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CEREBROSPINAL FLUID BIOCHEMICAL MARKERS IN CENTRAL NERVOUS SYSTEM METASTASIS: CLINICAL APPLICATIONS

Proefschrift

ter verkrijging van de graad van doctor in de geneeskunde aan de
Rijksuniversiteit Limburg te Maastricht, op gezag van de
Rector Magnificus, Prof. Dr. F.I.M. Bonke, volgens besluit van
het College van Dekanen in het openbaar te verdedigen op
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door

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Geboren te Meppel

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To the cancer patients

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Chapter 1.

Introduction

1.1. General value of biochemical markers in tumours

Cancer is the second most common cause of death in humans (1), and the incidence of nearly all neoplastic diseases is increasing. The increase in central nervous system (CNS) metastases from systemic cancer has been ascribed to prolonged survival caused by more aggressive forms of therapy and to the inability of most chemotherapeutic agents to cross the blood-brain barrier. Between 18% and 30% of all patients who die of systemic cancer have CNS involvement at autopsy (2).

Early detection of cancer may improve the chances of cure and survival; however, about two-thirds of all cancer patients already have metastases of the disease before cancer is diagnosed (3). For this reason, an urgent need exists for adequate screening methods which will permit diagnosis of cancer before the development of metastases. Biochemical markers, also called "tumour markers", may be important tools; such markers have been studied in various biological fluids, such as urine, plasma, pleural and peritoneal fluids, and cerebrospinal fluid (CSF) (4-11). Although these tumour markers, in general, have not proved to be very useful in the early diagnosis of cancer in the population at large (the high false positive rate and low true positive rate appear to be serious limitations), it may be different in the screening of subjects who run a high risk of developing cancer. At the moment, it is too early to make any definite conclusions.

Within this area of research, considerable interest has developed in identifying and measuring tumour-related substances in CSF. Recently, new techniques to measure the concentrations of tumour-specific or tumour-associated materials have been developed, and these techniques are the subject of this thesis.

A substance must meet certain criteria before it can be called a tumour marker. First the "ideal" tumour marker must be a molecule which is exclusively of tumour cell origin and it must be released by the tumour cell in quantities proportional to the tumour burden. For clinical purposes, a tumour marker should not be present in substantial concentrations in body fluids of non-tumour-bearing hosts. Finally, it

should be detectable before the tumour burden can be demonstrated with simple means or before it has progressed towards an incurable state.

The usefulness of a tumour marker can also be described in terms of parameters which are generally applied for the evaluation of clinical laboratory tests (12). These parameters include: (i) sensitivity "the ability of the test to detect all patients with cancer"; (ii) specificity "the ability of the test to identify individuals who do not have the disease"; (iii) positive predictive value "the probability that a person with a positive test actually has the disease"; and (iv) efficiency "the fraction of all results that are correct". Whereas sensitivity and specificity can be calculated directly from the data obtained in a clinical study, predictive values are dependent upon prevalence of the disease in the population to which the diagnostic test is administered.

CSF tumour markers may be divided into enzymes, tumour-associated antigens, and metabolic products. This overview will concentrate on the current and future role of tumour markers, particularly in relation to cancer diagnosis and the monitoring of the results of treatment.

1.2. Cerebrospinal fluid (CSF) formation and absorption

Cerebrospinal fluid provides the clinician with access to the brain, and variations in its composition allow him to pinpoint pathophysiological changes in brain function (13).

CSF is found in the ventricular system and in the subarachnoid spaces. The fluid is clear and colorless, the volume approximately 150 ml; it contains small amounts of glucose, protein, and potassium, large amounts of sodium chloride, and few or no cellular components (14-17).

The formation and absorption of CSF in humans has been measured by indicator dilution techniques. The rate of formation is approximately 0.35 ml per minute, or 500 ml per day (18). The choroid plexus is the principal source of CSF. The major absorptive sites are the arachnoid granulations (Pachioni), which penetrate the major dural venous sinuses in the cranium and epidural veins.

The chemical milieu of the CNS must be maintained within certain narrow limits to give all neurons the opportunity to function. The blood-brain barrier is directly responsible for this stable physical and chemical milieu. It acts as a differential filter, selectively

permitting the passage of some substances from the blood into the intracellular fluid. The epithelium of the choroid plexus may also be involved, actively secreting certain substances into CSF.

1.3. The route of entry of tumour markers into the CSF

Just how biochemical substances produced by malignant neoplasms, enter CSF is unknown. There are, however, several theories about the origin of these tumour products.

- (i) Local production and release of tumour products directly into CSF by tumour cells - "This may occur if the tumour cells are localised in the leptomeninges or are present within the CNS itself".
- (ii) Passage of tumour products from plasma into CSF, due to a rupture of the blood-brain barrier - "From electron microscopical studies, it appears that at the site of brain tumours, the blood-brain barrier is frequently open, allowing the free passage of relatively large proteins" (17). However, the observation that tumour products can be found in higher concentrations in CSF than in plasma strongly suggests that tumour cells contained within the subarachnoid space secrete their products directly into spinal fluid, making abnormal permeability of the blood-brain barrier a less plausible explanation.
- (iii) Access to CSF through cerebral, extracellular space - "It is known that proteins such as horse radish peroxidase (molecular weight 43,000 Daltons) can pass from the two compartments". Therefore, one cannot rule out the possibility of tumour products diffusing from a tumour deep within the parenchyma to the ventricular or subarachnoidal surface and then being secreted into CSF.

1.4. Potential use of cerebrospinal fluid markers in the diagnosis and follow-up of treatment of patients with central nervous system metastases

1.4.1. Screening the general population for presence of CNS tumours

In order to evaluate a new laboratory test, knowledge of the test characteristics are needed. Sensitivity and specificity, together with false negative and false positive rates, are the most important test characteristics to consider when analysing test results (12). Once a

cut-off limit has been chosen for a laboratory test, the sensitivity and specificity are fixed.

Determining the sensitivity and specificity of a test is, however, difficult, since an absolute standard (gold standard) against which to measure test performance, must first be chosen. In other words, the usefulness of a test is dependent upon its ability to analyse data to confirm the presence or absence of the disease. In our studies, standards were chosen according to the following criteria: In order to confirm a diagnosis of leptomeningeal metastasis, the presence of tumour cells in the CSF, or an autopsy revealing metastasis, was required. Yet, cytological analyses of CSF have also posed a serious problem, in that they sometimes give false positive and false negative results. In reviewing the literature, we found that these criteria have not always been used to evaluate a new laboratory test.

The usefulness of a tumour marker test in screening for the presence or absence of a disease can be determined from its positive or negative predictive value. These values are dependent not only on sensitivity and specificity of the test, but also on the prevalence of the disease within the population studied. For example, given an estimated prevalence in the general population of 4.5 cases of CNS tumours per 100,000 individuals, a test with a sensitivity and specificity of 99% would have a positive predictive value of only 0.44%; that is to say, it is quite likely that only one out of every 227 subjects with a positive test would actually have a CNS tumour.

One must bear in mind that a test has to have both a high sensitivity and a high specificity to be of value in this kind of screening; unfortunately, only a few such tests exist.

There are still other factors to consider with regard to mass screening by CSF tests. A test for general screening would have to be given to each member of the population, whether or not the person exhibited symptoms of the disease. Individuals in good health would most likely not agree to a test that involved obtaining CSF by lumbar puncture.

1.4.2. Determining the presence or absence of CNS metastasis in patients at risk

Acceptable predictive values can be obtained when a test group is limited to a population of patients with neurological diseases who have one or more defined symptoms indicating the possible presence of a CNS tumour.

The advent of computerized tomography (CT) has resulted in a major change in the detection, and follow-up to treatment, of CNS malignancy. However, small tumours and metastases in the posterior fossa and leptomeninges are difficult, if not impossible, to detect on CT scans. In such cases, tumour markers may prove quite helpful. They may also facilitate in the early detection of asymptomatic CNS metastases, such as an increase in the level of human chorionic gonadotrophin (B-HCG) in metastases from uterine chorion carcinoma (19, 20). In other words, elevation of tumour markers in CSF may indicate the presence of such tumours earlier than the methods currently available are able to.

1.4.3. Determining the site of CNS tumours

B-glucuronidase (B-gluc.) and carcinoembryonic antigen (CEA) in CSF seem to be accurate tumour markers for the detection of leptomeningeal metastases, but not for intraparenchymal or epidural metastases (4).

The diagnostic criterion for leptomeningeal metastasis is the presence of tumour cells in CSF, this points to the invasion of the leptomeninges by cancer. In one series of patients, 60% of the patients with leptomeningeal cancer had a positive cytology, indicating that failure to find malignant cells is relatively common among patients with leptomeningeal metastasis. In that case, other laboratory tests, such as identification of tumour markers within the CSF, can be of help (4, 22, 23).

1.4.4. Classification and grading of CNS tumours

The most reliable method for classifying CNS tumours is histological examination of a biopsy specimen of tumour tissue. However, surgical approaches often carry with them the risk of morbidity or mortality. Some CNS tumours, especially metastases of germ cell tumours (chorion carcinoma), may be highly vascular and prone to spontaneous hemorrhage (24). Surgical manipulation may provoke uncontrollable bleeding and

contribute to the patient's death. Measuring biological markers in CSF, such as alpha-fetoprotein (A-F.P.) and human chorionic gonadotrophin (HCG) makes it possible to obviate the need to perform a surgical biopsy. The detection of elevated A-F.P. or HCG in the CSF may be sufficient for diagnosis, so that therapy can be started.

Furthermore, some tumour markers may be of value in distinguishing benign from malignant primary CNS tumours (e.g. desmosterol polyamines and adenylate kinase in gliomas (25, 26)).

1.4.5. Assessment of therapeutic efficacy

Tumour marker measurements of CSF may be useful in monitoring the effects of treatment. Little data is available, however, concerning the serial measurements of biochemical markers during therapy (7, 22, 23, 27-29). In chorion carcinoma, regular measurements of HCG levels in lumbar CSF are very important in monitoring therapy (19, 20).

There is virtually no information in the literature on usefulness for tumour markers measured simultaneously in ventricular CSF, obtained from the Ommaya reservoir, and in lumbar CSF. Moreover, very little information is available concerning the usefulness of tumour marker determinations in the longitudinal follow-up of patients with leptomeningeal metastases.

1.4.6. Prediction of prognosis and long-term evaluation of tumour growth

Changes in CSF marker levels may be more reliable in predicting a decline in the clinical status of a patient, secondary to tumour regrowth, than those methods currently available. However, little information is available on fluctuations in tumour marker levels in relation to tumour growth. Long-term serial studies on patients who have been treated for CNS tumours are needed to correlate marker levels with clinical status.

1.4.7. The value of tumour markers in distinguishing leptomeningeal metastasis from infectious meningitis

Leptomeningeal metastases tend to mimic the clinical picture of infectious meningitis (30). The CSF findings in leptomeningeal metastasis may also tend to be identical to those found in cases of meningitis

arising from tuberculosis, cryptococcus, candida albicans, and sarcoidosis (31, 32). In patients with infectious meningitis, the tumour markers B-gluc., B2-microglobulin (B2-m), and lactate dehydrogenase (LDH) are elevated, possibly secondary to the presence of granulocytes in the CSF (33). In the differential diagnosis of leptomeningeal metastases from infectious meningitis, the combined determination of B-gluc., CEA, and LDH in CSF may be of even greater quantity measured; in meningitis, B-gluc. and LDH will be elevated, whereas CEA will be normal (20).

1.5. Classification of tumour markers in CSF

Tumour markers in CSF can be divided into three groups:

1.5.1. Tumour-associated antigens

1.5.2. Enzymes

1.5.3. Metabolic products

1.5.1. Tumour associated antigens

1.5.1.1. Carcinoembryonic antigen (CEA)

CEA, first isolated in 1965 by Gold and Freedman (34), is a B-1-glycoprotein with a molecular weight of approximately 180,000 Daltons. It is present in normal intestinal tissues, in various biological fluids, such as blood, urine, intestinal secretions, and peritoneal fluids (5, 6, 35), and in CSF (7, 36).

Raised serum levels of CEA are found particularly in patients with carcinoma of the gastrointestinal tract, breast, lung, ovaries, and pancreas (31, 37, 38), but have also been observed in patients with a variety of nonmalignant diseases.

In the diagnosis of CNS tumours, measurement of serum CEA appears to be of limited value. Only a small percentage of patients with primary or metastatic brain tumours have shown elevations of serum CEA (39, 40), whereas CEA levels are frequently elevated in the serum of patients with neuroblastoma (41).

Since the recent development of new techniques which permit measurement of nanogram concentrations of proteins, CEA has become detectable in CSF. This CEA from CSF is reported to be of value in the detection of CNS metastases of CEA-producing tumours, especially the leptomeninges from lung and breast carcinoma (7, 8, 39, 40, 42). There are no reports on CEA elevations in the CSF of patients with lymphoma

(8). Patients with primary brain tumours have a slight elevation, if any, of CEA in their CSF (7). The same is true of those with infectious meningitis (20).

1.5.1.2. Human chorionic gonadotrophin (HCG)

HCG is a glycoprotein produced by the placenta. It has two subunits - alpha and beta - which can be detected separately by radioimmunoassay methods. In chorion carcinoma, the quantitative assay of urine and/or serum HCG is essential for initial diagnosis and in monitoring the course of the disease; the higher the level, the larger the tumour mass (43). Chorion carcinoma is known to metastasize widely and at an early stage, with frequent involvement of the brain.

B-HCG is detectable in the CSF of patients with chorion carcinoma and embryonal carcinoma.

Analyses of CSF in series of patients with chorion carcinoma (44) showed that, in the absence of brain metastases, the level of HCG found in the CSF is proportional to the serum level. Therefore, a serum:CSF gradient for HCG is generally more meaningful than the actual CSF level. By use of this gradient, or ratio, it is possible to distinguish those patients with cerebral metastases from chorion carcinoma from those without it. In patients with cerebral metastases from chorion carcinoma, the serum:CSF gradient is usually below 60 (44). This method of diagnosing cerebral metastases is now routinely used in all patients with chorion carcinoma. Once cerebral metastasis has been diagnosed, CSF HCG measurements can be used in monitoring the efficacy of treatment (43, 44).

The origin of the elevated HCG levels in the CSF of patients with cerebral metastases has been puzzling. The observation that B-HCG is detectable in the CSF of subjects with normal pregnancy indicates that B-HCG enters the CSF only when a threshold value is excreted. Yet, there is also speculation that HCG from serum may enter an abnormally permeable blood-brain barrier. That this does, in fact, seem to be the case is suggested by the relationship between the HCG level in CSF and that in the serum. At the same time, there is some evidence of direct secretion of HCG from cerebral metastasis, particularly when the level of HCG in the CSF exceeds that found in the serum (19, 42, 43).

The HCG from CSF has also been used in the diagnosis and treatment of primary CNS tumours of germ cell origin. The marker profile for chorion carcinoma and embryonal carcinoma in CSF correlates positively with the histological diagnosis, that is to say, both tumours produce HCG (46). Their presence in CSF may be sufficient to confirm the diagnosis of cerebral metastasis, enabling specific chemotherapy or/and radiotherapy treatment to be started without surgical biopsy. The assessment of HCG in CSF is also a good indicator of the success of therapy (44).

1.5.1.3. Alpha-fetoprotein (AFP)

AFP is an oncofetal antigen found in fetal serum as early as the first month of human embryonic life. AFP is a glycoprotein synthesized by the yolk sac, liver, and gastrointestinal tract of the fetus.

Elevations of AFP levels in serum have been observed in patients with hepatocellular carcinoma, as well as in patients with viral hepatitis and liver cirrhosis. AFP levels may also be elevated in germ cell tumours, ovarian cancer, and a variety of gastrointestinal cancers.

Elevated AFP levels have also been found in cases of endodermal sinus tumour (46). The presence of a CSF:serum gradient of AFP may aid in the diagnosis of surgically inaccessible tumours. In that case, treatment may be started without an histological diagnosis.

The serial determination of the AFP level in CSF and serum in patients with metastases to the brain from endodermal sinus tumour and embryonal carcinoma may be a useful adjunct in determining response to therapy (44).

1.5.1.4. Beta-2-microglobulin (B2-m)

B2-m was isolated and described from human urine by Berggård and Bearn in 1968 (47). It is a protein with a molecular weight of 11,800 Daltons, and it is a small subunit of the human leucocyte antigens (HLA). B2-m is dissociated from the heavy chain of these antigens and is released in free form in extracellular fluid. Lymphocytes and macrophages are particularly rich sources of B2-m.

B2-m is present in various biological fluids, such as serum, urine, amniotic fluid, ascites, and CSF (48-50). Serum levels of B2-m increase in a variety of states, including various neoplastic, inflammatory, and immunologic disorders, and conditions like renal failure, in which

catabolism is diminished (49, 50). Raised serum B2-m levels were reported in the serum of patients with multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's disease, and chronic lymphatic leukemia (51). However, because of the multitude of factors that can affect its concentration, B2-m levels in serum are not of great diagnostic value (52).

It has been suggested that measurement of B2-m in CSF may be more promising for the diagnosis of CSF involvement by hematological tumours (28). However, CSF B2-m also appears to be elevated in leptomeningeal metastasis from solid tumours and in bacterial meningitis (29, 50). Therefore, the exact contribution of B2-m to the diagnosis of leptomeningeal metastasis remains to be determined.

1.5.2. Enzymes

Much attention has been paid to enzymes as tumour markers; nevertheless, the clinical significance of CSF enzymes in the diagnosis of CNS tumours is still poorly defined (53, 54).

One observation that has been made regarding CSF enzymes and malignancy is that the level of lysozyme muramidase present in the CSF of subjects with brain tumours appears to be raised (55), not unlike that of serum muramidase in the case of leukemia patients.

Tests have been carried out to determine the levels of many other enzymes in CSF, such as isocitrate dehydrogenase, aspartate transaminase, alanine transaminase, pyruvate kinase, aldolase, and adenylate kinase (53, 54); unfortunately, none of these tests has a high enough degree of specificity to be of real use since raised levels have been observed in both CNS tumours and many nonmalignant diseases, such as vascular and infectious lesions.

Measurements of yet other enzymes in CSF have been proposed as CSF markers of malignancy in the past several years (56, 58), among them, creatine kinase BB isoenzyme, enolase, beta-glucuronidase, and lactate dehydrogenase. The significance of the activities of these enzymes, as related to various neurological conditions, has been interpreted in various, and sometimes conflicting, ways by different investigators (57-61).

1.5.2.1. Creatine kinase BB isoenzyme (CK BB)

Concentrations of creatine kinase BB isoenzyme in CSF were measured by radioimmunoassay and showed striking elevations in acute CNS injury (8, 56). The CSF CK BB levels do not appear elevated in chronic neurological disease (58). Further study is needed to determine the significance of CSF CK BB measurements in metastatic CNS malignant neoplasms.

1.5.2.2. Enolase

Enolase is a dimeric enzyme with three subunit types: α , β , and γ , which give rise to five isoenzymes: $\alpha\alpha$, $\beta\beta$, $\gamma\gamma$, $\alpha\beta$, and $\alpha\gamma$, which show differential cellular distributions. Of particular interest are the isoenzymes patterns in the nervous system. Glia cells contain only $\alpha\alpha$ enolase, whereas $\gamma\gamma$ is confined to the neurons. Outside the nervous system, γ enolase occurs predominantly in the cells of the neuroendocrinological system. The measurement of isoenzyme levels, rather than of total activities, reflects more precisely the nature of the lesion. Elevated levels of γ enolase in CSF reflect neuronal damage.

Alpha and gamma enolase isoenzymes were studied in 212 patients with various neurological diseases. The results showed that these isoenzymes are sensitive markers of tissue damage and that they were especially raised in the CSF of patients with low grade astrocytomas (58, 62). Assay of the α - and γ -enolase isoenzymes seemed to reflect damage to glia cells and neurons, respectively. The neuron-specific enolase may also be a useful tumour marker in the diagnosis of CNS metastasis in small cell lung cancer (SCLC), especially in patients with leptomeningeal metastases (63).

