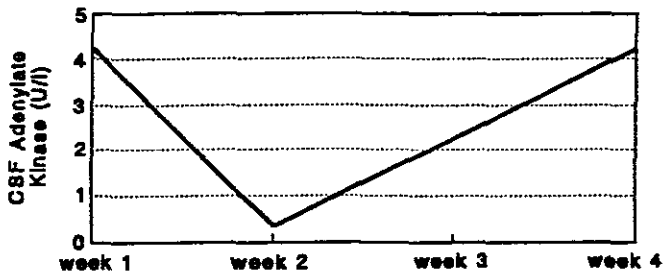


Comatose, spastic quadroplegia, Patient had to have a ventriculo peritoneal shunt, patient died.

Graphs illustrating Ak values increased coinciding with major complications The patients in Figures 33 and 34 developed infarctions. In Figure 35, the patient had to have a ventriculo peritonial shunt, patient died.

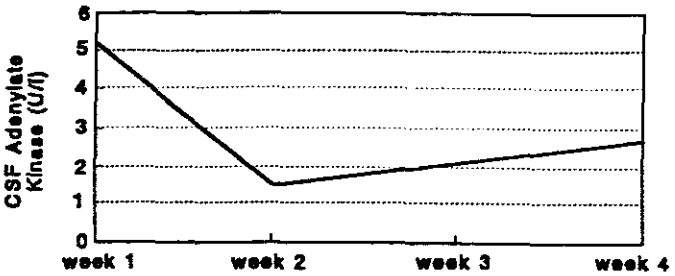
Available serial CSF AK values in children with Stage III TBM at Week 1, 2, 3 and 4

Figure 36



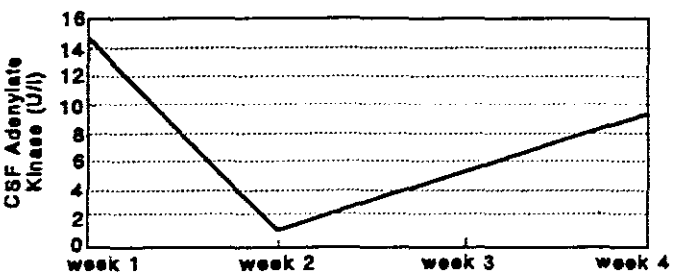
Patient severely handicapped, vegetative, NVII

Figure 37



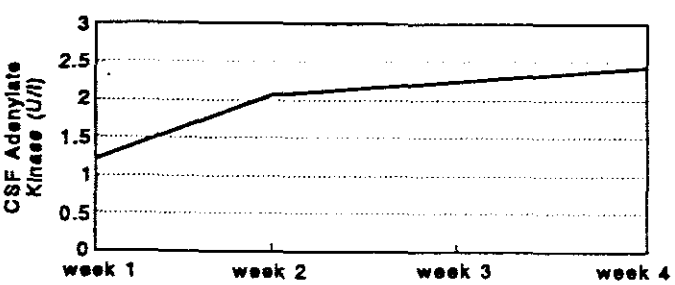
Patient had to have a ventriculo peritoneal shunt after 4th week. IQ 30, optical atrophy, hydrocephalus, infarction.

Figure 38



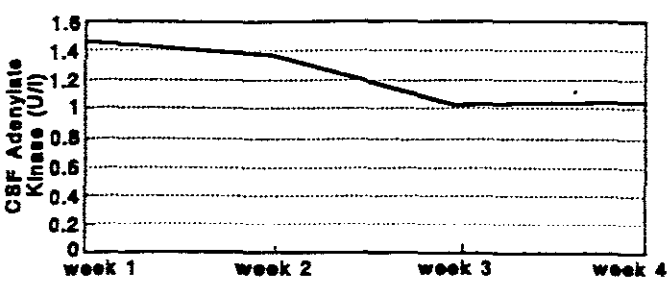
IQ 78, hydrocephalus, ventricles still enlarged

Figure 39



IQ 50, bilateral infarction, hemiparesis

Figure 40



Infarction, milestones normal at 6 months. AK values decreased but did not reach <1 u/l.

Graphs illustrating AK values increased coinciding with major complications: Figures 36, 37, 38 and 39. In Figure 40, the AK values were not very increased and the the patient had normal milestones at 6 months follow-up.