1.5.2.3. Beta-glucuronidase (B-gluc.)

B-gluc. is an intracellular enzyme, localized in lysosomes and microsomes, which hydrolyzes the B-glycoside bond between glucuronic acid and an aglucone (60). It is normally found in the brain, in both the white and the gray matter (59). Relatively high concentrations exist in pia-arachnoid tissue and choroid plexus (60, 64). However, the activity of B-gluc. within CNS is lower than in the viscera.

B-gluc. is usually found in the CSF of individuals without any disease of the nervous system (normals) in a concentration below 27 mU/l (64).

The concentration of the B-gluc. enzyme in lumbar CSF is approximately twice that of ventricular CSF (57).

As a result of malignant transformation, levels of B-gluc. in the brain, as well as in other tissues, may increase (65). Elevated B-gluc. activity in CSF has been found in patients suffering from bacterial meningitis or leptomeningeal metastases (4, 66). Malignant cells derived from adenocarcinoma and myelogeneous leukemias are very rich in B-gluc., lymphoma cells moderately so (65).

In patients with leptomeningeal metastases from hematological malignancies, B-gluc. activity was found to be less elevated than in leptomeningeal spread from solid tumours (4, 23, 65).

A slight increase in B-gluc. activity in CSF has been observed in patients with parenchymal or epidural metastases from solid tumours (59). Slight elevations have also been reported in some primary CNS malignant tumours and in fungal and tuberculous meningitis. Viral meningitis does not appear to cause elevation of B-gluc. activity in CSF (64, 66).

When patients are successfully treated for leptomeningeal metastases, the B-gluc. concentrations approach control levels in both lumbar and ventricular CSF (4).

1.5.2.4. Lactate dehydrogenase (LDH)

LDH is an enzyme that catalyzes the final step in the metabolic chain of anaerobic glycolysis. This enzyme has a molecular weight of 135,000 Daltons and is located predominantly intracellularly. In humans, this enzyme exists in five molecular forms, or isoenzymes (61), all of which are tetramers of two different subunits. LDH is a normal constituent of brain tissue, in which the total activity of LDH, as well as the LDH isoenzyme distribution, can be measured. Extracts from brain tumours may show changes in total LDH and isoenzyme activities, as compared to the activities in a normal brain. Various alterations of LDH isoenzyme patterns have been described in different types of tumours (67- 72).

These findings may be of diagnostic significance, since knowledge of the LDH isoenzyme distribution may help in evaluating tumours otherwise difficult to classify.

Low activities of total LDH and of isoenzymes are present in normal CSF and are found to be age-related (73, 74). Reports concerning the presence of this enzyme in the CSF of patients with various neurological

diseases first began to appear in 1956, and numerous publications have since documented changes in the LDH activity of CSF (10, 74-76).

LDH has been found in the CSF of patients suffering from a variety of neurological diseases, including primary and metastatic CNS tumours (72, 74-77). In the literature, reports on LDH activity in the CSF of control subjects and of patients with CNS tumours are not uniform (8). Several studies have indicated that LDH activity in CSF is normal in patients with primary tumours (15, 32); others have noted a rise in LDH activity in CSF, with benign, as well as malignant, tumours (10, 72, 77). Although most researchers have found no correlation between increased enzyme activity within CSF and the degree of malignancy of a tumour, the results of some reports do suggest that such a correlation does exist (71, 74). In one study, it was shown that, with time, the tissue culture medium of malignant cells contained ever increasing amounts of LDH, whereas culturing of benign cells did not produce an increase in the LDH content of the medium (72). The major source of LDH in CSF is almost certainly the tumours themselves. However, the destruction of nervous tissue by cerebral hypoxia caused by cerebral edema and elevated intracranial pressure, associated with very malignant tumours, may very well contribute to greater activities of LDH in CSF.

Much work has been done on LDH in the CSF of patients with systemic cancer (22) for the express purpose of detecting leptomeningeal metastases. Given their direct contact with the subarachnoid space, one might almost expect CNS tumours to cause a rise in LDH activity in CSF. Earlier studies, however, have reported quite different results (15, 17).

A same high elevation of LDH isoenzymes in CSF was found in patients with leptomeningeal metastases from solid tumours (22), as well as, though to a lesser degree, in patients with leptomeningeal metastases from hematological tumours. Many studies have also reported high LDH activity in the CSF of patients with cerebral and epidural metastases (8, 10, 74). Moreover, it is known that LDH activity in CSF increases in a number of nonmalignant conditions. Diagnostic significance has been ascribed to elevations of LDH activity in CSF in cases of cerebrovascular disease (10, 68, 71), bacterial meningitis (33, 70, 75), and head injury (10).

In some studies lead one to conclude that serial measurement of LDH in CSF is needed, of value in monitoring the efficacy of treatment (8, 22).

1.5.3. Metabolites

1.5.3.1. Polyamines

The levels of the polyamines putrescine, spermidine and spermine have been reported to be elevated in the serum and urine of many patients with cancer (78). The exact function of the polyamines under normal circumstances has not yet been determined, but they appear to be related to cellular growth and differentiation. Increases in polyamines in serum and urine have been reported in leukemia, cancer of the ovaries, lung, colon, rectum, prostate, kidney, testes, and bladder, and malignant melanoma (78).

Polyamines have also been measured in CSF, in neurological patients without CNS neoplasms, and low levels have generally been found. By contrast, the levels of polyamines in the CSF of a group of patients with malignant primary brain tumours have been found to be rather high (76, 79, 80). The assay of CSF polyamines may be useful in the evaluation of the effectiveness of chemotherapy in patients with primary brain tumours (78).

Monitoring of CSF for polyamines has been reported to allow early diagnosis of meningeal leukemia (79, 80). Further studies, particularly those including cases of inflammatory CNS lesions, are required to establish the exact value of polyamines as tumour markers.

1.6. Purpose of this study

As has been stated in 1.4.1, CSF tumour markers are not presently being used to screen the population at large for the presence of primary brain tumours. However, the potential role of such tumour markers in the diagnosis of malignancies in groups of high risk patients may be quite large. Indeed HCG and AFP have already been in use for many years as markers for a number of relatively uncommon sorts of cancer, such as chorion carcinoma and germ cell tumours. The serial measurement of these tumour markers in CSF is essential for the diagnosis and management of these diseases, and it has also appeared to be a useful adjunct in determining response to therapy.

The routine use of other tumour markers in CSF has been investigated in several studies. However, many of these studies have not included patients with CNS tumours or other neurological diseases. Information on these patients is necessary in order to evaluate the sensitivity and specificity of the test used; the analytical precision and the time, ease, and cost of the test should also be taken into account.

Little data is available concerning the sensitivity and specificity of the measurement of multiple markers in CSF samples. In many instances, there is no data concerning serial studies of patients undergoing treatment to evaluate the use of markers in predicting the efficacy of treatment. Nor have the effects of prophylactic radiation and chemotherapy on tumour marker levels been studied.

Our decision to make B-gluc., B2-m, CEA, and LDH the subjects of the present study was, in large part, related to the conflicting and incomplete nature of the results of past studies of these tumour markers in the literature. These markers had never before been studied in populations including patients with malignant and nonmalignant neurological diseases and, until now, no data has been available concerning their sensitivity and specificity. Another reason for choosing these markers was our contention that these four markers belong to routine laboratory determinations, and that they must, therefore, be measured rapidly, precisely, and economically.

1.7. Outline of the study

In this thesis, we describe the results of other studies on tumour markers in CSF. In chapters 2, 3, 4, and 5, the potential value of the tumour markers B-gluc., B2-m, CEA, and LDH in the diagnosis of CNS metastases is evaluated.

In these studies, CSF samples were taken from patients with CNS tumours and from patients with a broad spectrum of neurological diseases. Levels of the four tumour markers within that CSF were measured simultaneously in order to calculate the sensitivity and specificity of the markers.

In chapter 6, we evaluate the role of single and combined tumour marker tests in the diagnosis of leptomeningeal metastasis from breast carcinoma.

In chapter 7, we measure simultaneously the levels of the four tumour markers in the CSF obtained via lumbar and ventricular (i.e. Ommaya) taps. We investigate the levels of these tumour markers in lumbar CSF in relation to the extent of leptomeningeal disease. We also attempt to determine the role that serial tumour marker patterns in ventricular and spinal fluid have in monitoring the success of therapy in patients with leptomeningeal metastases.

In chapter 8, we discuss the various ways of diagnosing leptomeningeal metastasis. We summarize the results of several clinical tests which may be relevant to this diagnosis and evaluate the potential contributions of the four tumour markers studied.

In the summary and conclusions, we finally report how our results improve the possibilities of diagnosis and treatment of leptomeningeal metastasis.

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Chapter 2

Cerebrospinal Fluid Beta-glucuronidase Activities in Patients with Central Nervous System Metastases *

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2.1. Introduction

The micro- and lysosomal enzyme beta-glucuronidase (EC 3.2.1.31) is found in relatively high concentrations in the arachnoid and the choroid plexus. However, in cerebrospinal fluid (CSF) it occurs only in low concentration. Because leptomeningeal involvement in brain diseases often increases the CSF activity of beta-glucuronidase, its determination may be used as a marker (1-4), but there are insufficient data available concerning sensitivity and specificity of beta-glucuronidase when used as a marker in this context.

In the present study, beta-glucuronidase and the more conventional determinations of CSF levels of lactate dehydrogenase (LDH, EC 1.1.1.27) activity and total protein and glucose concentrations were investigated in a group of patients with metastatic and nonmetastatic diseases of the central nervous system (CNS). A group of patients with normal CNS and suffering from diseases other than malignancy served as a control group.

2.2. Patients and methods

2.2.1. Control group

The control group consisted of 73 subjects with an average age of 51.3 years, range 23-85 years (Table 1). There were 19 females and 54 males. None had structural lesions of the CNS or symptoms of polyneuropathy. Sixteen controls had low back pain or brachialgia without disc prolaps. In 16 out of the 73 controls CSF levels of protein were elevated (mean 0.61 g/l, range 0.52-0.84 g/l), in one patient CSF LDH activity was elevated (32 U/l) and in four CSF levels of glucose were subnormal (mean 2.3 mmol/l), range 2.2-2.4 mmol/l).

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Table 1. CSF beta-glucuronidase levels in groups of control subjects and patients.

Group	N	B-glucuronidase				Significance (p)
		Activity (mU/l)		Log activity		
		$\bar{x}$	range	$\bar{x}$	SD	
Control subjects	73	16.0	8-38	1.1875	0.1201	
Patient groups						
Solid tumours:						
without nervous system metastasis	23	16.8	11-30	1.2079	0.1268	
with brain metastasis	26	20.4	8-40	1.2806	1.1612	< 0.005
with epidural metastasis	17	20.1	12-39	1.2779	0.1494	< 0.01
with leptomeningeal metastasis	25	89.3	17-318	1.8288	0.3401	< 0.001
Hematological tumours:						
without nervous system metastasis	8	13.1	6-18	1.0962	0.1576	
with epidural metastasis	2	20.0	14-26	1.2806	0.1901	
with leptomeningeal metastasis	9	34.2	15-70	1.4689	0.2473	< 0.001
Viral meningitis	4	16.8	8-24	1.1923	0.2036	
Bacterial meningitis	4	61.5	30-81	1.7579	0.2009	< 0.001
Cerebrovascular accident	29	16.3	4-42	1.1823	0.1673	
Neuropathy	6	16.0	11-21	1.1964	0.0912	
Cerebral concussion	4	18.2	12-31	1.2287	0.1867	
Various neurological diseases	19	17.7	9-26	1.2347	0.1102	

2.2.2. Patient group

The patient group consisted of 176 patients with an average age of 56.3 years, range 14-92 year (Table 1). There were 106 females and 70 males. One hundred ten had malignant diseases consisting of solid tumours in 91 and hematological malignancies in 19 patients. In 23 patients of the solid tumour group and in eight of the hematological tumour group neither neurological examinations, including CSF analyses, nor CT brain scans were indicative of CNS metastases. In 26 patients with solid tumours the clinical diagnosis of cerebral metastasis was confirmed by CT brain scans. In 17 patients with solid tumours and in two with hematological malignancies myelography confirmed the diagnosis of spinal cord compression by epidural metastasis.

In 34 patients, 25 with solid and nine with hematological tumours, demonstration of tumour cells in lumbar CSF confirmed the diagnosis of leptomeningeal metastasis. Histopathology of the brain was obtained in 14 autopsies and confirmed the clinical diagnosis of leptomeningeal metastasis in five and brain metastasis in nine patients.

Patients with malignancies were admitted to the Antoni van Leeuwenhoekhuis during 1981-1983. Patients without malignancies were admitted to the Department of Neurology of the Slotervaart Hospital during 1981-1982.

Table 2. Within run and day-to-day imprecision.

Activity	N	mean $\pm$ SD (mU/l)		CV (%)
Intra-assay	29	254.9	(4.6)	1.8
Inter-assay	11	260.1	(16.7)	6.4
Analysis of duplicates	20	13.8	(0.61)	4.4
	20	70.6	(1.84)	2.6

2.2.3. Sample handling

CSF was removed by puncture and centrifuged for 10 min. at 1,500 Xg. CSF total protein, glucose and LDH were determined immediately after centrifugation. For CSF beta-glucuronidase measurement supernatants were frozen at -20°C until they were analysed, within 1 week after the date of sampling.

2.2.4. Reagents

All chemicals used were of the highest purity obtainable and were stored according to the manufacturer's instructions. For the determination of CSF beta-glucuronidase the following solutions were used:

1. Acetate buffer solution 0.15 mol/l, pH 4.5 (37°C).
2. Acetate buffer solution 0.10 mol/l, pH 4.5 (37°C).
3. Glycine buffer solution 0.40 mol/l, pH 10.7 (25°C).
4. Phenolphthalein glucuronide solution 0.010 mol/l. This solution was prepared freshly each day, by dissolving the calculated amount of phenolphthalein-mono-beta-glucuronic acid in 10.0 ml of distilled water. Note: the water content of phenolphthalein-mono-beta-glucuronic acid may vary depending upon

storage conditions, so determination of water content by the Karl-Fisher titration is recommended.

5. Working substrate solution, 65.0 ml solution 1 was added to 10.0 ml solution 4 (prepared also freshly).
6. Phenolphthalein standard solutions: (a) stock standard (108 $\mu\text{mol/l}$); 34.4 mg phenolphthalein was dissolved in 10.0 ml ethanol 96% (v/v) and made up to 1 litre with solution 2; (b) working standard solutions (54, 27, 18, 10.8 and 4.3 $\mu\text{mol/l}$). The stock standard was diluted with solution 2 to the given concentration.

2.2.5. Procedures

CSF beta-glucuronidase 2 tubes were used for each sample to be assayed, one labeled "blank", the other "test". To the tubes labelled "test" 1.5 ml working substrate solution (5) was added and 0.5 ml CSF. To the tubes labeled "blank" 1.5 ml working substrate solution (5) was added. The tubes were sealed, incubated in a water bath at 37°C for exactly 18 h. Immediately after the incubation period 2.0 ml glycine solution (3) was added to each tube labeled "test" and 2.0 ml glycine buffer solution (3) and 0.5 ml CSF to the tubes labeled "blank". The absorbances of the "blanks" and "tests" were read against distilled water at 555 nm, and using the corrected absorbance readings ($A_{\text{test}} - A_{\text{blank}}$) the beta-glucuronidase activity was determined using the calibration curve. Each analysis was performed in duplicate.

Calibration curve 2.0 ml working standard solution (6b) was added to 2.0 ml glycine buffer solution (3).

Blank 2.0 ml acetate buffer solution (2) was added to 2.0 ml glycine buffer solution (3). After mixing the absorbances were read at 555 nm vs the blank. A calibration curve of absorbance readings was plotted against beta-glucuronidase activities. The standard solution of 54 $\mu\text{mol/l}$ corresponds with 200 mU/l.

Beta-glucuronidase activities in serum were assayed, using phenolphthalein glucuronide as substrate according to Fishman (5). Total protein and lactate dehydrogenase was determined in CSF using the test methodologies of the ACA (Dupont Company, Clinical Systems Div., Wilmington, DE, USA). The LDH methodology was calibrated to give values

similar to results obtained with the methodology of the German Society for Clinical Chemistry (6). Glucose was determined using a Beckman glucose analyser (Beckman Instruments Inc., Fullerton, CA, USA). Spectrophotometric readings for the determination of beta-glucuronidase activities were made with Zeiss DM4 spectrophotometer (Carl Zeiss, Oberkochen, FRG).

2.2.6. Statistical methods

Standard methods of statistical analysis were used to evaluate the results, including Student's t test and analysis of variance and covariance. Before analysis CSF beta-glucuronidase activities were log-transformed. The sensitivity, specificity and the receiver-operator characteristics (ROC) curve were calculated according to Griner et al (7). Normal values for CSF glucose, total protein and LDH were estimated in patient data using a Bhattacharya plot as described by Naus et al (8).

Table 3. Reference values for CSF beta-glucuronidase activities.

Control subjects	N	Reference ^a range (mU/l)	Mean (mU/l)	Ref.
Patients without CNS disease	42	8-34 ^b	17.2	9
Patients undergoing spinal anaesthesia	30	7-23	14.8	1-3
Patients with systemic cancer but without CNS metastasis	50	0-43	24.3	4
Patients without CNS disease (for details, see text)	73	9-27	16.0	This study

^a A unit of enzyme activity is expressed in terms of μmol phenolphthalein liberated/min.

^b Measuring temperature 38°C.

2.3. Results

Linearity of the assay system was evaluated by determining beta-glucuronidase activities in serial dilutions of the beta-glucuronidase preparation Sigma G-0258. A linear range up to 1.000 mU/l was found. The imprecision of the CSF beta-glucuronidase assay was evaluated by calculation of the standard deviation from duplicates and from repeated assays of a frozen preparation (-20°C) of beta-glucuronidase (Sigma G-0258) in a solution containing 1 mmol/l 1 disodium EDTA and 1 mmol/l L(+) cysteine (Table 2). Reference values for CSF beta-glucuronidase activity were estimated from the results in the control group. Results appeared to be reasonably log normally distributed. No statistically significant differences ($p < 0.10$) between beta-glucuronidase activities in controls with or without elevated CSF total protein content could be demonstrated. Therefore, all patient data from this control group were used for estimating the reference range of CSF beta-glucuronidase activities. No relationship was found between log (beta-glucuronidase activity) and age or sex. The reference range was established as 9-27 mU/l, based on mean log (beta-glucuronidase activity) ± 2 SD.

The mean CSF beta-glucuronidase level was increased in patients with leptomeningeal metastases from solid tumours (mean 89.3, range 17-318 mU/l) and hematological malignancies (mean 34.2, range 15-70 mU/l). Abnormally high values were also found in patients with bacterial meningitis. The mean value of beta-glucuronidase was slightly increased in patients with brain or epidural metastases. The remaining patients showed normal CSF beta-glucuronidase activities (Table 1).

In 15 patients with various neurological diseases beta-glucuronidase activities were measured in serum as well as CSF. No correlation could be demonstrated.

Normal values for CSF glucose, total protein and LDH were estimated from the results of routine analysis of CSF from patients admitted to the Slotervaart Hospital during the past 2 years. We found glucose 2.5-4.2 mmol/l ($n=1997$), total protein 0.14-0.51 g/l ($n=2784$) and LDH 0-26 U/l ($n=1949$).

Fig. 1

Receiver operator characteristic curves for beta-glucuronidase ———, LDH, glucose -----, and total protein oooo for patients at risk for developing leptomeningeal metastasis from solid tumours.

2.4. Discussion

The determination of beta-glucuronidase in CSF has already been described by Anlyan and Starr (9), using a method developed by Fishman et al (10). At the present, the glucuronides of p-nitrophenol, 8 hydroxyquinoline and phenolphthalein are widely used as substrate in the assay of beta-glucuronidase (5). We adapted the assay of Talalay and Fishman (11) using phenolphthalein glucuronide as substrate. Our reference range agrees rather well with those reported by other groups using phenolphthalein glucuronide as substrate (Table 3).

Our results confirm the previous findings of high CSF levels of beta-glucuronidase activities in patients suffering from bacterial meningitis or leptomeningeal metastases. A small but significant increase

in CSF beta-glucuronidase activities was observed in patients with parenchymal or epidural metastases from solid tumours.

The most impressive result of this investigation was the discriminative value of this assay in cases of leptomeningeal spread from solid tumours. In analysing the usefulness of the beta-glucuronidase assay in this respect, ROC curves of the different tests, i.e. CSF beta-glucuronidase, glucose, total protein and LDH were calculated in the group of patients at risk for developing leptomeningeal metastases from solid tumours (Fig. 1).

Table 4. Sensitivity and specificity for different chemical tests in CSF, used in a patient group at risk in developing leptomeningeal spread from lymphoma and leukemia.

Test	sensitivity	specificity
Beta-glucuronidase ^a	0.4	1.0
LDH ^a	0.8	0.9
Glucose ^b	0.6	1.0
Total protein ^a	0.7	0.6

Test criteria for a positive test:

^aresult above upper limit of normal

^bresult below lower limit of normal.

These ROC curves correlate true and false positive ratios (sensitivity and 1 minus specificity) for a series of cutoff points for the respective test. Good clinical performance of a test is characterized by a high true positive value and a low false positive value. In this way, Fig. 1 as well as the frequency distribution of test results in the patients groups "leptomeningeal dissemination from solid tumours" and "no meningeal dissemination from solid tumours" (Fig. 2), as calculated from the patient data incorporated in Table 1 show the value of the determination of CSF beta-glucuronidase activities in the diagnosis of leptomeningeal metastasis from solid tumours. At the moment, diagnosis of leptomeningeal metastasis is based on clinical signs and symptoms

suggesting neoplastic infiltration of the leptomeninges and the finding of tumour cells in the CSF (3, 13, 14). Our patients with leptomeningeal metastasis met these criteria. The diagnosis of bacterial meningitis can be confirmed by the demonstration of the micro organism involved.

Fig.2: Frequency distributions of results of different tests for patients at risk for developing leptomeningeal metastasis from solid tumours. WMD, patients without meningeal dissemination; MD, patients with meningeal dissemination.

For meningeal dissemination from tumours arising from the hemopoetic system, we could not make reliable receiver operator characteristic curves, due to the limited number of patients. Our results regarding sensitivity and specificity for this patient group are summarized in Table 4. They show the limited value of CSF beta-glucuronidase activities with respect to other tests in use, especially CSF LDH. In some cases, we were able to monitor CSF beta-glucuronidase during treatment. A representative curve is demonstrated in Fig. 3.

In conclusion, based on the data presented we recommend the determination of CSF beta-glucuronidase activities in the diagnosis of patients with leptomeningeal metastases from solid tumours. Serial measurements of beta-glucuronidase may prove valuable in assessing the efficacy of treatment. However, the number of patients represented is very small. Therefore, additional clinical trials are necessary.

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Fig.3: Time course of CSF beta-glucuronidase activities in a patient with breast cancer after diagnosis of leptomeningeal metastasis has been made and treatment started.

2.5. Summary

Beta-glucuronidase activities were determined in cerebrospinal fluid from 249 patients suffering from various neurological diseases. Reference values were established as 9-27 mU/l. Marked elevations of cerebrospinal beta-glucuronidase activities were observed in patients with bacterial and carcinomatous meningitis. Slight elevations of cerebrospinal beta-glucuronidase activities were observed in epidural and parenchymal metastases from solid tumours. Comparison was made with the determination

of total protein, glucose and lactate dehydrogenase in cerebrospinal fluid. Cerebrospinal beta-glucuronidase activity appeared the most useful test in monitoring patients at risk in developing meningeal metastases from solid primary tumours.

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Chapter 3

Cerebrospinal Fluid Beta-2-Microglobulin: a Study in Controls and Patients with Metastatic and Non-Metastatic Neurological Disease *

Albert Twijnstra, Anton P. van Zanten, Willem J. Nooyen, Augustinus A.M. Hart and Bram W. Ongerboer de Visser.

3.1. Introduction

Beta-2-microglobulin (B2-m) has been isolated and characterized from human urine by Berggård and Bearn in 1968 (1, 2). It is a small subunit of the Human Leucocyte Antigens (HLA) on the surface of nucleated cells. B2-m is dissociated from HLA heavy chain and released in free form in extracellular fluid. Lymphocytes and macrophages are particularly rich in B2-m.

B2-m is present in various biological fluids, such as serum, urine, amniotic fluid, ascites, and cerebrospinal fluid (CSF) (3-6).

From blood B2-m is removed by glomerular filtration. The serum concentration may increase in various circumstances such as in newborns, in the elderly, in renal disorders, in malignancies, in auto-immune and inflammatory disorders (7).

The multiple factors that affect B2-m concentration in the serum, make it questionable in diagnostic procedures (8). Raised serum B2-m values nevertheless are to occur reported in serum of patients with multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's disease and chronic lymphatic leukemia (9).

Measurement of CSF B2-m seems promising in the diagnosis of CNS involvement by hematological tumours (5). Recently it has been reported that CSF B2-m may increase before the CSF becomes positive for tumour cells (9, 10). This may lead to earlier and more specific diagnosis of CNS leukemia and lymphoma. As little data are available concerning: (i) CSF B2-m values in normal subjects, (ii) CSF B2-m values in metastatic and nonmetastatic diseases, (iii) the sensitivity and specificity of CSF

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B2-m, we set up this present study, to investigate the possible application of this assay. Without knowledge of the CSF B2-m from patients with neoplastic and non-neoplastic neurological diseases, CNS involvement by solid and hematological tumours cannot be diagnosed with reasonable confidence.

Other investigators reported high level CSF B2-m in bacterial meningitis (5, 10). Because this infiltration can be diagnosed by culture, bacterial meningitis was excluded from this study.

3.2. Patients and methods

We collected CSF samples from 197 patients with normal renal function. The CSF specimen were coded and stored at -20°C until analyzed by radioimmunoassay. We performed lumbar puncture as a part of clinical diagnostic procedures, and never for the measurement of B2-m only. CSF examinations included protein concentration, glucose content, LDH, culture and cytology with the cytocentrifuge technique. We compared the CSF B2-m level in 10 patient groups (Table 1). The 48 patients with low back pain with or without leg pain served as a control group. They were otherwise healthy. Myelography was normal in 36 of them and showed a lumbar disc herniation in 12 subjects. Age and sex distributions of the controls and patients are given in Table 1. Patients with malignancies were admitted to the Antoni van Leeuwenhoekhuis during 1981-1983, and patients without malignant diseases to the Department of Neurology of the Slotervaart Hospital during 1981-1982. The criteria used for the diagnosis leptomeningeal metastases was the presence of tumour cells in the CSF. In 12 patients B2-m levels were measured in serum as well as in CSF. We determined total protein and lactate dehydrogenase (LDH) in cerebrospinal fluid using the test methodologies of the ACA (Dupont Company, Clinical Systems Division, Wilmington, DE 19898, USA). LDH methodology was calibrated to give values similar to results obtained with the method of the German Society for Clinical Chemistry (11). Glucose was determined using a Beckman glucose analyzer (Beckman Instruments Inc., Fullerton, CA 92634, USA).

We measured both serum and CSF B2-m by radioimmunoassay using the Phadebas B2-m microtest (Pharmacia Diagnostics, Uppsala, Sweden). Briefly, aliquots of 200-fold diluted serum and CSF were incubated for 3 hours at room temperature with 125-I labeled B2-m and anti-B2-m

antibodies coupled to sephadex particles. Thereafter, the bound and free B2-m were separated by centrifugation. The radioactivity bound to sedimented sephadex particles was then measured. This radioactive uptake varied inversely with the quantity unlabeled B2-m present. From the results obtained in the controls, we calculated reference values for CSF B2-m (Table 1).

Table 1. CSF Beta-2-Microglobulin levels (mg/l) in groups of control subjects and patients.

Group	N	Age		Male/Female	$\bar{x}$ mg/l	SD	skewness	kurtosis
		Mean	SD					
Low back pain (controls)	48	48	16	34/14	1.33	0.48	0.77	1.17
Trauma capitis	4	38	26	3/1	1.85	1.12	1.11	-0.70
Neuropathy	6	52	18	5/1	1.56	0.55	0.98	-0.41
Cerebrovascular accident	29	71	12	17/12	1.74	0.48	-0.21	-0.28
Epidural metastases	19	58	11	1/18	1.85	0.97	1.38	1.39
Brain metastases	26	52	17	11/15	1.49	0.67	2.69	8.55
Leptomeningeal metastases solid tumours	25	53	11	6/19	3.22	1.79	0.93	0.55
Leptomeningeal metastases hematological tumours	9	49	17	7/2	4.42	2.13	0.45	-1.16
Solid tumours without CNS metastases	23	55	16	3/20	1.57	0.70	2.47	5.92
Hematological metastases without CNS metastases	8	46	22	5/2	1.43	0.86	1.61	1.37

3.2.1. Statistics

From the results of a preliminary analysis, it was decided to transform the B2-m logarithmically prior to the final analysis in order to get a closer approximation of the variation of observed values by the normal distribution in all groups. Ordinary linear regression analysis was used to determine the relationship between log B2-m and sex and age. To test the statistical distribution of values for normality the test of Shapiro and Wilk was used (11).

Skewness indicates that data distribution was not symmetrical.

Kurtosis indicates the degree of steepness of the middle part of the data distribution; it measures the concentration around the mean value.

3.3. Results

In Table 1 we show the mean and SD of CSF B2-m levels, as well as skewness and kurtosis. The skewness and kurtosis indicate measurements for the shape of the distribution. In the control group a relationship was found between CSF log B2-m and age ($p < 0.0001$) (Fig. 1). We found no difference between females and males with respect to the slope of log B2-m against age ($p = 0.45$) nor with respect to the intercept ($p = 0.91$). Because of the age dependence of log B2-m, age standardization is needed in comparing the different groups. From the regression equation in the control group we defined the following quantity as standardized log B2-m at 50 years:

$$- \log (\text{B2-m}) \text{ AS} = \log (\text{B2-m}) - 0.00595 (\text{age} - 50).$$

We found no deviation from normal in groups at a level of $p < 0.10$. Deviation from normal occurred in the patient groups with cerebrovascular accident (CVA) ($p < 0.05$), brain metastases ($p < 0.02$), and solid tumours without metastases ($p < 0.01$). We defined the reference range of B2-m standardized at the age of 50 as 0.65-2.20 mg/l.

From Table 2 it can be seen that B2-m used as the sole diagnostic tool leads to a sensitivity of 70% (95% confidence interval: 47-87%) for leptomeningeal metastases from solid tumours and of 90% (52-99%) for leptomeningeal metastases from hematological tumours. Adding all groups with leptomeningeal metastases together we would find a specificity of 93% (87-96%). Counting only the tumour group without leptomeningeal involvement would lead to a specificity of 87% (76-94%). All four patients with spinal epidural metastases and raised CSF B2-m showed a gross elevation of CSF protein and a complete block by myelography, so clinically seeding was suspected. Of the 2 patients with intraparenchymal metastases and elevation of CSF B2-m levels, the metastases were on CT-scans adjacent to leptomeningeal surface, but we did not find tumour cells in the CSF. The serum B2-m and CSF B2-m levels did not correlate in twelve patients of the different groups.

Table 2. Beta-2-Microglobulin in Cerebrospinal Fluid.
Elevated Values in Different Groups.

Group	Total	B2-microglobulin elevated	total % elevated
Control subjects	48	0	0
Trauma capitis	4	1	25
Neuropathy	6	1	17
Cerebrovascular accident	29	0	0
Epidural metastases	19	4	21
Brain metastases	26	2	8
Leptomeningeal metastases solid tumours	25	17	68
Leptomeningeal metastases hematological tumours	9	8	89
Solid tumours without CNS metastases	23	2	9
Hematological tumours without CNS metastases	8	2	25
Totals	197	37	

3.4. Discussion

In recent years malignant disease appears to be more frequently diagnosed (12-14). This increase has generally been attributed to longer survival of patients because of more effective drug treatment of the primary tumour and the failure of almost all currently used chemotherapeutic agents to cross the blood-brain barrier.

Early diagnosis of leptomeningeal metastases is a common diagnostic problem (12, 15, 16). The false negative rate of CSF cytology varies from 5 to 40% of the patients with leptomeningeal tumours. The yield of positive cytology may be enhanced by examination of successive CSF samples (6, 17). We found raised CSF B2-m in 2 autopsy proven cases with leptomeningeal tumours, with negative cytological findings in CSF. Tumour associated proteins in CSF, or so-called tumour markers, have been

described previously (6, 9, 10, 18-20), for the early identification of metastases to the leptomeninges.

From these studies insufficient data are available on patients with parenchymal and epidural metastases and non-neoplastic neurological diseases. Such information is necessary to evaluate sensitivity and specificity of the CSF B2-m assay for the diagnosis of leptomeningeal tumours (21, 22). In our control group CSF B2-m increased significantly with increasing age. This complicates the comparison of different groups of diseases. To overcome this difficulty we standardized CSF B2-m values for age using the regression equation found in the control group. We defined the reference range of B2-m standardized at the age of 50 from 0.65 to 2.20 mg/l. Other investigators described similarly reference ranges (2, 10).

Like Schaub et al, we have not observed any correlation between corresponding serum and CSF B2-m levels in a group of neoplastic patients, which suggests an autonomous production of B2-m in the two compartments (23).

Both Mavligit et al (9) and Koch et al (10) recently published results of a study about CSF B2-m in CNS metastasis. They did not mention an effect of age on CSF B2-m.

We found that B2-m as a sole diagnostic tool leads to a sensitivity of 70% for leptomeningeal carcinomatosis and of 90% for leptomeningeal dissemination of hematological tumours (21, 22).

A specificity of 93% was calculated adding all patients together with nonmalignant and malignant neurological diseases, but without malignant leptomeningeal dissemination. We detected a raised B2-m level in two patients with cerebral and four with epidural metastases. These elevations may be due to the invasion of submeningeal or subependymal parenchym, so that malignant cells may not be detected. On CT-scan we noted subependymal brain metastases in two patients.

From the present study we conclude that the determination of age standardized B2-m in CSF affords a valuable adjunct in the diagnosis for leptomeningeal tumour, more appropriate for lymphoma than for solid tumours.

Further studies will be needed to determine the validity of the test in monitoring progression of the disease and evaluating tumour response to therapy.

Fig.1: The relationship between log B2-m in CSF and age of the 48 control subjects.

Log B2-m levels increased significantly with age for the overall group ($p = 0.0004$) independent of sex difference ($p = 0.86$).

Regression equation: $\log B2-m = 3.081 - 0.00595 (\text{age} : 50)$.

3.5. Summary

We studied cerebrospinal fluid beta-2-microglobulin (CSF B2-m) in 197 patients with a variety of neurological diseases to evaluate the usefulness of B2-m in the detection of meningeal dissemination of malignancy. In the control group we found a relationship between CSF log B2-m and age ($p < 0.0001$).

Age standardized reference values were established as 0.65-2.2 mg/l. The results show that CSF B2-m was elevated in the majority of cases with leptomeningeal metastases from solid and hematological tumours.

We observed slight elevations of CSF B2-m in epidural and parenchym metastases from solid tumours. Our study shows that B2-m in CSF is a sensitive marker for leptomeningeal metastases especially from hemopoietic tumours.

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Chapter 4

Cerebrospinal Fluid Carcinoembryonic Antigen in Patients with Metastatic and Non-Metastatic Neurological Diseases *

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4.1. Introduction

Carcinoembryonic antigen (CEA), first isolated in 1965 by Gold and Freedman (1), is a beta-1-glycoprotein with a molecular weight of approximately 180,000 Daltons. It is present in normal intestinal tissues, in various biological fluids such as serum, urine, intestinal secretions, pleural and peritoneal fluids (2-4) and in the cerebrospinal fluid (CSF) (5, 6).

Raised serum levels of CEA are found particularly in patients with carcinoma of the gastrointestinal tract, breast, lung, ovaries, and pancreas (7-9), but have also been established in patients with a variety of normal malignant diseases.

The CSF CEA level is valuable in the detection of CNS metastases of CEA producing tumours especially to the leptomeninges (5, 10-12). There are few data available concerning CSF CEA values in normal subjects, CSF CEA values in metastatic and nonmetastatic diseases, the sensitivity and specificity of the CSF CEA test, and the CSF CEA relation to the CSF protein, glucose, and lactic dehydrogenase (LDH) levels. Therefore, the present study was set up to investigate further this possible application of the assay.

4.2. Patients and methods

Cerebrospinal fluid was obtained by lumbar puncture that was performed as a part of the regular clinical diagnostic procedure and never for the measurement of the CEA level alone. The CSF examinations included protein concentration and glucose content measurements, LDH, culture, and cytologic studies with the cytocentrifuge technique.

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The control group consisted of 45 patients (11 men and 34 women) with an average age of 53 years (range, 23 to 85 years). They had low back pain with or without leg pain and were otherwise healthy. Myelography showed a lumbar disc herniation in 35 subjects and no abnormalities in the remaining ten subjects.

Table 1. Distribution of patients with malignant and nonmalignant diseases

Diseases		No
Malignant (solid tumours)		96
Epidural metastases	19	
Brain metastases	28	
Leptomeningeal metastases	26	
Without central nervous system involvement	23	
Malignant (hematological tumours)		19
Epidural metastases	2	
Leptomeningeal metastases	9	
Without central nervous system metastases	8	
Nonmalignant		40
Trauma capitis	4	
Neuropathy	6	
Cerebrovascular accident	30	

The patient group consisted of 155 patients with an average age of 56.1 years (range, 16 to 92 years) (Table 1). There were 98 female and 57 male patients. The CNS metastases were diagnosed by computerized tomographic brain scans, myelography and the presence of tumour cells in the CSF. The demonstration of malignant cells in the CSF was a requisite for the diagnosis of leptomeningeal metastases. In two patients with clinical signs and symptoms of leptomeningeal involvement the CSF remained persistently clear of tumour cells. The diagnosis was confirmed at a postmortem examination. Histopathologic studies of the brain were obtained at 15 autopsies and confirmed the clinical diagnosis of brain metastases in nine and leptomeningeal metastases in six patients, respectively.

Patients with malignant neoplasms were admitted to the Antoni van Leeuwenhoekhuis during 1981-1983, and patients without malignant diseases were admitted to the Department of Neurology of the Slotervaart Hospital, Amsterdam, during 1981-1982.

Total protein and LDH level were determined in CSF using commercially available tests. The LDH measurement was calibrated to give values similar to results obtained by the method of German Society for Clinical Chemistry (13). The glucose level was determined using a commercially available glucose analysis. The CSF CEA levels were estimated by the method of Egan et al (14), with several modifications. The 4 cm, plastic incubation tubes (inside diameter 8 mm with a round bottom) contained a sample of 50 μ l of CSF and 20 μ l of goat antiserum diluted with phosphate buffered saline solution (0.07 M: pH 7.4) containing rabbit serum dilution (1:150,000).

After overnight incubation at 37°C, 20 μ l of radioactive iodine 125 I CEA solution (3 μ g/l) was added. Incubation was continued for two hours at 37°C. Separation of antibody bound CEA from unbound CEA was performed as follows: 50 μ l of normal goat serum at the appropriate dilution (with phosphate buffered saline solution containing rabbit serum), followed by 50 μ l of rabbit antigoaat serum (usually diluted with phosphate buffered saline solution containing rabbit serum) was added. The tubes were stored overnight at 4°C.