3.1.5 *Relationship between other cerebrospinal fluid findings, intracranial pressure and AK values in children with TBM*

There was no significant relationship between CSF AK values and CSF cell count, total protein, CSF glucose or ICP measurements during the first month of treatment. CSF AK values were however significantly related to CSF lactate concentrations during the first week after diagnosis (P=0,001). (See Figure 41)

3.1.6 *CSF AK values during the first week of treatment and outcome of 6 months treatment*

In Figure 42 CSF AK values obtained during the first week of treatment are illustrated for different outcomes on completion of 6 months treatment. Values in the small number of children found to be normal at follow-up were significantly lower than those in each of the other categories.

Figure 41 AK versus Lactate values Week 1.
Available CSF AK activity and CSF lactate concentrations determined during the first week of treatment were significantly related (p=0,001)
● = Stage II TBM Δ Stage III TBM

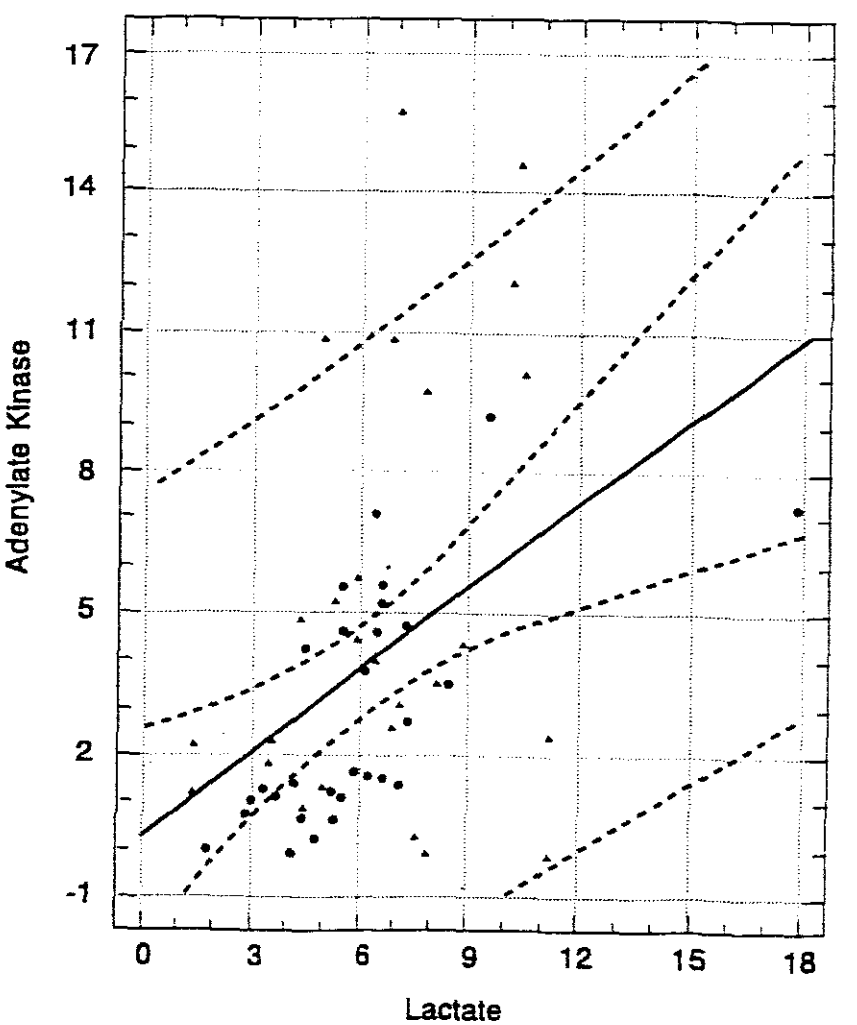
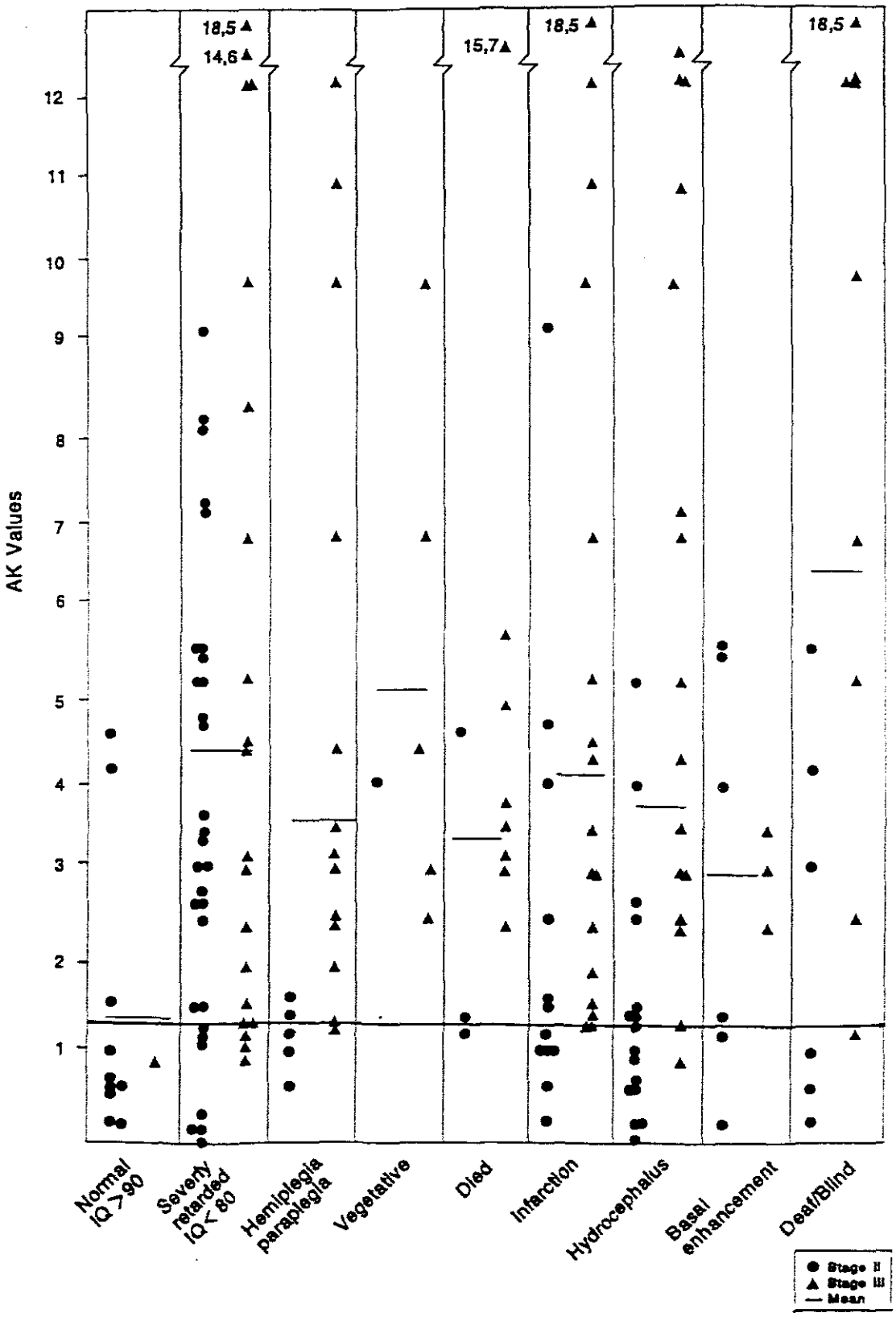


Fig. 42 Week 1 AK values with outcome at 6 months follow-up.
If an AK activity of 1,27 U/l is accepted as the upper limit of normal, then 73% of children considered to be normal at 6 months follow-up had a normal AK value during the first week of treatment. Conversely the majority of children with serious complications at 6 months follow-up had raised AK values during week 1 of treatment.



3.2 BACTERIAL MENINGITIS

The CSF cell count, chemistry, glucose and protein concentration results of the 33 patients with bacterial meningitis are presented in Table 9. AK ranged from 1,12 U/l to 28,81 U/l with a mean of 7,73 U/l.