After the addition of 300 μ l of polyethylene glycol solution, centrifugation was carried out. The supernatant was carefully removed by aspiration, and the residue was counted. For the construction of standard inhibition curves, aliquots (50 μ l) of diluted CEA solutions were used.

4.3. Statistics

From the results of a preliminary analysis, we decided to transform the CEA values prior to the final analysis. Ordinary linear regression analysis was used to determine the relationship between the log CEA level and sex and age. To test the statistical distribution of values for normality, the Shapiro and Wilk test (15) was used. Skewness indicates that the data distributions are not symmetric. Kurtosis indicates the degree of steepness of the middle part of the data distributions. It measures the concentration around the mean value.

Table 2. Cerebrospinal fluid Carcinoembryonic Antigen (CEA) Levels in Groups of Control Subjects and Patients.

Group	No of subjects	CEA ng/ml		log CEA ng/ml		skewness	kurtosis	p ¹⁾
Control subjects	45	1.86	0.58	0.25	0.14	-0.32	0.02	>0.10
Trauma capitis	4	2.35	0.39	0.37	0.69	0.68	-0.94	>0.10
Neuropathy	6	1.87	0.30	0.27	0.07	-0.34	-1.14	>0.50
Cerebrovascular accident	30	1.99	0.59	0.28	0.11	0.87	1.08	>0.10
Epidural metastases*	21	2.81	2.38	0.38	0.22	1.74	3.88	<0.01
Brain metastases	28	3.92	9.06	0.38	0.28	3.97	16.20	<0.01
Leptomeningeal metastases	26	21.91	57.93	0.78	0.59	1.34	0.84	<0.01
solid tumours								
Leptomeningeal metastases	9	2.21	0.56	0.33	0.12	-0.55	-0.97	>0.10
hematological tumours								
Solid tumours without	23	2.07	0.59	0.29	0.18	-2.63	8.56	<0.01
CNS metastases								
Hematological tumours without	8	1.62	0.23	0.21	0.06	-0.65	0.33	>0.10
CNS metastases								

* Two patients had a hematological tumour

1) p value from Shapiro-Wilkes test for normality.

4.4. Results

Reference values for CSF CEA levels were calculated from the results obtained in the control subjects. No difference in the mean and distribution of CSF CEA levels were observed in control subjects with a normal total protein content (<0.51 g/l) or abnormal protein content (>0.51 g/l). Therefore, we used all data of the controls for the calculation of the reference range of CSF CEA levels.

Table 3. Control subjects and patients with elevated cerebrospinal fluid Carcinoembryonic Antigen (CEA) levels.

Group	No of subjects	No (%) with abnormal CEA*
Control subjects	45	1 (2)
Trauma capitis	4	0
Neuropathy	6	0
Cerebrovascular accident	30	1 (3)
Epidural metastases	21	3 (14)
Brain metastases	28	2 (7)
Leptomeningeal metastases solid tumours	26	11 (42)
Leptomeningeal metastases hematological tumours	9	0
Solid tumours without CNS metastases	23	0
Hematological tumours without CNS metastases	8	0

* CEA level > 4 ng/ml was considered abnormal.

We found no relationship between the CSF log CEA level and age ($p=.62$). No difference between female and male subjects was found with respect to the slope of the log CEA level against age ($p = 1.00$), nor with respect to the intercept ($p = .09$). The mean $\pm$ SD of the CEA level and the log transformation of the mean, SD, skewness, and kurtosis are given in Table 2. The skewness and kurtosis indicate measurements for the shape of the distribution.

Normal probability curves of the log CEA level for the different groups are given in the Figures (1-3). From these data an upper limit of 4 ng/ml was chosen for the normal range of CSF CEA levels. Raised levels (≥ 4 ng/ml of CEA) were detected in 18 of 200 subjects (controls and patients) (Table 3). Eleven of the 18 subjects were in the group with leptomeningeal spread from solid tumours. None of the nine patients with leptomeningeal metastases from hematological tumours had CSF CEA values of ≥ 4 ng/ml. Three patients with spinal epidural metastases and two patients with cerebral metastases had CSF CEA levels of ≥ 4 ng/ml. In 23 patients with leptomeningeal seeding, the CEA level was measured in serum as well as in CSF. No correlation could be demonstrated. The CSF CEA level correlated with LDH, glucose, and protein levels in patients with leptomeningeal metastases from solid tumours. There was also a relationship between CSF CEA and the histologic type of the primary tumour. Metastatic lung and breast cancer showed the highest CSF CEA levels.

From Table 3 it can be seen that the use of the CEA level as the sole diagnostic tool leads to a sensitivity of 31% for leptomeningeal metastases from solid and hematological tumours, for only solid tumours we found a sensitivity of 42%. Adding all groups without leptomeningeal metastases together, we found a specificity of 90%. Counting only the tumour group without leptomeningeal involvement would lead to a specificity of 91%.

Fig.1: Normal probability plots of carcinoembryonic antigen (CEA) concentration in cerebrospinal fluid: no malignant neoplasms.

Fig.2: Normal probability plots of carcinoembryonic antigen (CEA) concentration in cerebrospinal fluid: metastatic disease.

Fig.3: Normal probability plots of carcinoembryonic antigen (CEA) concentration in cerebrospinal fluid: no metastatic disease.

4.5. Discussion

Carcinoembryonic antigen is the most studied and the best known of the circulating tumour-associated antigens, which can be measured in various biological fluids by means of radioimmunoassay techniques (2, 7, 9). Contrary to previous findings (5, 6), in the CSF of most control subjects we found detectable levels of CEA using an assay obtained by the directed technique (14).

The explanation for the mode of entry of CEA into CSF from patients with leptomeningeal disease has been widely discussed in the literature (5, 6, 10-12, 14). Some authors argue that CSF CEA originates from a local production of CEA by metastatic tissue, while others suspected a leakage of plasma CEA through the blood CSF barrier (5). In the present study we found no elevated levels of CEA in the CSF of patients with cerebrovascular diseases with raised plasma CEA levels of up to 26 ng/ml, suggesting that production of CEA rather than opening the blood CSF barrier is a causative factor of abnormal CEA levels.

In some studies, cutoff levels have not been used to define abnormal levels. There is also insufficient information concerning the levels of CSF CEA in populations that included both metastatic and nonmetastatic neurological diseases, which is necessary to evaluate the sensitivity and specificity of tumour associated antigen (16).

We selected a CSF CEA cutoff point of 4 ng/ml to differentiate nonmalignant from malignant neurological diseases. Only a high cutoff point was specific of malignant CNS involvement. However, in this case the sensitivity will decrease (15). In our study, using 4.0 ng/ml as the upper limit of normal, two (2%) patients of those with nonmalignant neurological diseases and 16 (14%) of the patients with cancer with or without CNS metastases had elevated CSF CEA levels.

Our results confirm previous findings of raised CSF levels of CEA in patients suffering from leptomeningeal carcinomatosis (5, 6, 12, 17), the highest in patients with leptomeningeal metastases from lung and breast carcinoma. A small but significant increase in CSF CEA levels was observed in two patients with parenchymal and three patients with epidural metastases from solid tumours without CSF tumour cells. These elevations may have resulted from submeningeal or subependymal infiltration of tumour cells. The CSF CEA level correlated positively

with protein, glucose, and LDH levels in patients with leptomeningeal carcinomatosis.

Early diagnosis of leptomeningeal metastases is a common diagnostic problem (11, 16-19), because of false negative results of cytologic studies of CSF samples. We found raised CSF CEA levels in two autopsy-proved cases with leptomeningeal metastases from solid tumour, in which the cytology study results remained persistently negative. This finding indicates that CEA level measurement may be valuable when CSF cytology fails to confirm the presence of malignant cells.

In other work, CEA level elevations in plasma are related to the extent of metastatic disease (20). We also found the highest CSF CEA levels in our patients with leptomeningeal spread at all levels of the neuraxis.

We conclude that CSF CEA assays do possess a low sensitivity (31%) to establish the diagnosis of leptomeningeal seeding from solid tumours. However, the clinical use of CSF CEA assay should be undertaken when CSF cytological studies fail to confirm the presence of malignant cells. The CSF CEA level seems to be of limited value as a marker of leptomeningeal infiltration from lymphoma.

4.6. Summary

Cerebrospinal fluid (CSF) carcinoembryonic antigen (CEA) values were determined in 200 patients suffering from various neurological diseases. We found no relationship between CEA levels and age or sex. A positive test was defined as an upper limit of at least 4.0 ng/ml of CEA. We found raised CSF CEA levels in patients with leptomeningeal spread from carcinoma, but not in some patients with leptomeningeal metastases from lymphoma. We also found high values of CSF CEA in three of 21 patients with epidural and in two of 28 patients with cerebral metastases from solid tumours. Comparison was made with the CSF levels of total protein, glucose, and lactate dehydrogenase. The sensitivity of the CSF CEA determination in patients for the presence of leptomeningeal involvement of solid tumours is 42% and the specificity is 90%.

4.7. References

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Chapter 5.

Serial Lumbar and Ventricle Cerebrospinal Fluid Lactate Dehydrogenase Activities in Patients with Leptomeningeal Metastases from Solid and Hematological Tumours *

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5.1. Introduction

Changes of biochemical markers in tissues, body fluids and serum may be used in the diagnosis of some types of cancer (1-3). However, the clinical significance of biochemical marker tests in the cerebrospinal fluid (CSF) in the diagnosis of central nervous system (CNS) tumours is still poorly defined (4). Moreover, various CSF biomarkers were found to be elevated in a substantial number of non malignant disorders.

In this article we will discuss the change in the lactate dehydrogenase enzyme (LDH) activity in the CSF, found in patients with metastatic and non metastatic diseases of the CNS. The choice to study the CSF LDH enzyme is related to the fact that LDH belongs to routine laboratory determinations and with modern equipment, the catalytic activity of LDH in the CSF can be measured rapidly, precisely and economically.

LDH is an enzyme that catalyzes the final step in the metabolic chain of anaerobic glycolysis. Its molecular weight is 135,000 Daltons and it is predominantly located intracellular. LDH is a normal constituent of brain tissue and low activity can be measured in normal CSF. CSF LDH activity may increase in a number of conditions. Diagnostic significance has been ascribed to elevations of CSF LDH activities in cases of cerebrovascular diseases (5-7), bacterial meningitis (8-10), head injury (6), primary brain tumours (7, 11, 12) and CNS metastases from solid (7, 8, 11, 12) or hematological malignancies (8, 9). Little is known, however, about the quantitative meaning of such elevations, especially regarding the usefulness of this marker in the diagnosis of lepto-

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meningeal metastases.

To our knowledge the serial measurements of LDH in lumbar and ventricular CSF before, during and after treatment, have hitherto been unreported. In the present study we analyzed the results of CSF LDH measurements at different levels of the neuraxis in patients with metastatic and non metastatic neurological diseases to determine: (i) the sensitivity, specificity and the predictive values of the LDH level in the CSF as tumour marker test, (ii) the relationship between the pretreatment level of LDH in the CSF and the extent of the disease, and between that level and the histological type of the primary tumour, (iii) the concentrations of the LDH in lumbar CSF related to ventricular CSF simultaneously, and (iv) the correlation of serial ventricular CSF LDH changes with changes in the clinical disease status.

5.2. Patients en methods

CSF was obtained by lumbar puncture, which was performed as a part of the regular clinical diagnostical procedure, and never for the measurement of the LDH activity only. All the CSF examinations included protein concentration and glucose content, bacteriological culture, and cytological studies with the cytocentrifuge technique. The 350 patients (Table 1) with an average age of 54.4 years, range 15-92 years, were admitted to the Department of Neurology of the Slotervaart Municipal Hospital and to the Antoni van Leeuwenhoekhuis, both in Amsterdam, during 1981 to 1983. There were 198 females and 152 males. The CNS metastases were diagnosed by computerized tomography (CT) brain scans, myelography and the presence of tumour cells in the CSF. The demonstration of malignant cells in the CSF was required for the diagnosis of leptomeningeal metastasis. In two patients, with characteristic clinical manifestations of leptomeningeal metastases, repeated lumbar CSF examinations failed to demonstrate malignant cells. The diagnosis was confirmed at a postmortem examination. In two other patients CSF cytology became positive later on in the course of the disease. Histopathological studies of the brain were obtained at 15 autopsies and did confirm the clinical diagnosis of brain metastases in nine patients and leptomeningeal metastasis in six patients, respectively.

In 23 patients of the solid tumour group and in eight patients of the hematological tumour group, neither neurological examinations including

Table 1. CSF LDH levels in groups of control subjects and patients, combined positive test results according to assay for CSF LDH.

Group	No	Activity (U/l)			Significance of difference with control group (p)	Number of positive tests LDH > 26 U/l	
		$\bar{x}$	SD	range		N	%
Control subjects	110	10.2	6.3	0 - 34		3	2.7
Patient groups							
Solid tumours							
without nervous system metastasis	23	11.0	6.7	0 - 26		0	0
with brain metastasis	26	22.6	28.4	0 - 136	< 0.02	6	23.1
with epidural metastasis	17	18.8	21.2	0 - 96	< 0.02	2	11.8
with leptomeningeal metastasis	25	147.4	171.2	6 - 711	< 0.001	20	80
Hematological tumours							
without nervous system metastasis	8	13.0	10.7	4 - 36		1	11.1
with epidural metastasis	2	20.0	8.5	14 - 26		0	0
with leptomeningeal metastasis	9	122.3	98.6	6 - 292	< 0.001	7	77.8
Benign primary CNS tumours (meningioma)	7	20.0	15.0	8 - 52	< 0.05	1	14.3
Malignant primary CNS tumours (glioma grade III-IV)	11	30.8	29.9	6 - 115	< 0.001	4	36.4
Viral meningitis	10	14.6	12.0	0 - 38		1	10.0
Bacterial meningitis	9	236.0	950.3	34 - 1432	< 0.001	9	100
Cerebrovascular accident	41	31.3	33.7	2 - 150	< 0.001	13	31.7
Polynuropathy	10	18.6	7.8	0 - 35	< 0.01	2	20
Head injury	42	23.3	23.8	0 - 132	< 0.001	13	31
	34					27	55

CSF analysis, nor CT brain scans or myelograms were indicative of CNS metastases. In 26 patients with brain metastases from solid tumours, the diagnosis was confirmed by CT brain scans. In 17 patients with solid tumours and in two with hematological malignancies, myelography confirmed the diagnosis of spinal cord compression by epidural metastasis. In 34 patients with clinical evidence of leptomeningeal metastases, 25 with solid and nine with hematological tumours, this diagnosis was confirmed by positive CSF cytology or positive postmortem studies. In nine patients bacterial meningitis was demonstrated by gram stain and culture. In ten patients the diagnosis of viral meningitis was established by usual clinical and laboratory criteria such as meningeal irritation, uncomplicated clinical course, CSF mononuclear pleocytosis, negative bacterial cultures, gram stains and normal CSF glucose and CSF total protein levels. The lumbar CSF of seven patients with meningioma and 11 with malignant primary brain tumours (glioma grade III-IV) were also studied.

In 24 (Table 2) of the 34 patients with leptomeningeal metastases from solid and hematological malignancies we were able to measure the LDH in lumbar and ventricular CSF subsequently, in the same session. These patients were treated with intraventricular methotrexate (MTX) via an Ommaya reservoir (13). The CSF was withdrawn from the lumbar and ventricular spaces before treatment was initiated.

The patients with leptomeningeal metastases were divided into three anatomical categories based on their neurological signs and symptoms (Table 2): I. brain, II. cranial nerves, and III. spine. The extent of the tumour growth was indicated by different neurological signs and symptoms, belonging to one or more anatomical category. We have treated the patients with leptomeningeal metastases with intraventricular MTX or with whole brain radiation followed by intraventricular MTX (13).

The criteria: improved, stable and progression were used for tumour response. Improved was defined as the disappearance of all signs and symptoms: disappearance of malignant cells was not necessary. Stable was defined as no changes in neurological signs and symptoms during therapy.

Progression was defined as an increase of neurological signs and symptoms or the appearance of new lesions. The duration of the response was determined from the onset of the treatment to relapse.

Table 2. CSF data from all patients with leptomeningeal metastases from solid and hematological tumours.

Patient number	Sex	Age	histology	neurological evaluation**	protein concentration (g/l)	glucose level (mmol/l)	CSF evaluation tumour cells	pretreatment		
								WBCs/cumm	lumbar LDH level (U/l)	ventricular LDH level (U/l)
1	F	71	lymphoma	I + II + III	0.59	1.2	+	186	20	8
2	M	35	lymphoma	I	0.33	3.3	+	300	6	6
3	F	70	lymphoma	I + II	2.60	2.2	+	1532	152	80
4	M	40	lymphoma	I + II + III	5.30	2.1	+	133	292	43
5	M	34	lymphoma	I + II	2.30	1.4	+	210	179	30
6	M	51	lymphoma	I + II + III	4.50	0.4	+	1515	228	48
7	M	20	lymphoma	I + II + III	0.79	4.9	+	700	44	16
8	M	62	lymphoma	I + II + III	0.34	3.8	+	675	62	42
9	M	52	lymphoma	I	2.00	2.0	+	80	118	-
10	M	31	melanoma	I + II + III	4.00	0.2	+	61	184	-
11	M	64	small cell lung carcinoma	III	1.05	3.1	+	4	32	-
12	F	49	melanoma	I + II	1.80	2.2	+	80	40	2
13	F	33	breast carcinoma	I	0.77	0.7	+	42	118	46
14	F	62	breast carcinoma	I + II + III	5.10	0.9	+	10	276	86
15	F	53	breast carcinoma	I + II + III	8.80	1.7	+	20	532	-
16	F	48	breast carcinoma	I + II + III	5.90	2.5	+	22	138	4
17	F	71	breast carcinoma	I	0.56	3.0	+	1	42	-
18	F	46	breast carcinoma	I	0.41	3.8	+	13	26	16
19	F	63	breast carcinoma	I + II + III	2.10	0.5	+	7	346	2
20	F	56	breast carcinoma	III	0.77	1.7	+	3	42	-
21	F	59	breast carcinoma	III	2.80	4.2	+	24	88	16
22	F	50	breast carcinoma	I + II + III	1.50	3.3	+	27	98	14
23	F	43	breast carcinoma	I + II + III	4.40	3.3	+	3	482	94
24	F	72	breast carcinoma	I	0.20	3.5	+	3	20	12
25	F	59	breast carcinoma	I + II + III	1.32	1.8	+	22	174	12
26	F	53	non-small cell lung carcinoma	I + II + III	0.16	4.1	+	38	6	-
27	F	55	small cell lung carcinoma	I + II + III	1.58	2.3	+	20	142	29
28	F	47	breast carcinoma	I	2.11	0.5	+	15	70	-
29	M	40	melanoma	I	2.91	2.7	+	12	80	-
30	M	52	teratoma	III	2.19	2.6	+	87	90	8
31	F	50	head & neck cancer	I + II + III	4.20	0.3	+	5	711	8
32	F	50	breast carcinoma	I	1.05	0.6	+	125	511	273
33	M	60	small cell lung carcinoma	I	0.63	5.6	+	-	44	-
34	F	45	non-small cell lung carcinoma	I	0.60	2.9	+	6	26	20

* Autopsy studies revealed leptomeningeal metastases

§ CSF cytology became positive later on

** Neurological evaluation:

I = cerebral signs and symptoms

II = cranial nerve signs and symptoms

III = spinal signs and symptoms

LDH levels were determined in CSF using commercially available tests (14). The test methodology of the ACA (Dupont Company, Clinical Systems, Wilmington DE, USA) is a modification of the enzymatic lactate to pyruvate procedure modified by Gay, McComb and Bowers (15). As the method described by the Deutsche Gesellschaft für Klinische Chemie (14) in our laboratory is considered to be the reference method, the ACA method was calibrated to give results comparable with this method.