Table 9
Adenylate kinase values, mean cell count and chemistry - bacterial meningitis

	MEAN	RANGE	N=
Adenylate Kinase u/l	7,73	1,12 - 28,81	33
Lymphocytes $x10^6$/l	1159	0- ≥500	33
Polymorphonuclear leucocytes $x10^6$/l	1438	50- ≥500	33
Protein g/l	2,6	0,4- 6,6	32
Glucose mmol/l	2,7	0,8- 9,3	33
Globulin (qualitative) (turbidity)	3+	0-4+	33

Only 1 patient (3%) with bacterial meningitis had a cranial nerve palsy, with a CSF AK value of 8,98 U/l. The CSF AK levels of the patients who did not have cranial nerve palsies ranged from 1,11 U/l to 28,8 U/l with a median of 4,28 U/l.

The 4 patients (12%) who developed hemi- or paraplegia had CSF AK values of 2,31 U/l, 8,98 U/l, 1,20 U/l, and 1,84 U/l. The AK values of patients who did not develop hemi- or paraplegia ranged from 1,11 U/l to 28,81 U/l with a median of 4,24 U/l.

Fifteen patients (45%) with bacterial meningitis had a CT scan at presentation. Four patients (12%) with CSF AK values of 6,57 U/l, 1,80 U/l, 8,60 U/l and 28,81 U/l had hydrocephalus. The patients who did not have hydrocephalus had a median CSF AK value of 4,11 U/l (range 1,11 - 28,12 U/l).

Six patients (18%) with subdural effusions had CSF AK values of 6,57 U/l, 8,98 U/l, 3,43 U/l, 1,20 U/l, 1,84 U/l and 17,64 U/l. The patients without subdural effusions had a median AK value of 5,06 U/l (range 1,11 U/l to 28,81 U/l) One patient (3%) developed brain infarction with a AK value of 28,12 U/l. Too few patients had abnormal CT scans for reliable statistical analysis.

Of the 33 patients initially enrolled in the study, 3 (9%) died in the acute phase. Their initial AK values were 28,12U/l, 25,24U/l and 1,20 U/l. The median AK value of the survivors was 4,54 U/l (range 1,11 to 28,81 U/l).

After letters were twice sent to the parents or caretakers of the 33 patients diagnosed with meningitis and treated at Tygerberg Hospital, a disappointing number of only 14 parents or caretakers responded and brought the children fortlb: neurological examinations by appointment as part of the follow-up. Three patients institutionalized after contracting meningitis, were regarded as mentally handicapped by the psychologist at the Dominican Convent for the Deaf.

Of the survivors, 40% were evaluated neurologically. Six (20%) of these had normal motor development (median AK 5,83 U/l, range 4,24 U/l to 25,24 U/l). The 2 patients (7%) with abnormal motor development had CSF AK values of 4,45 U/l and 28,81 U/l respectively. Only 10 patients (33%) had their IQ tested. Of these 3 (30%) had a normal IQ of more than 90. The AK values were 1,93 U/l, 4,24 U/l and 11,91 U/l. The remaining 7 patients (70%) were handicapped and had AK values ranging from 1,76 U/l to 25,24 U/l (median 5,83 U/l).

Hearing was tested in nine patients. Seven patients (78%) were normal. The two patients (22%) with abnormal hearing had AK values of 5,83 U/l and 12,50 U/l.

CHAPTER 4

DISCUSSION

4.1 Tuberculous meningitis

This study has shown that Adenylate Kinase CSF concentrations were significantly raised in children with TBM, particularly during the first week of therapy compared to patients without neurological disease. During this first week the values were also significantly higher in children at stage III TBM than in those at stage II TBM. Other CSF investigations reflecting the inflammatory response of the meninges were also abnormal during this first week with a tendency to normalise during the following month. None-the-less, neither the CSF cell count, CSF glucose nor CSF protein concentrations were related to CSF AK values. CSF lactate concentrations, generally considered to reflect hypoxic brain metabolism (Eross J *et al* 1981) were however significantly related to CSF AK values.

AK activity values, when compared from week, to week did not predict whether patients would develop granulomas, cranial nerve palsies, hemi- or paraplegia or abnormal audiograms. All the patients at stage II had hydrocephalus and therefore CSF AK could not be used to predict this complication.

AK activity in children with stage III TBM was not indicative of death, granuloma development, cranial nerve palsies, hemi- or paraplegia or hearing impairment. Although there was a definite difference in median values of 10,84 U/l to 3,51 U/l for impaired hearing, compared to normal hearing, and 9,74 U/l to 3,20 U/l for impaired vision compared to normal vision, statistical analysis found was not of significant value. However 19 (95%) of the 20 surviving patients at stage III TBM had an IQ of less than 90. Only one patient with stage III TBM had an AK value of less than 1 U/l during the first week.

CSF AK values during the first week of therapy in 5% of children considered to be normal at 6 month follow-up, were significantly lower than those in any group of children with TBM with specific neurological deficits. With very few exceptions, children with values higher than the upper limit of normal CSF AK values (1,27 U/l) had developed at least one neurological deficit.

Despite the above relationships, it was disappointing that in follow-up specimens there were several instances of a lack of correlation between clinical deterioration and CSF AK values. On the one hand CSF AK values rose in children who on clinical evaluation did not deteriorate in any way, while on the other hand several children experienced clinical deterioration and developed complications without any change or rise in the CSF AK values.

However, in this and other studies (Ronquist G *et al*, 1985) the elevated AK values found in patients may have been the result of a relative ischemia in the brain due to a reduced cerebral blood flow. We found raised AK values in patients with increased intracranial pressures and vasculitis. Bollensen E *et al*, 1989 found increased AK values only in patients with large and recent brain infarctions. This could also be a possibility why we could not always find increased AK values in some patients.

After the acute destruction of brain cells that occurs within the first 16-24 hours or is likely up to 48 hours after a brain infarction, lowered AK values were found afterwards. (Frithz G *et al*, 1982) We obtained CSF weekly from the TBM patients because pressure studies had to be done on them. We may have gained a false impression of sensitivity, because AK values were lower after 24-48 hours following acute insults to the brain. In some patients we could not obtain CSF at certain weeks, which could possibly have given a complete different picture of development of the illness. We must presume that the AK values did not increase during those specific weeks. In two children with TBM the AK values were within normal range over the four week period. At six months follow-up lumbar punctures were done because the patients' conditions were not satisfactory. Both AK values were increased, 1,37 U/l and 1,88 U/l respectively.

To be able to attempt to predict neurological outcome in children with meningitides with AK values, we should ideally have obtained the CSF within 24-48 hours after onset of illness. Because of the difficulties to diagnose TBM, this devastating sickness had already developed over days or weeks before patients were admitted. This could be the reason why in some patients the AK values and clinical picture did not correlate. Another problem was that only one or two CSF samples could be obtained from quite a number op patients which made the AK values useless.

With regard to conventional CSF investigations in TBM, at, or close to the time of diagnosis, it has been suggested that they bear no relationship to prognosis (Humphries MJ *et al*, 1990), so that the lack of correlation of CSF AK values and conventional CSF investigations such as CSF glucose, protein concentrations, and cell count is not unexpected. In TBM the detection of clinical signs is dependent upon the precise area of the brain which is involved in the pathological process. It is not unlikely that even extensive brain damage in an area of relatively insignificant neurological importance might not lead to clinically detectable signs, but could lead to rising CSF AK values during follow-up in the absence of clinical signs. It would however seem most unlikely that clinical deterioration could occur without brain damage, which should theoretically have been reflected in CSF AK values, however this was not always the case.

In another study in this department (Pretorius *et al*, 1995), CSF AK values correlated well with EEG-background patterns and neurological outcome in asphyxiated neonates. There was a statistically significant difference between the mean CSF AK levels of the control and axphyxiated group of neonates ($p < 0,01$). Simultaneous correlation with respective EEG background patterns substantiated the predictive merit in its own right. This study is still ongoing.

The study reported in this thesis confirmed the findings of Jonsson *et al*, 1979 that the AK values were significantly related to the CSF lactate concentrations. However, we were the first workers to confirm a definite correlation between CSF AK values and CSF lactate concentrations in children with TBM. The AK values which should be absent or low (< 1 U/l) in normal patients, were raised in all patients with TBM. This suggests that AK is a sensitive marker for cerebral ischaemia not detected by conventional laboratory methods in children with TBM.