Fig.1: CSF log (LDH) in 110 control subjects ranging in age from 23-81 years. Log LDH levels increased significantly with age for the group aged 70 years and older ($p < 0.002$).

5.3. Statistics

From the results of preliminary analysis, it was decided to transform the LDH logarithmically prior to the final statistical analysis in order to get a closer approximation of the variation of observed values by the

normal distribution in all groups. Ordinary linear regression analysis was used to determine the relationship between the log LDH level and sex and age. To test the statistical distribution of values for normality, the Shapiro and Wilk test was used. Standard methods of statistical analyses were used to evaluate the results, including Student's t test and analysis of variance and covariance. The sensitivity and specificity were calculated according to Griner et al. (16). Normal values were estimated from patient data using a Bhattacharya plot as described by Naus et al. (17).

Table 3. Sensitivity, Specificity and Predictive Value Examinations for Leptomeningeal Metastases.

		<u>Disease</u>			
		<u>Present</u>	<u>Absent</u>		
Test	Positive	27	55	82	Positive test = $\frac{27}{82} = 33\%$
	Negative	$\frac{7}{34}$	$\frac{261}{316}$	$\frac{268}{351}$	Negative test = $\frac{261}{268} = 97\%$
Sensitivity = $\frac{27}{34} = 79\%$					Specificity = $\frac{261}{316} = 83\%$

5.4. Results

The data obtained from controls and patients are summarized in Tables 1 and 2. Reference values for lumbar CSF LDH were calculated from results obtained in routine CSF analyses during a period of 34 months. Based upon 1949 patients we found a reference range from 0-26 U/l (18). The mean CSF LDH levels of the control subjects were 10.2 U/l with a standard deviation of 0.63 units. In this group statistical analyses were carried out to detect relationships between CSF LDH activities and age or sex. From ordinary linear regression analyses we found a non linear relationship between CSF log LDH and age (p quadratic 0.036) (figure 1). Inspection of these figures suggests a difference between CSF LDH and the age groups of <70 years and ≥ 70 years. In subjects aged 70 years and older we measured no CSF LDH less than 5 U/l. This estimated difference

between the two age groups was not related to sex ($p = 0.94$). We found no relationship between females and males and CSF log LDH levels ($p = 0.067$), and we calculated a deviation from normal in the control subjects at a level of $p < 0.01$. The upper normal limit for CSF LDH activity was established as 26 U/l. In the controls the CSF LDH was elevated in three cases. Table 1 lists the positive results in patients with metastases and primary CNS tumours and non malignant neurological diseases according to assay for CSF LDH. The catalytical activity of total LDH was elevated in 27 of the 34 patients with leptomeningeal metastases from solid and hematological tumours, but also in 55 of the 317 patients without leptomeningeal metastases. On the basis of these data, the sensitivity of the test for leptomeningeal metastases was 79%, and the specificity 83%. The predictive values of positive and negative LDH tests were 33% and 97%, respectively (Table 3).

We simultaneously measured the LDH activities in lumbar and ventricular CSF (Table 2) and found a mean concentration of LDH in the lumbar CSF of 177.88 U/l and in the ventricular CSF of 38.13 U/l. The calculated mean lumbar LDH activity was approximately five times greater than the ventricular CSF LDH activity.

Table 4. Relationship between the lumbar and ventricular CSF LDH and the anatomical site of the leptomeningeal metastases.

Neuroanatomical site neurological evaluation	number of patients	mean LDH lumbar CSF	mean LDH ventricular CSF
I	11	102.29	48.5
I + II	3		
III	4	63.00	12.0
I + II + III	16	233.44	26.92

In Table 4 we show the relationship between lumbar and ventricular CSF LDH and the different area of the neuraxis involved in the leptomeningeal disease. Greater tumour size (including the anatomical area I + II + III) correlated with higher levels of the CSF LDH at the lumbar space. There was no association with the ventricular CSF level. When only the area of the spine is clinically involved (III), the lumbar CSF LDH concentration

was greater than the ventricular CSF LDH concentration ($p < 0.03$). In clinical involvement of the leptomeninges of the brain and cranial nerves (I + II) the ventricular CSF LDH level was higher than the ventricular CSF, taken from patients with spinal leptomeningeal metastases (area III).

The CSF LDH activities in several types of primary tumours are summarized in Table 5. There was no relationship between the CSF LDH level and the histology of the primary tumours.

Table 6 gives the relationship of serial LDH levels in the ventricular CSF to the pretreatment value and clinical response to therapy. A good correlation between decreasing LDH levels and response to chemotherapy was suggested. CSF LDH levels returned to normal values in 11 (69%). In five patients with decreased CSF LDH levels we found a progression of the clinical signs and symptoms. The CSF LDH increased in three of the 11 non-responders. In five unchanged CSF LDH levels there was a progression of the neurological disease in three cases.

Table 5. Relationship between the lumbar LDH CSF level and the histological type of the primary tumour in patients with leptomeningeal metastases

primary tumour	No	LDH			
		elevated		mean	range
breast	15	12/15	80%	192.87	20-582
non-Hodgkin's lymphoma	9	7/9	78%	122.33	6-292
lung	5	3/5	60%	50.00	6-142
melanoma	3	3/3	100%	101.00	40-184
various	2	2/2	100%	405.50	90-711
reference range		0-26 U/l			
mean $\pm$ SD					

5.5. Discussion

Leptomeningeal metastasis has been reported with an increasing frequency, especially in breast and lung cancer and lymphoma (19-23), probably because of better control of early systemic disease and accompanying prolongation of survival by combination chemotherapy (24).

Table 6. Relation of serial ventricular CSF lactate dehydrogenase (LDH) change with criteria of response to therapy in patients with leptomeningeal metastases.

relation with pretreatment LDH	number of patients	correlation with clinical disease status		
		improved	stable	progression
ventricular LDH level decreased	16	11	0	5
unchanged	5	1	1	3
increased	3	0	0	3
	24	12	1	11

The diagnosis of leptomeningeal metastasis is based upon the demonstration of malignant cells in the CSF. However, the incidence of positive CSF cytology findings in the first sample may only be 40-60% (25) and in approximately 4-5% of patients even repeated lumbar CSF examinations fails to demonstrate malignant cells. Because of negative cytological findings, the diagnosis may be delayed. Early diagnosis, however, is particularly important since there is evidence that early treatment may improve results of treatment (1, 13).

The application of tumour markers has been discussed for the diagnosis leptomeningeal metastasis (4, 26-28). We have previously reported on the usefulness of the biochemical CSF markers B-glucuronidase, B2-microglobulin and carcinoembryonic antigen (29-31) in the detection of leptomeningeal metastasis. Reports concerning the presence of LDH in the CSF of various neurological diseases, including primary and metastatic CNS tumours, have been published since 1956 (6, 9, 11, 33). However, those studies are not uniform. Methodologies of measurements do not always meet with the optimal recommendations of to-day (34, 35).

More recent studies (36, 37) only dealt with CSF LDH activities of affected patients and did not incorporate an adequate control group. For a proper diagnostic test control subjects should reflect the group of patients with a high probability of having the disease in question. In patients with leptomeningeal metastases the differentiation must be made

from cerebral metastases (38-40) and several types of infectious meningitis. In patients with spinal symptoms caused by leptomeningeal involvement from malignancies the differentiation must be made from bone and epidural metastases and non malignant conditions such as polyneuropathy, root lesions caused by herniated intervertebral disc and the Guillain Barré syndrome.

Galen and Gambino (41) have published criteria for the evaluation of clinical laboratory tests used for diagnostic purposes. This model may only be applied when sufficient data are available for the calculations of sensitivity, specificity and predictive value of the test.

In the previous reports, the reference populations used to calculate the limits, were not always free of disease. Moreover, few authors adjusted the normal range for the factors that might influence the test results other than disease. Normal values of many variables are affected by the age of the subjects. Although the earliest studies of CSF LDH did not separate normal values by age (6, 42), later studies (43, 45) showed, that normal values were age related. Hain et al. (44) reported a linear increase of LDH with age. This increase was linear to the age of 70 and then it remained constant through the age of 90 years. Spolter et al. published a study of age related LDH activities and found a CSF LDH increase with increasing age of patients. We found, however, a non linear relationship between CSF LDH activities and age. This relationship was based upon the absence of CSF LDH activities < 5 U/l in the patients aged 70 years and older. In the patients aged 70 years and older the CSF LDH increased significantly ($p = 0.002$). Because our group consisted of three patients over 70 years of age only, we did not standardize for age.

We found elevated CSF LDH levels in leptomeningeal disease from solid and hematological malignancies and in malignant primary brain tumours. The number of patients with leptomeningeal metastases, who had negative cytologic results, have been evaluated. We found two patients with persistently negative cytology but highly elevated LDH activities. The diagnosis leptomeningeal metastasis was confirmed at autopsy. In non malignant conditions the CSF LDH was elevated in cerebrovascular accidents, in head injuries and in bacterial meningitis. Except the patients with bacterial meningitis, the degree of elevation of CSF LDH activity in leptomeningeal metastases was usually more than five times

the non malignant conditions. These data indicate that an elevated CSF LDH level of approximately five times the mean activity of the control group is highly related to the diagnosis leptomeningeal metastasis.

The differentiation of leptomeningeal metastasis and bacterial meningitis might be made by the CSF white blood cell counts and culture. The CSF white blood cell counts in leptomeningeal metastases from solid tumours are usually less than 100 per cubic millimeter, while in bacterial meningitis they are greater than 100, usually in the range of 1000 to 10,000 per cubic millimeter (46).

We found that the lumbar CSF LDH concentration was about five times greater than ventricular CSF LDH concentration. These differences in lumbar and ventricular CSF LDH at these different levels of the neuraxis have not previously been described. A possible explanation might be a higher concentration of LDH in the CSF in proximity to the leptomeningeal site of involvement.

Virtually no data are available regarding changes in CSF LDH activity as a function of clinical status or treatment in patients with leptomeningeal metastases. Efficacy of treatment can be evaluated by clinical parameters and following the presence of tumour cells in the CSF. Frequently the clinical evaluation may be difficult especially if neurological improvement cannot be expected to occur due to irreversible damage to the nervous tissue. In these patients tumour response will be assessed by following CSF parameters including tumour cells. However, CSF levels of protein, glucose and malignant cells can differ at different anatomical levels of the neuraxis in patients with leptomeningeal metastases. This means that ventricular CSF may be negative while at the same time lumbar CSF contains tumour cells. We therefore analyzed the relationship between the serial ventricular CSF LDH in patients with leptomeningeal metastases and the results of treatment. Our analysis shows a moderate correlation between serial ventricular CSF LDH level changes and positive response to therapy or progression of disease.

This study indicates that the LDH assay of cerebrospinal fluid can be used as an adjunctive diagnostic test for leptomeningeal metastases if the clinical condition is carefully evaluated and appropriate caution is exercised in interpretation. Furthermore, serial ventricular CSF LDH measurements may be useful for monitoring the response to therapy.

More studies are needed to evaluate the use of multiple sequential tumour markers in the CSF, in monitoring patients with leptomeningeal metastases from solid and hematological tumours.

5.6. Summary

Lactate dehydrogenase activities were measured in cerebrospinal fluid in 350 patients with various neurological diseases to establish the sensitivity and specificity of the CSF LDH as a marker for the diagnosis of leptomeningeal metastases. Slight elevations of CSF LDH were observed in nonmalignant diseases, while marked elevations were observed in a considerable number of patients with bacterial meningitis. We calculated a sensitivity of 79% and a specificity of 83%. In the 34 patients with leptomeningeal metastases from solid and hematological tumours, we measured the LDH in lumbar and ventricular CSF simultaneously. The lumbar CSF LDH concentration in patients with leptomeningeal metastases was about 5 times greater than that in the ventricular CSF. No relationship was found between the CSF LDH and histology of the primary tumour. A good correlation was demonstrated between the lumbar CSF LDH and the effected area of the neuraxis. Serial determinations of CSF LDH showed a relationship between level changes and responses to therapy or progression. The findings of this study indicate that measurement of LDH in CSF can be used as an adjunctive diagnostic test for leptomeningeal metastases and in monitoring the efficacy of treatment.

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Chapter 6

Sensitivity and Specificity of Single and Combined Tumour Markers in the Diagnosis of Leptomeningeal Metastasis from Breast Cancer *

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6.1. Introduction

In recent years the diagnostic incidence of leptomeningeal metastases from breast carcinoma has steadily increased (1, 2, 10, 15). This has been attributed to longer survivals achieved with more effective treatment and to better neurological diagnostic procedures. Cytologic evaluation of the cerebrospinal fluid (CSF) is the principle modality to confirm the diagnosis of leptomeningeal metastases. With the cytocentrifuge technique, however, cytology will only be positive in 60% of the patients and in 4% there is a false positive result (3). This has greatly contributed to the inability to make an early diagnosis of leptomeningeal metastases in breast cancer (3-9).

In previous studies we measured the levels of CSF B-glucuronidase (B-gluc.), B2-microglobulin (B2-m), carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH) in CSF of patients with a variety of metastatic and nonmetastatic diseases (11-13).

The most important points to consider in the evaluation of a tumour marker are the specificity and sensitivity of the various tests. By specificity is meant the ability of the test to discriminate those individuals who do not have the disease. Sensitivity refers to the ability of the test to detect all patients with the disease.

CSF B-gluc. appeared to be an accurate marker for leptomeningeal metastases from solid tumours and B2-m the most useful for the detection of leptomeningeal spread from hematological tumours (11, 12). CSF CEA assay possesses a low sensitivity but a high specificity in establishing the diagnosis of leptomeningeal metastasis from solid tumours (13).

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We also found that CSF LDH was a useful marker for leptomeningeal metastases, however, in contrast to the other tests the CSF LDH was elevated in various nonmetastatic diseases (11).

The purpose of the present study was to determine whether in patients with breast cancer a combination of B-gluc., B2-m, CEA and LDH measurements is more effective than the use of one of these single tests in the detection of leptomeningeal metastasis.

Table 1. Positive test results in breast cancer patients with and without CNS metastases, according to assay for B-gluc., B2-m, CEA and LDH.

Group	No of Patients	No of positive tests			
		B-gluc.	B2-m	CEA	LDH
Breast cancer with CNS metastases	41	16	13	12	17
leptomeningeal	15	13	9	9	14
spinal	14	1	3	3	1
parenchymatous	12	2	1	0	2
Breast cancer without CNS metastases	16	0	1	0	0
neuropathy/plexopathy	8	0	1	0	0
lumbar disc disease	4	0	0	0	0
headache	3	0	0	0	0
cerebrovascular accident	1	0	0	0	0
reference range $\pm$ 2 SD		9-27 mU/l	0.65-2.2 mg/l	0.8-4 ng/ml	0-26 U/l

6.2. Patients and methods

CSF levels of B-glucuronidase, B2-m, CEA and LDH were measured in 57 woman with breast cancer and neurological disorders referred to the Antoni van Leeuwenhoekhuis during 1981-1983. Their ages ranged from 30 to 78 years (mean, 57 years). In all patients diagnostic procedures included: CT brain scan, bacterial culture and cytologic examinations of CSF.

They were divided into two groups (Table 1). Group I comprised 41 patients with CNS metastases, of whom 15 had leptomeningeal metastases confirmed by positive CSF cytology in 13 and by postmortem findings in two. Group II comprised 16 patients with neurological disorders, but without demonstrable metastases to the central nervous system (CNS).

6.2.1. Samples

CSF samples were centrifuged for cytological examinations and immediately analysed for LDH. The additional CSF samples were coded and stored at -20°C until analyses. Lumbar puncture was performed as a part of clinical diagnostic procedures, and never for the measurement of the markers only.

6.2.2. Assay methods

CSF B-gluc. was measured as described earlier (11), normal values ranged from 9-27 mU/l (Table 1). CSF B2-m was measured by radioimmunoassay using the Phadebas B2Micro Test (Pharmacia Diagnostics, Uppsala, Sweden). B2-m increased significantly ($p < 0.0001$) with increasing age (12). No relationship was found for sex. Normal values standardized for age ranged from 0.65 to 2.20 ng/l (Table 1). CSF CEA was measured by the method of Egan et al (19) with several modifications (13, 16); the upper limit of normal is 4 ng per millilitre (Table 1). For B-gluc. and CEA no relationships were found between test results and age or sex (11, 13).

LDH determinations were performed using a Dupont ACA, calibrated to give values corresponding with the method of the German Society for Clinical Chemistry (18).

Our test results related to age ($p = 0.036$) and not to sex (11). The upper limit of normal is less than 26 U/l (Table 1).

6.2.3. Statistics

The analysis in this paper depends upon data from previous studies to estimate test sensitivity and specificity.

The receiver operator curve is a graph, which has been constructed to correlate true- and false-positive rates for a series of cutoff points for four biochemical marker tests. This graph is used to decide the optimum cutoff point according to the purpose of the test. When two or

more tests are available to pursue a diagnostic consideration, the comparison of the receiver operator characteristic (ROC) of each will often show, where one test has an advantage over the other test.

For B2-m and LDH values an unequal deviation was found, so that results were analysed using normal quadratic analysis (17, 18). The sensitivity, specificity and the ROC curves were calculated according to Griner et al (14). Galen and Gambino published criteria for evaluating markers in diagnosis and screening (16). The reference ranges for the four markers in normal CSF were based on mean $\pm$ 2 SD (Table 1) (11-13).

Table 2. True and false positive rates for four markers of leptomeningeal metastases from breast cancer.

Test	cutoff value	sensitivity (true pos.rate)	1- specificity (false positive rate)
B-glucuronidase (mU/l)	27	87	7
B2-microglobulin (mg/l)	2.2	60	12
CEA (ng/ml)	4	60	7
LDH (U/l)	26	93	7

6.3. Results

Table 1 shows the positive results in groups of subjects with and without breast cancer for B-gluc., B2-m, CEA and LDH.

True-positive i.e. sensitivity and false-positive rates for the four tumour markers using the upper limit of normal as cutoff point are presented in Table 2. The sensitivities and specificities were established for the diagnosis leptomeningeal metastasis. B-gluc., CEA and LDH were found to be relatively specific giving a false positive rate of 7%. CEA and B2-m had a similar sensitivity but CEA was slightly more specific than B2-m; the false positive rates are 7 and 12%, respectively. Both LDH and B-gluc. were the most sensitive markers. LDH gave a true-positive rate of 93% and B-gluc. of 87% taking the mean plus standard deviation as the upper limit of normal as cutoff point. LDH showed a higher true-positive rate than the other tests, but it is quite difficult to compare these tests directly.

We are only able to do this under comparable conditions of the four tests. Therefore, we changed the decision level for each test to give

specificities (true-negative rates) of 93% and 100% respectively (Table 3). We then compared the tests on the basis of the resulting true-positive rates; LDH was more sensitive than the other tumour markers at a specificity rate of 93%. At a specificity of 100%, however, B-gluc. is considerably more sensitive than LDH.

Table 3. Test characteristics for leptomeningeal metastases from breast cancer.

Test	specificity 93%		specificity 100%	
	cutoff value	sensitivity	cutoff value	sensitivity
B-glucuronidase (mU/l)	28	87	39	87
B2-microglobulin (AS) (mg/l)	2.9	47	4.4	13
CEA (ng/ml)	4.05	60	12.05	33
LDH (U/l)	24	93	96	47

If we chose decision levels needed to give sensitivity rates of 93% or 100% respectively (Table 4), then we found that LDH was more specific than each of the other CSF markers. Table 5 shows data concerning the operating characteristics of B-gluc. and LDH when used alone or in combination. The combined tests are most helpful when both markers are abnormal, the combined specificity is then higher, but the sensitivity is lower. In Figure 1 we constructed a graph that correlates all true- and false-positive rates for a series of cutoff points for different tests, i.a. B-gluc., B2-m, CEA and LDH. Such a graph is known as the receiver operating curve (ROC). The curve can be used to decide the optimum cutoff point according to the purpose of the test. A comparison of the ROC of each test will often show where one test has an advantage over the other test.

Table 4. Test characteristics for leptomeningeal metastases from breast carcinoma.

Test	sensitivity 93%		sensitivity 100%	
	cutoff value	specificity	cutoff value	specificity
B-glucuronidase (mU/l)	19	69	17	45
B2-microglobulin (AS) (mg/l)	1.16	38	1.00	14
CEA (ng/ml)	2.05	72	2	26
LDH (U/l)	24	93	23	78

Table 5. Operating characteristics of combination testing.

Test	sensitivity	specificity
	%	
B-glucuronidase (A)	87	93
LDH (B)	93	93
A or B positive	99	86
A and B positive	81	99

6.4. Discussion

A number of CSF markers have been suggested as potential tumour markers for leptomeningeal metastases in solid tumours (3, 4, 11-13). For interpretation of these laboratory tests, sensitivity, and specificity calculations are necessary.

Our results regarding sensitivity and specificity for this patient group are summarized in table 2. It shows the limited diagnostic value of CSF CEA and B2-m with respect to other tests. We standardized the four tests by choosing cutoff points with which all tests had the same true-positive rates, the tests could then be directly compared with regard to their true-negative rates. When we fixed the specificity for the four tests at 93%, then B-gluc. and LDH showed sensitivity rates from 87% and 93% respectively. However, when choosing decision levels needed to give a 100% specificity, then LDH was markedly less sensitive (47%) than B-gluc. (Table 3).

A sensitivity of 13% at 100% specificity for B2-m makes B2-m of little value as a marker for leptomeningeal metastases from breast carcinoma. Its usefulness was not altered by the combination with the other tests. An elevated CSF CEA value had a 100% specificity, but the ability of this test to detect meningeal spread from breast carcinoma (1, 2, 10) is low (sensitivity level 32%) and was unaltered by combination of the test. The ROC curves represented in the figure correlate true- and false-positive rates (sensitivity and 1- minus specificity) for a series of cutoff points for the respective test. Good clinical performance for a test is characterized by a high true-positive rate and a low false-positive rate.

B-gluc. was the only marker with a high sensitivity at a specificity level of 100%. LDH on the other hand, appeared to have in this patient

group a higher sensitivity when the specificity was fixed at 93%, but it had a markedly reduced sensitivity of 47% at 100% specificity. To our knowledge no other study provided statistical data of the usefulness to combine CSF markers. In patients at risk for leptomeningeal metastases, using the combination of the markers with the highest sensitivity and specificity i.e. B-gluc. and LDH respectively, we found an increase in specificity from 93 to 99% at a sensitivity of only 81% (Table 5).

In conclusion, when taking into account the factors of sensitivity and specificity in evaluating the four markers, the combined tests of CSF B-gluc. and LDH seem to be the most reliable markers for the detection of leptomeningeal metastases in patients with breast cancer.

Fig.1: A receiver operator characteristic for B-glucuronidase ———, B2-m ooooo, CEA and LDH **** for patients with leptomeningeal metastases from breast carcinoma.

6.5. Summary

We assessed the clinical efficacy of four laboratory tests in detecting leptomeningeal metastases in 57 patients with breast carcinoma. The sensitivity and specificity of B-glucuronidase (B-gluc.), B2-microglobulin (B2-m), carcinoembryonic antigen (CEA) and lactate dehydrogenase (LDH) in cerebrospinal fluid were determined. As a single test B-gluc. was the most sensitive (93%) and specific (93%) for discriminating between leptomeningeal metastases and other CNS metastases from breast cancer. LDH was the next most useful marker. Both B2-m and CEA had a sensitivity of 60%. More specific results were achieved by combining B-glucuronidase and LDH. CSF B-glucuronidase may be useful by itself and in combination with LDH in the detection of leptomeningeal metastases from breast carcinoma.

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Chapter 7.

Serial Lumbar and Ventricular Cerebrospinal Fluid Biochemical Marker Measurements in Patients with Leptomeningeal Metastases from Solid and Hematological Tumours *

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7.1. Introduction

In previous studies (1-4), we measured the levels of B-glucuronidase (B-gluc.), B2-microglobulin (B2-m), carcinoembryonic antigen (CEA), and lactate dehydrogenase (LDH) in the cerebrospinal fluid (CSF) of 197 patients with disorders of the central nervous system (CNS). We concluded that, of these CSF biochemical markers, B-gluc. and LDH were the most accurate markers of CNS metastases, especially in the detection of leptomeningeal metastases from solid tumours. The B2-m in CSF was found to be the most accurate in the detection of leptomeningeal metastases from hematological tumours. The diagnostic approach that was used could prove to be helpful to patients with leptomeningeal metastases.

The most effective treatment to date for patients with leptomeningeal metastases from solid or hematological tumours consists of intrathecal administration of methotrexate (MTX) (5). The method of choice is injection of the drug directly into the ventricular system via an Ommaya reservoir (5, 6).

A routine examination of the protein, glucose, and tumour cells within CSF is valuable in the follow-up of patients with leptomeningeal metastases. However, the composition of CSF can differ markedly between different CSF sources, such as the Ommaya reservoir or lumbar space (7), even in the absence of an extradural block. It has therefore been suggested that the response to treatment by serial examination of CSF obtained from the sites with the most marked pretreatment abnormality be monitored (7, 8). The now routine clinical use of intraventricular

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Table 1. CLINICAL DATA FROM 28 PATIENTS WITH LEPTOMENINGEAL METASTASES FROM SOLID AND HEMATOLOGICAL TUMOURS WHO WERE FOLLOWED LONGITUDINALLY WITH SERIAL MEASUREMENTS OF BIOCHEMICAL MARKERS IN CSF.

CSF Pretreatment Marker Assays													
Number	Sex/Age	Histology	B-glucuronidase		B2-microglobulin		CEA		LDH		Treatment	Response	
			Lumbar	Ventricular	Lumbar	Ventricular	Lumbar	Ventricular	Lumbar	Ventricular			
1	F/68	NHL	20	10	2.5	0.7	2.8	1.6	20	8	MTX	progressive	
2	M/35	NHL	20	10	2.9	1.3	1.3	1.8	6	6	MTX	progressive	
3	M/70	NHL	56	33	3.2	1.4	3.0	2.2	152	80	MTX	improved	
4	M/49	NHL	56	11	2.8	0.9	2.3	2.5	292	43	MTX	progressive	
5	M/33	NHL	70	23	2.0	1.8	2.6	2.6	179	30	MTX	progressive	
6	M/51	NHL	20	8	7.5	4.9	2.4	1.8	228	48	MTX	progressive	
7	M/20	NHL	15	6	4.5	2.7	1.8	1.7	78	22	MTX+RT	progressive	
8	M/61	NHL	30	14	7.6	1.6	1.5	1.6	62	42	MTX	progressive	
9	M/64	SCLC	36	20	3.5	1.7	6.0	4.2	42	32	MTX+RT	progressive	
10	F/49	Melanoma	41	12	2.3	1.0	2.2	1.9	40	2	MTX	progressive	
11	F/33		Breast carcinoma	54	24	2.2	0.9	0.3	2.8	118	46	MTX	progressive
12	F/62		Breast carcinoma	61	32	4.2	0.8	3.8	2.0	267	86	MTX+RT	progressive
13	F/48		Breast carcinoma	219	14	0.9	0.3	4.2	2.4	138	4	MTX	improved
14	F/46		Breast carcinoma	18	11	1.1	0.9	2.7	3.9	26	16	MTX+RT	improved
15	F/63		Breast carcinoma	195	23	4.0	1.9	3.1	4.6	246	2	MTX	progressive
16	F/56		Breast carcinoma	108	17	2.0	0.6	17.5	2.3	88	16	MTX	improved
17	F/50		Breast carcinoma	40	8	1.8	0.8	3.2	2.0	98	14	MTX	improved
18	F/41		Breast carcinoma	150	65	3.9	1.5	39.0	14.7	482	94	MTX	improved
19	F/70		Breast carcinoma	18	23	1.8	2.1	2.2	3.0	12	24	MTX	improved
20	F/69	Breast carcinoma	71	11	3.1	2.1	7.0	2.4	174	12	MTX	stable	
21	M/54	SCLC	96	36	1.7	0.9	2.0	2.0	142	29	MTX+RT	progressive	
22	F/45	Non-SCLC	49	21	1.5	1.3	46.5	37.5	26	20	MTX	improved	
23	M/53	SCLC	17	8	3.9	0.9	2.0	1.8	20	8	MTX	progressive	
24	F/51	Breast carcinoma	157	21	6.9	0.9	2.5	1.8	721	8	MTX	progressive	
25	F/50	Breast carcinoma	111	51	3.7	2.5	295.0	38.0	500	273	MTX	improved	
26	F/56	Breast carcinoma	164	35	2.5	1.1	4.5	4.6	240	60	MTX	progressive	
27	M/54	SCLC	137	25	2.4	1.9	18.0	20.0	425	47	MTX	progressive	
28	F/63	Non-SCLC	57	22	3.4	1.2	68.0	34.0	182	51	MTX	stable	

Normal value: B-glucuronidase 0-27 U/l B2-microglobulin 0.65-2.2 mg/l CEA 0.8-4.0 ng/ml LDH 0-26 U/l (lumbar CSF)

SCLC = Small Cell Lung Carcinoma

NSCLC = Non-Small Cell Lung Carcinoma

NHL = Non-Hodgkin's Lymphoma

administration of chemotherapy via the Ommaya reservoir has made ventricular CSF more readily available for analysis. In this way, efficacy of treatment may be monitored by serial examination of ventricular CSF. Although numerous biochemical markers have been associated with leptomeningeal disease (9-11), as yet, none has proven to be an adequate marker with clinical utility in monitoring this disease. We, therefore, studied the CSF levels of various potential biochemical markers in relation to treatment. To improve the utility of biochemical markers in the clinical setting, we investigated: (i) marker levels in ventricular and lumbar CSF simultaneously, (ii) the correlation of marker levels with histology of the primary tumour, (iii) the correlation of marker levels with the most symptomatic area along the neuraxis, (iv) the correlation of marker levels with CSF cytology in the ventricular and lumbar spaces, and (v) the correlation of marker levels with the disease status.

Attention was also directed to the potential influence of prophylactic chemotherapy on the level of the markers in the CSF examined.

In the current study, we utilized all of these approaches by simultaneous analysis of four biochemical markers in patients with leptomeningeal metastases from solid and hematological tumours.

7.2. Patients and methods

Between January 1981 and December 1983, 28 consecutive, unselected patients with leptomeningeal metastases from solid and hematological tumours, who had been referred to the Antoni van Leeuwenhoekhuis for treatment, had at least two assays of one of the following biomarkers: B-gluc., B2-m, CEA, and LDH. When clinically possible, serial assays from ventricular and lumbar CSF were performed just prior to each treatment, at intervals of one day or longer. Concurrently, other routine biochemical, cytological, and clinical reassessments of tumour activity were performed. The ages of the patients with leptomeningeal metastases ranged from 20 to 70 years (patients with lymphoma were between 20 and 70, and those with carcinoma between 37 and 70). All of the patients were treated for their leptomeningeal disease with intraventricular MTX, five patients in combination with cranial radiotherapy. The median length of survival was three months and the range, 1 to 10 months; no patients are still being treated at this time. The diagnosis of leptomeningeal

Table 2. BIOCHEMICAL MARKER ASSAYS RESULTS.

	B-glucuronidase		B2-microglobulin		CEA		LDH	
	Lumbar	Ventricular	Lumbar	Ventricular	Lumbar	Ventricular	Lumbar	Ventricular
Number of patients	28	28	28	28	28	28	28	28
Total number of assays	72	127	73	134	73	137	74	139
Average number of assays/patients	2.57	4.54	2.61	4.79	2.62	4.89	2.64	4.96
Number (%) of patients with ≥ 2 assays	22 (79)	25 (89)	20 (71)	26 (93)	20 (71)	27 (96)	20 (71)	27 (96)

metastasis was made on the basis of clinical signs and symptoms, and was confirmed by CSF cytology or autopsy findings. The initial staging work-up included, in some patients, computerized tomography (CT) scanning and myelography. The diagnosis of primary tumours was: breast carcinoma (13 cases), lymphoma (eight cases), lung carcinoma (six cases), and melanoma (one case). There was no other documented reason for any of the patients to have had abnormal biochemical marker levels. (Bacterial meningitis was ruled out by culture).

The criteria "improved", "stable", and "progressive" were used for tumour response. "Improved" signified the disappearance of all signs and symptoms (although not necessarily the disappearance of malignant cells from CSF). "Stable" meant that there had been no changes in neurological signs and symptoms during therapy. "Progressive" indicated an increase of neurological signs and symptoms or the appearance of new lesions. The duration of the response was defined as the period of time from the onset of treatment to relapse.

The same tumour markers were also measured in CSF from nine patients, five of whom had acute leukemia and four, a non-Hodgkin's lymphoma. These patients ranged in age from 16 to 59 years. All had been treated with prophylactic central nervous system intrathecal MTX.

7.2.1. Cerebrospinal fluid sampling

To obtain ventricular CSF, the Ommaya reservoir was entered with a 23-gauge butterfly needle; 3 ml cerebrospinal fluid which had passed through the reservoir and tubing, was withdrawn and discarded. An additional 7 ml was then removed and set aside for assay. Thus, no more than 10 ml of cerebrospinal fluid was removed from the ventricle for purposes of sampling. One hour after the Ommaya sampling, a total of 10 ml of CSF was also removed from the lumbar region. Both CSF samples were centrifuged for cytological examinations and immediately analysed for LDH. Additional samples were coded and stored at -20°C for future analysis. Ventricular and lumbar punctures were performed as a part of clinical, diagnostic, and treatment procedures, and never for the measurement of the markers alone.

7.2.2. Assay methods

Total protein and LDH levels in CSF were determined using commercially available tests (i.e. the testing methodologies of the ACA, Dupont Company, Clinical System Division, Wilmington, DE. 19898, U.S.A.). The LDH measurement was calibrated to give values similar to the results obtained by the method used by the German Society for Clinical Chemistry (12). There was a nonlinear relationship between CSF, LDH, and age over 70 years. LDH increased significantly ($p < 0.001$) among patients 70 years and older (4). Since few cases were older than 70 years, normal values were not standardized for age. Glucose level was determined enzymatically by using commercially available glucose analysis systems. The levels of CEA in CSF were estimated by the method used by Egan et al. with several modifications (13), and the upper normal limit was 4 ng per milliliter. B-gluc. in CSF was measured as described earlier (1). B2-m in CSF was measured by radioimmunoassay, using the Phadebas B2-microtest (Pharmacia Diagnostics, Uppsala, Sweden), and it was found to increase significantly ($p < 0.001$) with age (2). No relationship was between B2-m level and gender. Normal values were standardized for age. The respective normal values for these tumour markers in our laboratories were: B-gluc.: 9-27 mU/l; B2-m: 0.65-2.2 mg/l; CEA: 0.8-4.0 ng/ml; and LDH: 0-26 U/l of CSF obtained by lumbar puncture (1-4).

Table 3. PRETREATMENT BIOMARKER ASSAYS IN LEPTOMENINGEAL METASTASES*.

	B-glucuronidase	B2-m	CEA	LDH
Breast Carcinoma	11/13 (85)	7/13 (54)	6/13 (46)	11/13 (85)
Small Cell Lung Carcinoma (SCLC)	3/4 (75)	3/4 (75)	2/4 (50)	3/4 (75)
Non-Small Cell Lung Carcinoma (NSCLC)	2/2 (100)	1/2 (50)	2/2 (100)	1/2 (50)
Non-Hodgkin's Lymphoma (NHL)	4/8 (50)	7/8 (88)	0/8 (0)	6/8 (75)

* Values = No elevated/total tested. The percentage is given between parentheses.

7.2.3. Statistical methods

Standard methods of statistical analysis were used to evaluate the results. One-way analysis of variance was performed on biochemical marker levels grouped by response category. The same analysis was also performed to determine the relationship between markers and the major symptomatic area and cytology of the CSF.

7.3. Results

The clinical characteristics of the patients with leptomeningeal metastases are shown in Table 1. We found no correlation between normal pretreatment levels of the four biochemical markers and response to chemotherapy.

The total number of assays performed for each biochemical marker in the lumbar and ventricular CSF, and the number of patients from whom these assays were drawn, are shown in Table 2. The number of patients having two or more assays of a given marker are indicated separately. For each marker, more than 70% of the patients had two or more assays.

Table 4. BIOCHEMICAL MARKER LUMBAR/VENTRICULAR CSF RATIOS IN LEPTOMENINGEAL METASTASES.

	Lumbar/Ventricular Ratio			
	mean	median	SE	range
B-glucuronidase	3.7	2.5	0.58	0.78-15.6
B2-microglobulin	2.5	2.3	0.3	0.86- 6.6
Carcinoembryonic Antigen	1.7	1.2	0.3	0.11- 7.8
Lactate Dehydrogenase	12.9	8.8	5.2	50-123

The proportion of patients with increased levels of biochemical markers is shown in Table 3. Eleven of the thirteen patients with breast carcinoma had pretreatment levels moderately elevated over the control value; all but two had elevated B-gluc. and LDH. Of the eight NHL patients, none had elevated pretreatment CEA levels, and the pretreatment elevations of B-gluc. were, in general, slight. In all lung carcinoma patients (SCLC and NSCLC), an increase in B2-m coincided with an increase in B-gluc. or LDH, or both. The mean, median, standard error, and range of the lumbar:ventricular CSF ratio are shown in Table 4. Almost all ratios were greater than 1, except the CSF:CEA ratio, which had nine values less than 1, indicating that CEA was relatively more often elevated in the ventricular CSF than it was in the lumbar CSF. The site

of involvement was correlated with the pretreatment marker concentrations in lumbar and ventricular CSF, respectively (see Table 5).

The CSF:LDH ratio showed a significant correlation with the site of CNS involvement (i.e., at the spinal roots; $p < 0.03$). For the other markers, no correlation was found.

Studying pretreatment levels of B-gluc., B2-m, CEA, and LDH in CSF with respect to the different histologic types, we found that patients with non-small cell lung carcinoma (NSCLC) had highly significant elevations of CEA in lumbar CSF ($p < 0.01$) (see Table 6). We did not find other significant differences among the various histological types. The small numbers, however, preclude definitive conclusions.

During treatment, the disappearance of tumour cells from the lumbar and ventricular CSF was correlated with the four marker concentrations in the lumbar and ventricular CSF. CSF cytology, during and after treatment, was described as one of the following: I. positive cytology in both lumbar and ventricular CSF, II. negative lumbar and positive ventricular cytology, III. positive lumbar and negative ventricular cytology, or IV. negative cytology in both lumbar and ventricular CSF. The results are shown in Table 7. Group II contained only one patient and, therefore, was not evaluated. In each of the remaining groups, tumour cells and marker levels were determined simultaneously. Statistically significant correlations were found only for the LDH value in ventricular CSF ($p < 0.01$). No correlation was found between the presence or absence of malignant cells and levels of LDH in lumbar CSF or of B-gluc., B2-m, or CEA in lumbar and ventricular CSF.

Table 8 shows the means, standard errors, and numbers of patients for the respective biochemical markers which were divided into the three disease activities and pretreatment categories. A steady increase in the numerical values of means with increasing disease activity is apparent for ventricular B2-m (using Kruskal-Wallis correlations of statistical significance; $p < 0.08$). In addition, ventricular LDH showed a significant positive correlation with decreasing disease ($p < 0.05$). For all other markers, there was no correlation.

Clinical diagnoses of the nine prophylactically treated patients with acute leukemia and lymphoma but without CNS disease are shown in Table 9. All of these patients received, in addition to systemic chemotherapy, CNS prophylaxis with intrathecal MTX. The CSF was examined just before CNS

Table 5. CORRELATION BETWEEN BIOCHEMICAL MARKER CONCENTRATION AND SITE OF DISEASE.