4.2 Bacterial meningitis

In this and another study (Jonsson *et al*, 1979) AK values were raised in all patients with bacterial meningitis. In some patients the AK values were extremely high with devastating results and were accompanied by serious complications. In a few patients the AK values were not very high - some of these patients were not admitted within 48 hours after onset of sickness which could be the reason for the lower AK values in some cases.

When AK activities were roughly correlated with the reports from the local health authority clinic sisters, patients with AK values of < 4 U/l were considered "normal" by them and patients with AK values between 5,0 U/l and 7,0 U/l were regarded as clumsy and having deficient control of muscular movement and a deterioration in school work.

None-the-less, CT scans were performed on 15 (15%) of those patients studied. 20% had hydrocephalus, 13 % had cerebral oedema and 27% had subdural effusions. 18% of the bacterial meningitis patients were on steroids. Not a single patient had a normal AK activity of less than 1 U/l. Three patients (9%) died of whom 2 had a very high AK activity. As a rule IQs are not evaluated on patients with bacterial meningitis. Out of 10 patients evaluated, 7 (70%) had a markedly decreased IQ (mean 79,6 range 55-89). It should be considered to evaluate IQs on all children whom had meningitis in this hospital.

The 15 patients with *H.influenzae* meningitis had the most serious complications like subdural effusions, hemiparesis, hypotonia, hydrocephalus and deafness. The 13 patients with *N.meningitidis* were not affected as seriously as the *H.influenzae* patients although their IQs were all decreased.

The results of the evaluation of CSF AK activity in the children with bacterial meningitis were disappointing due to the poor response of parents or caretakers to bring the children for follow-up evaluations.

CHAPTER 5

CONCLUSIONS

This study has established for the first time that CSF adenylate kinase activity is increased in children with tuberculous meningitis, and that this increased activity bears some relation to neurological damage and prognosis, in that significantly higher activity is found in children at stage III tuberculous meningitis who are acknowledged to have a worse prognosis than in children at stage II tuberculous meningitis. There is also a significant relationship found between CSF lactate concentrations, an acknowledged indicator of hypoxic cerebral metabolism, and CSF adenylate kinase activity, suggesting that increased CSF adenylate kinase activity does indeed reflect hypoxic cerebral metabolism.

This study has also for the first time delineated the range of CSF adenylate kinase activity in otherwise normal children investigated, because of the clinical suspicion of meningitis but subsequently considered not to have meningitis. Adenylate kinase activity ranged from 0 - 1,27 U/l in these children [mean 0,59 U/l].

On completion of 6 months therapy, 11 children (13%) were considered to be neurologically normal and were found to have an I.Q. > 90. Only three of these children (27%) had CSF adenylate kinase activity higher than 1,27 U/l before or during the first week after starting antituberculosis treatment.

The majority of children who were mentally retarded (I.Q. < 80), or who had one or more serious neurological deficits, had CSF adenylate kinase activity of more than 1.27 U/l during or before the first week after starting therapy. Of the 6 children (7%) who were in a vegetative condition 6 months after starting treatment, and the 10 children (11%) who died, only 1 had CSF adenylate kinase < 1,27 U/l.

When those children who had more than three consecutive determinations of CSF adenylate kinase activity were considered, four groups could be identified. Firstly those in whom initially increased CSF adenylate kinase activity decreased onver 2 - 4 weeks and remained low. Secondly those in whom increased adenylate kinase activity was detected at the same time as an alteration in the child's clinical condition or CT findings. Thirdly those in whom CSF adenylate kinase activity increased without any detectable alteration in clinical condition or CT findings, and fourthly those whose clinical condition or CT findings deteriorated without any increase in CSF adenylate kinase activity.

In the light of the above findings it can be stated that it is unlikely that a child with tuberculous meningitis who has CSF adenylate kinase activity greater than 1,27 U/l during the first week after starting therapy, will escape without any neurological damage and that, although there are exceptions, the finding of increasing levels of CSF adenylate kinase activity following the start of antituberculosis therapy, will frequently be associated with the development of fresh neurological lesions or the failure of existing complications, for example hydrocephalus, to resolve satisfactorily.

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