Site of Disease	Lumbar / Ventricular Ratio									
	B-glucuronidase		B2-microglobulin		CEA		LDH		SE	
	mean	SE	mean	SE	mean	SE	mean	SE		
I brain and cranial nerves	10	3.6	1.4	2.0	0.2	1.8	0.7	7.8	3.5	
II spinal roots	6	5.4	1.1	2.9	0.8	2.2	1.1	38.7 *	21.7	
III brain, cranial nerves and roots	12	3.0	0.5	2.8	0.4	1.5	0.2	4.7	3.6	

* p < 0.03

Table 6. LUMBAR AND VENTRICULAR CSF BIOCHEMICAL MARKER LEVELS IN PATIENTS WITH LEPTOMENINGEAL METASTASES FROM SOLID AND HEMATOLOGICAL TUMOURS.

	B-glucuronidase						82-microglobulin						Carcinoembryonic Antigen						Lactate Dehydrogenase					
	Lumbar			Ventricular			Lumbar			Ventricular			Lumbar			Ventricular			Lumbar			Ventricular		
	mean	SE	range	mean	SE	range	mean	SE	range	mean	SE	range	mean	SE	range	mean	SE	range	mean	SE	range	mean	SE	range
1 Breast Carcinoma	103.5	18.4	18-219	26.2	5.0	8-65	2.3	0.4	0.9-5.9	1.3	0.2	0.3-2.5	6.5	2.8	1.6-38	2.3	2.2	0.3-2.2	238.4	57.7	12-711	50.4	20.4	2-273
2 Small Cell Lung Carcinoma	71.5	27.6	17-137	22.2	5.8	8-36	2.9	0.5	0.7-3.9	1.4	0.3	0.9-1.9	7.0	4.4	1.8-20	0.7	0.3	0.2-1.8	154.7	94.2	20-425	25.0	8.5	8-47
3 Non-Small Cell Lung Carcinoma	53.0	4.0	47-57	21.5	1.5	21-22	2.5	1.0	1.5-3.4	1.3	0.05	1.2-1.3	36.0*	1.8	34-38	5.7	0.8	4.6-6.8	104	78	26-182	35.5	15.5	20-51
4 Non-Hodgkin's Lymphoma	35.9	7.6	15-70	14.4	3.2	6-33	2.1	0.8	2.0-7.6	2.0	0.5	1.7-4.9	2.0	0.1	1.6-2.6	2.2	0.2	1.3-3.0	125.9	36.8	6-292	34.8	8.5	6-80

* The CEA level in lumbar CSF is significantly elevated in Non-Small Cell Lung Carcinoma (<0.01)

Table 7. CORRELATION OF CYTOLOGIC RESULTS WITH BIOCHEMICAL MARKER CONCENTRATIONS.

CSF Cytology	B-glucuronidase				B2-microglobulin				CEA				LDH			
	Lumbar		Ventricular		Lumbar		Ventricular		Lumbar		Ventricular		Lumbar		Ventricular	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
I Lumbar + Ventricular +	62.1	9.8	24.5	4.0	2.6	0.2	1.5	0.2	20.4	9.1	8.9	3.3	171.1	34.4	51.3	14.9
§ II Lumbar - Ventricular +	(1)				(1)				(1)				(1)			
III Lumbar + Ventricular -	62.9	16.8	16.5	3.5	2.9	0.4	1.5	0.2	(10)	6.6	4.2	1.5	(11)	76.8	13.9	4.8
IV Lumbar - Ventricular -	(17)				(12)				(13)				(13)			
	58.6	14.0	17.1	1.8	2.6	0.4	1.0	0.1	4.6	1.5	3.5	1.2	130.8	54.9	19.0	6.8

*Figures in parentheses represent number of patients.

+ =presence of malignant cells

- =absence of malignant cells

§ group II only consists of one patient.

Table 8. CSF BIOCHEMICAL MARKER RESULTS BY DISEASE ACTIVITY.

	Pretreatment		Progressive Disease		Stable Disease		Improved Disease	
	Lumbar	Ventricular	Lumbar	Ventricular	Lumbar	Ventricular	Lumbar	Ventricular
B-gluc. (mU/l)								
Number of patients	28	28	9	6	17	13	17	23
Mean	73.8	21.2	61.4	29.0	64.6	14.9	43.6	17.0
SE	14.9	2.6	12.4	4.9	14.6	3.1	12.3	2.7
B2-m (mg/l)								
Number of patients	28	28	5	8	7	13	15	23
Mean	3.2	1.5	2.0	9.7*	1.3	3.1	0.5	0.9
SE	0.3	0.2	7.4	4.2	3.6	1.5	2.8	1.8
CEA (ng/ml)								
Number of patients	28	28	5	8	7	13	15	24
Mean	19.5	7.2	23.5	5.9	29.4	14.3	190.3	9.0
SE	10.6	2.1	2.3	2.7	13.3	23.0	186.2	19.8
LDH (U/l)								
Number of patients	28	28	5	8	7	13	17	24
Mean	177.6	39.5	133.6	52.6	114.1	17.2	103.3	10.2**
SE	137.81	9.9	47.2	13.2	24.7	8.3	23.5	9.6

*p < 0.08

** p < 0.05

Table 9. CSF BIOCHEMICAL MARKERS DURING CHEMOTHERAPY PROPHYLAXIS IN PATIENTS WITH HEMATOLOGIC TUMOURS.

Primary tumour histology *	Age	Number of samples	Mean protein + range (g/l)	Mean glucose + range (mmol/l)	Mean B-gluc. + range (mg/l)	Mean CEA + range (ng/ml)	Mean LDH + range (U/l)	CSF cytology
NHL	41	7	0.67 (0.42-0.90)	2.9 (2.6-3.4)	24 (20-28)	1.6 (1.4-2.0)	19 (15-21)	-
ALL	23	3	0.41 (0.25-0.50)	2.7 (2.6-2.9)	13 (10-16)	2.2 (2.0-2.7)	18 (17-20)	-
AML	45	3	0.44 (0.32-0.60)	2.8 (2.1-3.8)	16 (12-18)	2.2 (0.6-4.5)	32 (20-45)	-
AML	50	6	0.45 (0.25-0.60)	3.5 (2.8-4.2)	23 (18-28)	1.8 (1.4-2.1)	42 (25-91)	-
NHL	37	2	0.58 (0.56-0.60)	2.9 (2.4-3.4)	18 (16-20)	1.7 (1.2-2.2)	36 (32-40)	-
ALL	59	2	0.55 (0.50-0.60)	4.0 (3.3-4.7)	14 (12-16)	0.5 (0.3-0.7)	16 (12-20)	-
ALL	18	2	0.25 (0.20-0.30)	4.0 (3.9-4.1)	6 (2-10)	1.6 (0.3-2.9)	46 (30-62)	-
NHL	16	2	0.26 (0.22-0.30)	3.8 (3.1-5.5)	15 (12-18)	1.8 (1.6-2.0)	28 (20-36)	-
NHL	32	2	0.62 (0.61-0.63)	2.7 (2.3-3.1)	27 (21-33)	0.9 (0.8-1.0)	20 (18-22)	-

*NHL = Non-Hodgkin's Lymphoma

ALL = Acute Lymphoblastic Leukemia

AML = Acute Myeloid Leukemia

prophylaxe and at least one time during the treatment with intrathecal MTX. These results are also shown in Table 9. The glucose level in the CSF of these patients was normal, but protein content rose during CNS chemotherapy. In some patients the LDH activities during CNS treatment were higher than normal. None of the patients had CNS symptoms or signs during treatment. Our results show that prophylactic CNS chemotherapy does not conclusively produce biochemical changes of the CSF.

7.4. Discussion

There have been a number of studies showing lumbar CSF measurements of biochemical markers as an aid in the diagnosis of leptomeningeal metastasis (9-11). These markers, however, have less often been measured in ventricular CSF. Relatively few studies (10, 11, 14) in leptomeningeal metastases have focussed specifically on the value of biochemical markers in CSF in monitoring and predicting the progression of disease. Those that have generally indicated that biochemical marker levels vary in accordance with changes in disease status; yet, little data has been analysed to indicate correlations between ventricular and lumbar CSF marker levels. To our knowledge, no studies in leptomeningeal metastasis have evaluated the role of multiple, sequential biochemical markers in monitoring the efficacy of chemotherapy.

Intraventricular administration of chemotherapeutic agents via the Ommaya reservoir is now the treatment of choice. Routine use of this device has made ventricular CSF readily available for analysis. However, it is difficult to evaluate treatment for patients with leptomeningeal metastases because standard criteria for response are lacking. Efficacy of treatment is determined by clinical parameters and with serial CSF examination. Yet, clinical response to treatment cannot always be obtained, since irreversible damage to nervous tissue cannot be corrected by anti-tumour therapy. Therefore, ventricular CSF is also used to assess response of CSF composition after treatment. Other points that should be considered during monitoring are false negative (15) and false positive cytologies in CSF. One such example is when brain tissue is transversed by a catheter (Ommaya device). Ventricular CSF can become contaminated by neurons or glia cells and these cells can be mistaken for malignant cells (16).

The composition of ventricular CSF can differ dramatically from that of lumbar CSF. It is known that a CSF protein gradient exists (7). Normal ventricular protein levels in adults range from 0.06 to 0.15 g/l, as compared with normal values of 0.20 to 0.50 g/l in lumbar CSF. This difference in protein concentrations has not been adequately explained. In addition, other CSF constituents, such as electrolytes, show no concentration gradients in normal subjects. Differences in CSF composition make monitoring the efficacy of chemotherapy by routine CSF examinations unreliable.

Schold et al. (11) reported that the levels of B-gluc. and CEA in ventricular CSF were always lower than those in lumbar CSF, with ratios ranging from 0 to 0.5 and from 0.2 to 0.5, respectively. Our results are consistent with these findings. To date, in the literature, CSF ratios for B2-m and LDH have not been described; nor is the explanation for the marked variation in CSF from various regions along the neuraxis (ventricular:lumbar) clear. Obstructions to the flow of CSF were ruled out by myelograms. No relationship to age or specific diagnosis was apparent. However, the extent of active leptomeningeal involvement of the CNS may have been important. A review of our own data reveals that most of the marker levels in CSF obtained near the major symptomatic area, were no higher than those obtained from other more distant sites. Only the level of LDH in the lumbar CSF was significantly higher when the spinal roots were involved. This means that when using the concentration of LDH in CSF to monitor disease activity, it is best to obtain CSF from the site along the neuraxis in which the most marked abnormality is detected.

Also analysed in our study was the relationship between malignant cells in the different spaces and the marker levels. Only the LDH level in ventricular CSF showed a positive correlation with the CSF cytology. Although it has yet to be proven that CSF marker level elevation precedes other symptoms of CNS disease (cell shedding, neurological signs), it seems logical that marker levels could, indeed, precede the actual shedding of cells, which has been revealed through cytological study, the most effective test to date.

Drugs may very well influence the production, release, or elimination of the biochemical markers in CSF. Therefore, we also analysed B-gluc., B2-m, CEA, and LDH in the lumbar CSF of patients with acute

leukemia or lymphoma who received systemic and intrathecal MTX as prophylaxis. In some patients only LDH showed slight to marked elevations. Thus, the validity of the test in monitoring progression of disease was not affected by intrathecal MTX.

As stated earlier, relatively few studies have focussed specifically on the value of serial biochemical marker levels in monitoring the progression of disease. Yet, a number of those which have indicated that B-gluc. and CEA levels vary in accordance with changes in disease status (10, 11).

In our study, the B2-m and the LDH levels in ventricular CSF exhibited a positive relation with disease status. Such was not the case with any of the other markers. We agree with Mavligit et al. (14) that the B2-m level in CSF in monitoring treatment for patients with leptomeningeal metastases; however, he neglected to mention the source of the CSF B2-m.

7.5. Conclusion

It appears that only the serial measurements of changes in the levels of B2-m and, LDH in ventricular CSF are valuable in the follow-up of patients with leptomeningeal metastases. Our findings indicate that routine measurements of B2-m in ventricular CSF may be reliable in monitoring the efficacy of chemotherapy and are independent of the most abnormal site, histology of the primary tumours, and CSF cytology. This study has also shown that the levels of these markers may vary in accordance with changes in disease status. The serial measurements of B2-m and LDH in ventricular CSF may be of use in indicating the need for a change in therapy; however, prospective studies are needed to substantiate this hypothesis.

7.6. Summary

The serial biochemical markers B-glucuronidase (B-gluc.), B2-microglobulin (B2-m), carcinoembryonic antigen (CEA), and lactate dehydrogenase (LDH) were measured in lumbar and ventricular cerebrospinal fluid (CSF) in 28 patients with leptomeningeal metastases from solid and hematological tumours. All of the patients were treated with methotrexate (MTX); some also received adjunctive cranial radiotherapy. The level of at least one pretreatment biochemical marker was elevated. Response rates were similar in patients with normal and abnormal pretreatment levels.

The lumbar and ventricular marker levels were measured simultaneously. CSF levels of the four markers differed markedly at different sites along the neuraxis. Only LDH had a significant correlation with specific metastatic site ($p < 0.03$). Pretreatment CEA correlated well with the histology of the primary tumour for Non-Small Cell Lung Carcinoma ($p < 0.01$). LDH level in ventricular CSF was significantly higher in CSF with positive cytology.

Increasing levels of B2-m ventricular CSF correlated with progressive disease ($p < 0.08$), and ventricular LDH showed a significant positive correlation with decreasing disease ($p < 0.05$).

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Chapter 8

Diagnosis of Leptomeningeal Metastasis

8.1. Introduction

This chapter consists of two parts. In the first part we present the diagnostic procedures for detection of leptomeningeal metastasis with particular emphasis on those advances that have increased the accuracy of the diagnosis.

In the second part of this chapter we analyze the usefulness of several tests in the diagnosis of leptomeningeal metastasis from solid and hematological tumours. The patients reported here were referred to the Antoni van Leeuwenhoekhuis Hospital, Amsterdam, and to the Department of Neurology at the University Hospital, Maastricht, from January 1981 to September 1985. We accepted those patients who had either the typical clinical findings (i.e. neurological dysfunction at multiple levels of neuraxis without radiographic evidence of either brain or epidural spinal metastases) or conclusive laboratory evidence supporting the diagnosis, i.e. malignant cells in the CSF.

Early diagnosis of leptomeningeal metastasis is particularly important because early treatment with intralumbar or intraventricular chemotherapy with or without radiotherapy may lengthen and improve the quality of survival in such patients (1).

8.2. Definition of leptomeningeal metastasis

Leptomeningeal metastasis is a form of metastasis of systemic cancer with focal, multifocal, or diffuse infiltration of the leptomeninges by cancer cells.

8.3. Pathogenesis of leptomeningeal metastasis

Tumour cells may infiltrate the subarachnoid or ventricular cavity by various routes. It is thought that in the majority of cases tumour cells reach the leptomeninges via the blood stream. The cells pass the endothelium of leptomeningeal vessels and enter into the CSF. This mechanism has been demonstrated in leukemia (2). In addition, this

hematogenous entry into the leptomeninges is suggested by the presence of tumour cells in and around superficial cortical vessels (3).

Meningeal infiltration from cerebral metastases to the leptomeninges is rare (4) because the fibrous reaction provoked by the invading tumour prevents free dissemination of tumour cells into the CSF. Subependymal metastases may, however, reach the ventricular surface because there is no fibrous scarring of the subependymal tissue (5).

Choroid plexus metastases (6, 7) form an important source of dissemination of tumour cells to the leptomeninges. The direct spread of a primary tumour in proximity of the CNS is a rarity because it is thought to be unusual for tumour cells to invade the dura (5, 8-10).

The spread from more distant foci along perineural lymphatic vessels is controversial (11, 12). The infiltration of the leptomeninges via vertebral or paravertebral routes may occur in some cases, but is probably rare (7, 13).

8.4. Pathology

Tumours which have seeded the leptomeninges may grow in a linear pattern, creating a thin layer of cells spread diffusely over the brain and spinal surfaces or leptomeningeal tumour may assume a nodular growth pattern involving the leptomeninges in a multifocal pattern with intervening tumour free areas.

In most cases there is a slight leptomeningeal opacity over the sulci which is often more marked on the basal surface of the brain than on the convexity, particularly the chiasmatic cistern, the interpenduncular fossa and the cerebellopontine angles. Sometimes the infiltration may be confined to the spinal subarachnoid space (7, 11).

Microscopically, tumour cells may be found in most parts of the subarachnoid and perivascular spaces. The leptomeninges of the brain, the cranial and spinal nerves may be invaded by tumour cells or may be surrounded by a single layer of cells or a thick cuff of tumour tissue. Invasion of the parenchyma of the nerve has been noticed in rare instances. The infiltration by tumour cells may also extend into the perivascular spaces of the perforating blood vessels.

8.5. Clinical incidence and features

The exact incidence of leptomeningeal metastasis is unknown, but the disease has been more frequently recognized in recent years, because of increased awareness, improved laboratory techniques and because of longer survival with combination chemotherapy.

The various series reported in the literature are not strictly comparable, as some include hematological tumours and even gliomas, while others confine themselves to carcinoma (7, 11, 14). Tumours of the lung and breast and leukemias are the most frequently reported primary site (1). Approximately 30% of patients who die from systemic cancer have evidence of involvement of the central nervous system (CNS) at the time of autopsy, and an estimated two-thirds to three-fourths have experienced symptoms of their CNS lesions (1).

As longer life spans are achieved in various specific types of leukemia, lymphoma and carcinoma by means of improved techniques of treatment, CNS relapse is becoming an increasingly common problem (11, 15, 16).

In breast cancer and in small cell carcinoma of the lung the frequency is stated to be from 4% to 27% (17-21). The diagnosis of leptomeningeal metastasis from solid and hematological tumours is often difficult to establish because of a multitude of indefinite signs and symptoms (7). A definite diagnosis of leptomeningeal metastasis during life is based upon the demonstration of malignant cells in CSF. Several lumbar punctures may be necessary before tumour cells can be demonstrated and commonly one has to rely on indirect evidence (pleocytosis, elevated protein, low glucose and elevated intracranial pressure) in conjunction with clinical findings.

Leptomeningeal metastasis may occur in patients with longstanding cancer after many years of apparently good health. On the other hand, the illness may be the first manifestation of cancer. The age of the patients mostly reflects the age-incidence of cancer. Myelography and computerized tomography have occasionally been helpful in establishing the diagnosis (26, 27).

Neurological signs and symptoms may be minimal and at times concealed by polyneuropathy due to chemotherapy (1). In patients with the intracranial form of leptomeningeal metastasis the differentiation must be made from cerebral metastasis (22-24), specific infections and

ordinary forms of meningitis (7, 23, 26), i.e. neurosyphilis, sarcoidosis and tuberculosis which are also important to consider in patients unknown to harbour a tumour (25). An aneurysm may be suspected when a patient presents with headache and ophthalmoplegia. In patients with spinal symptoms the differentiation must be made from bony and epidural metastases and nonmalignant conditions such as herniated intervertebral disc.

The neurological symptoms and signs may be divided into three anatomic levels: I. brain, II. cranial nerve and III. spinal nerve roots (1, 11, 12, 28). Patients may have symptoms from one anatomic site only, while others have complaints referable to lesions in two or in all anatomical sites.

Onset of brain signs is often preceded by a period of vague complaints such as dizziness, mental disorders, disturbed consciousness, nausea and apparently unimportant headache or backache. Intracranial symptoms are caused by meningeal irritation, hydrocephalus due to CSF passage disturbances and parenchymatous involvement.

The most frequent symptoms and signs referable to cranial nerve palsies, are diplopia, facial numbness, facial palsy and weakness and loss of hearing.

The spinal form of leptomeningeal metastasis often presents with back pain, symptoms including weakness, usually affecting the legs, bladder and bowel dysfunction and radicular pain as their leading complaints. The referred symptoms and signs develop owing to compression and invasion of the cranial nerve spinal root and cauda equina (11, 12).

8.6. Laboratory findings

8.6.1. Cytopathology of cerebrospinal fluid

Cytologic examination of CSF is the major tool utilized in the diagnosis of patients with leptomeningeal metastasis. The diagnostic criterion of the disease is the presence of tumour cells in the CSF and all patients suspected of leptomeningeal metastases have to undergo a lumbar puncture for CSF analysis.

Four techniques are in use for the preparation of the CSF cell smears:

(i) Simple centrifugation and coverslip smears of the sediment. This

method, however, does not yield high quality cytological preparations (29).

- (ii) The Sayk technique; in the sedimentation chamber developed by Sayk, sediment cells are under gentle pressure while liquid is removed by paper filtration (18).
- (iii) The cytocentrifuge technique has become one of the most widely used methods of handling several CSF samples simultaneously. Cell morphology, however, may be altered by centrifugation (30).
- (iv) The membrane filtration technique is a rapid method providing the best quantitative yield of cells but the cell morphology is not as well preserved as with the Sayk's sedimentation technique (31-33).

About sixty percent of the patients with leptomeningeal metastases have positive cytological findings. False positive results occur when inflammatory cells are mistakenly held for tumour cells or due to the contamination with peripheral blood or bone marrow from leukemic patients (34-36). Ventricular samples in which brain tissue is transversed by needles or catheters may also be contaminated with neuron or glia cells, which can be mistaken for malignant cells (34, 35). This problem can be overcome by repeated cytological examinations. The false negative cytologies are more common when tumour cells are masked by reactive leukocytosis. The yield of CSF positive findings is generally lower in cases of primary brain tumour (15% to 30%), than in brain metastases (20% to 50%) (37). Autopsy studies, correlated with CSF findings, showed that cells are rarely shed into the CSF by a deep parenchymal tumour. These results imply that the presence of tumour cells rather frequently indicates the invasion of the leptomeninges by a tumour (15).

8.6.2. The role of CSF biochemical markers in central nervous system (CNS) metastases

In chapter one we have extensively discussed the role of the biochemical markers in the CSF. With respect to leptomeningeal metastases, four potential markers in cerebrospinal fluid (CSF) have been studied, namely: B-glucuronidase (B-gluc.), B2-microglobulin (B2-m), carcinoembryonic antigen (CEA) and lactic dehydrogenase (LDH) (38-43).

8.6.3. The role of monoclonal antibodies in CSF cytology

The diagnosis of leptomeningeal metastases by routine CSF cytological staining methods is frequently unsatisfactory and reported rates of detection of malignant cells vary between 40% and 60% of all cases. This percentage may rise after multiple spinal taps (1, 33). In the literature, studies are reported on efforts to determine whether diagnostic accuracy in CSF examinations can be improved by performing monoclonal antibody immunocytology (44-46). Coakham et al. found that an important diagnostic contribution can be made by monoclonal reagents in CSF cytology. A diagnostic monoclonal antibody battery, which included markers for neural, epithelial and lymphoid differentiation antigens, was used in the detection of tumour cells in CSF. The monoclonal reagents were found to be of great value in the detection and characterization of neoplastic cells in CSF from patients with leptomeningeal metastases. Applying monoclonal immunocytology, the detection of malignant cells increased from 73% to 95% and the characterization of neoplastic cells from 16% to 95%. These findings suggest that patients with leptomeningeal metastases may be spared prolonged investigation and inappropriate management by using batteries of monoclonal antibodies.

8.6.4. Cytogenetic studies of cells in the CSF

Reports of cytogenetic studies of cells in the CSF are very scarce. However, chromosome analysis of peritoneal and pleural fluid has been recognized as a useful tool in the diagnosis of neoplasia (47).

Baughman and Hirsch (48) first reported chromosome abnormalities in a post-mortem study of CSF of a patient known with malignant glioma.

Mastrangelo et al. (49) demonstrated that chromosome analysis of CSF cells may be more accurate than cell cytology in the diagnosis of metastatic meningeal involvement of acute lymphoblastic leukemia. This technique has also been used as a diagnostic procedure in patients with leptomeningeal metastases from solid tumours where CSF cells with clearly abnormal karyotype were found (50). Sometimes chromosome analysis of CSF may be more accurate than cell cytology in the diagnosis of leptomeningeal metastasis, particularly in cases in which leptomeningeal metastases is the first clinical manifestation of malignancy.

8.7. Radiological studies

8.7.1. Computerized tomography of leptomeningeal metastasis

Computer tomography may be helpful in establishing the diagnosis of leptomeningeal metastasis. The characteristic CT-scan observations in leptomeningeal metastases have been described in 1976 (26, 51, 52). They include gyral enhancement, without edema, sulcal and basilar cisternal obliterations and ependymal-subependymal enhancement. Ventricular dilatation was a non-specific finding due to either communicative or non-communicative obstruction.

8.7.2. Myelography in patients with leptomeningeal metastases

Wasserstrom et al. (1) studied the role of myelography in patients suspected of leptomeningeal metastases. He performed myelography on a total of 49 patients with leptomeningeal metastases secondary to a variety of solid tumours. Twenty-two myelograms were normal, 14 showed spinal epidural metastatic lesions, whereas only 13 (26%) suggested leptomeningeal metastases. Pederson et al. (27) studied the use of myelography in diagnosing leptomeningeal metastases secondary to small cell carcinoma of the lung. Myelograms were suggestive of leptomeningeal metastasis in nine out of 20 patients.

The myelography is useful and complements CSF cytological examination in the evaluation of patients suspected of leptomeningeal metastases. The current policy is to perform a myelogram in patients suspected of leptomeningeal metastases and to regard a positive finding as diagnostic of the disease. Furthermore, therapy can be monitored by serial myelograms.

8.8. Clinical investigations and diagnosis in 41 patients with leptomeningeal metastases

The diagnosis of leptomeningeal metastasis is often difficult to establish despite the availability of various diagnostic tests.

In this part of the chapter we describe the results of these various tests in the aid of 41 patients with the diagnosis leptomeningeal metastasis from solid and hematological tumours.

8.8.1. Clinical findings

Table 1. Leptomeningeal metastases from solid and hematological tumours in 41 patients: Vital Data.

primary tumours	No	female/male	age range (years)
Breast	16	16/0	37-70
Lung	12	1/11	45-69
adeno	2	1/1	45-69
small cell carcinoma	6	0/6	53-62
epidermoid	4	0/4	50-69
Malignant melanoma	3	1/2	31-49
Non-Hodgkin's lymphoma	9	1/8	35-70
M.Kahler	1	1/0	64
Total	41	20/21	31-70

In table 1 we show the histology of the primary tumours. There were 20 women and 21 men, ranging in age from 31-70 years. Carcinoma of the breast was the most common primary tumour, accounting for 16 of the 41 patients. Carcinoma of the lung was the primary tumour in 12 patients (six small cell carcinomas, four epidermoid carcinomas and two adenocarcinomas). Malignant melanoma was the primary tumour in three patients, whereas lymphoma was the primary tumour in nine patients and multiple myeloma in one patient. The interval between the diagnosis of the primary malignancy and the diagnosis of leptomeningeal metastases varied from less than one day to more than ten years (Table 2). In one patient leptomeningeal involvement was the presenting complaint and the primary tumour was found subsequent to the CNS metastasis.

Fourteen patients had active disease outside the nervous system, including metastases to the lungs, liver and bone. Three patients had brain metastases, one had epidural metastasis and two had both brain and epidural metastases. In 21 patients metastases were limited to the leptomeninges.

Table 2. The interval after primary diagnosis in
41 patients with leptomeningeal metastases.

1: presenting signs	1
2: less than 6 months	12
3: 6-36 months	19
4: 3-9 months	8
5: 10 more years	1

8.8.2. Symptoms and signs of patients with leptomeningeal metastases

All patients suspected for central nervous system metastases underwent a clinical evaluation by a neurologist. The neurological symptoms and signs were categorized into three anatomic levels (Table 3).

8.8.2.1. Cerebral signs and symptoms (I)

Cerebral signs and symptoms occurred in 35 patients. Headache, mental change, nausea and vomiting were the most frequent. Three patients had severe headache, but no neurological signs.

8.8.2.2. Cranial nerve signs and symptoms (II)

Cranial nerve signs and symptoms occurred in 26 patients. The most frequent complaints were diplopia, facial numbness and weakness. Loss of hearing was found in three patients and nerve palsies were found in six patients.

8.8.2.3. Spinal signs and symptoms (III)

Spinal symptoms and signs were noted in 27 patients. The most frequent were weakness, usually affecting the legs, paresthesias in the lower legs, and bladder and bowel dysfunction. The most frequent complaint was backache and radicular pain. These complaints developed during weeks to months before the diagnosis was established.

When signs and symptoms were considered together, 15 patients had signs and symptoms limited to one area, 5 patients to two and 21 patients to all three.

Table 3. Neurological evaluation: CSF, myelography, CT-scan and autopsy findings in 41 patients suspected of leptomeningeal metastases.

patient number	neurological evaluation*	CSF evaluation** No results	Myelography*** results	CT-scan****	Autopsy ⁺
1	I + II + III	1 +	0	-	0
2	I	2 ± +	0	-	+
3	I + II	1 +	0	-	0
4	I + II + III	2 ± +	0	-	0
5	I + II	1 +	total stop	0	0
6	I + II + III	1 +	0	-	0
7	I + II + III	1 +	0	0	0
8	I + II + III	2 ± +	0	+	0
9	I	2 ± +	0	-	0
10	I + II + III	1 ±	0	met.	+
11	I + II + III	1 +	+	+	0
12	III	1 +	0	0	0
13	I + II	1 +	0	+	0
14	I	1 +	0	+	0
15	I + II + III	2 ± +	+	+	+
16	I + II + III	1 +	0	met.	0
17	I + II + III	1 +	0	+	0
18	III	1 +	0	+	0
19	I + II + III	4 - - -	+	0	+
20	III	2 ± +	-	0	0
21	I + II + III	3 ± ± +	+	+	0
22	I + II + III	3 ± ± +	0	+	0
23	I	2 ± +	0	0	0
24	I + II + III	1 +	0	+	0
25	I + II + III	1 +	0	+	0
26	I + II + III	1 +	+	+	0
27	I	1 +	-	0	0
28	I + II + III	1 +	0	+	0
29	I + II + III	4 - - -	total stop	+	+
30	I + II + III	3 ± ± +	+	met.	0
31	I	1 +	0	0	0
32	I	1 +		0	0
33	I	1 +	0	+	0
34	III	1 +	0	0	0
35	I + II + III	1 +	+	0	+
36	III	1 +	+	0	+
37	I	1 +	0	0	+
38	I + II	3 ± ± +	0	met.	+
39	III	2 ± ±	0	0	+
40	I + II + III	2 ± ±	0	met.	+
41	I + II	1 +	0	0	+

Legend to Table 3

* Neurological evaluation :

- I : cerebral signs and symptoms
- II : cranial nerve signs and symptoms
- III : spinal signs and symptoms

** CSF evaluation :

- + : malignant cells in the CSF
- ± : suspect cells or protein > 0.51 g/l, glucose < 2.0 mmol/l, pressure > 18 cm H₂O, leukocytes > 8 mm³
- : normal CSF

*** Myelography :

- + : irregular filling of the subarachnoid space due to metastatic deposits
- : normal
- 0 : myelography not done

**** CT-scan :

- + : contrast enhancement and hydrocephalus
- : normal
- 0 : CT-scan not done
- met : metastases

+ Autopsy: all autopsies are microscopic and include the spinal cord

- + : tumour cells have been found
- : no tumour cells have been found
- 0 : autopsy not done

8.8.3. Laboratory findings

8.8.3.1. Cytology of cerebrospinal fluid

All patients who had the typical findings of leptomeningeal metastases (i.e., neurological dysfunction at multiple levels of neuraxis without radiographic evidence of either brain or epidural spinal metastases) underwent a lumbar puncture. Cytological examination of CSF has become the most important diagnostical procedure in patients suspected of having meningeal spread of their tumour. CSF was examined in all patients of this study, many of them underwent several examinations before the diagnosis was established. The CSF findings were divided into three groups: a) a definite leptomeningeal metastasis if malignant cells are found (+); b) suspected leptomeningeal metastasis if atypical cells or changes compatible with leptomeningeal metastasis were found (protein > 0.51 g/l, glucose < 2.0 mmol/l, leukocytes > 8/mm³, pressure > 18 cm CSF) (+), c) not suspected in the absence of any of the above mentioned findings (-).

The initial lumbar puncture (Table 3) was abnormal in all but two patients. Three patients neurologically suspected of leptomeningeal metastases had dubious CSF. In 50% of the cases CSF pressure was above 18 cm. The cell count was elevated in 26 patients. The majority of these cells were mononuclear leukocytes, mostly lymphocytes. The protein concentration was above 0.51 g/l in 30 patients. In 19 patients the CSF glucose was less than 2.0 mmol/l. Among the 41 patients with meningeal neoplasia the initial lumbar puncture was abnormal in 25 (61%), the detection rate increased after multiple spinal taps (from 61% to 88%). The CSF cytology remained persistently negative in two patients throughout the course of the disease. However, at autopsy leptomeningeal involvement of metastases were found.

8.8.3.2. Biochemical markers in CSF as a diagnostic aid in leptomeningeal metastasis

We studied four biochemical markers in 41 patients with leptomeningeal metastases from carcinoma and lymphoma. B-gluc., B2-m, CEA and LDH were measured simultaneously on CSF samples from patients with CNS tumours and in patients with a broad spectrum of other neurological diseases.

CSF B-gluc., B2-m, CEA and LDH measurements were performed as reported

Table 4. Elevated biochemical tumour markers in CSF from 41 patients with leptomeningeal metastases from solid and hematological tumours.

primary tumour	Beta-glucuronidase	carcinoembryonic antigen	B2-microglobulin	lactic dehydrogenase	one abnormal marker
breast	16	10/16	10/16	15/16	15/16
lung	12	5/12	7/12	10/12	12/12
melanoma	3	0/3	3/3	3/3	3/3
Non-Hodgkin's lymphoma	9	0/9	8/9	7/9	9/9
M.Kahler	1	0/1	1/1	1/1	1/1
Total	41				
reference range: mean $\pm$ 2 SD	9-27 mU/l	0.8-4 ng/ml	0.65-2.2 mg/l	0-26 U/l	

previously (53-56). The reference ranges for the four markers in normal CSF were based upon mean $\pm$ 2 SD (Table 5). B2-m increased significantly with increasing age (18). Using these reference ranges B-gluc. and LDH were found to be the most frequent positive tests in patients with leptomeningeal metastases from solid tumours (breast carcinoma, melanoma). For leptomeningeal metastases from hematological malignancy (lymphoma and M.Kahler) B2-m was the most frequent positive (Table 4).

In one case with cytologic evidence of leptomeningeal metastases, the autopsy was negative and there were almost entirely normal values of the CSF tumour markers. More common than these so-called 'false positive' cytologies is the failure to find malignant cells, when the clinical findings strongly suggest and autopsy revealed leptomeningeal metastases, the so-called 'false negative' cytologies. Of four patients, strongly suspected of leptomeningeal metastases, two patients had persistently negative CSF and two patients had suspicious CSF for tumour cells, however measurements of biochemical markers revealed elevated values with positive autopsy findings. Table 5 illustrates the findings when the four markers are measured simultaneously. Because these markers are sometimes elevated in different situations, measuring all of them often gives more information about the patient's clinical diagnosis than measurement of either alone.

8.8.3.3. Autopsy studies

In 12 cases out of 41 patients with clinical leptomeningeal metastases a general autopsy and macroscopic neuropathological reports were investigated, and a large number of slides of sections of various levels of the CNS were microscopically examined. The infiltration of the leptomeninges by tumour cells was focal in three patients, multifocal in four patients and in five patients diffuse. In three patients the infiltration by tumour cells was visible to the naked eye.

8.8.4. Radiological studies

8.8.4.1. Computerized tomography of leptomeningeal metastasis

In establishing the diagnosis of leptomeningeal metastasis computerized tomography (CT) may be helpful. We performed CT-scan in 26

Table 5. Summary of CSF B-glucuronidase, B2-m, CEA and LDH levels in Neurological Diseases.

Disorder	B-glucuronidase (mU/l)	B2-microglobulin (mg/l)	CEA (ng/ml)	LDH (U/l)
Leptomeningeal metastasis (solid tumour)	Marked elevation	Normal to moderate elevation	Normal to marked elevation	Marked elevation
Leptomeningeal metastasis (hematological tumour)	Moderate elevation	Moderate to marked elevation	Normal	Moderate to marked elevation
Bacterial meningitis	Marked elevation	Marked elevation	Normal	Marked elevation
Viral meningitis	Normal	Normal	Normal	Normal
Patients who received pre- vious intrathecal pro- phylactic chemotherapy	Normal	Normal	Normal	Moderate elevation
Primary solid and hemato- logical tumours without CNS metastasis	Normal	Normal	Normal	Normal
Brain metastasis	Normal to moderate elevation	Normal	Normal	Normal to moderate elevation
Epidural metastasis	Normal	Normal	Normal	Normal
Cerebrovascular accident	Normal	Normal	Normal	Normal to moderate elevation
Head injury	Normal	Normal	Normal	Moderate to marked elevation
Polynuropathy	Normal	Normal	Normal	Moderate elevation
Malignant Primary CNS Tumour	Normal	Normal	Normal	Moderate to marked elevation
Benign Primary CNS Tumour	Normal	Normal	Normal	Normal to moderate elevation
Reference ranges mean $\pm$ SD	9-27 mU/l N < 27 mU/l Moderate elevation 27-54 mU/l Marked > 54 mU/l	0.65-2.2 mg/l N < 2.2 mg/l Moderate elevation 2.2-3.0 mg/l Marked > 3 mg/l	0.8-4 ng/ml N < 4 ng/ml Moderate elevation 4-5 ng/ml Marked > 5 ng/ml	0-26 U/l N < 26 U/l Moderate elevation 26-52 U/l Marked > 52 U/l

patients (Table 3) and found characteristic CT-abnormalities in 15 patients (58%).

Findings were borderline or suggestive in three patients (12%). CT was normal in eight patients (31%). Enlargement of the entire ventricular system without an obstructing mass lesion was present in 15 of the CT-scans. Contrast enhancement in the basal cistern or cerebral gyri-sulci was present in 17 patients. Five scans showed evidence of parenchymal brain metastases.

8.8.4.2. Myelography in patients with leptomeningeal metastasis

In the present study we performed a myelography in 12 patients with leptomeningeal metastases and spinal root symptoms: all patients had malignant cells in their CSF (Table 3).

Two myelograms were normal, two showed spinal epidural metastatic lesions, giving rise to a total stop, whereas 8 patients (64%) had a positive myelogram, with irregular fillings of the subarachnoid space due to thickening and nodosity of nerve roots, particularly of the cauda equina.

8.8.5. Concluding remarks

Leptomeningeal metastases are a clinically important complication of solid and hematological malignancies occurring with an increasing incidence.

The diagnosis can be established by the clinical signs and symptoms and by appropriate laboratory and radiological examination. It has been suggested that symptoms more often stabilize than improve upon treatment; therefore early diagnosis may be important before symptoms are devastating.

The usefulness of the biochemical markers B-gluc., B2-m, CEA and LDH have been described as an aid in the diagnosis of leptomeningeal metastasis.

Further studies are needed to establish other diagnostic methods such as the value of magnetic resonance for imaging leptomeningeal lesions and flow cytometry to measure the DNA index in CSF cells in cases of suspected leptomeningeal metastases.

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Samenvatting

In dit proefschrift worden de resultaten van het onderzoek over tumor merkstoffen in de liquor cerebrospinalis bij patienten met een leptomeningeale metastasering beschreven. De leptomeningeale metastasering is een ernstige neurologische complicatie van kanker, die vroeger zeldzaam was, maar tegenwoordig in stijgende frequentie wordt herkend.

Bij leptomeningeale uitzaaiing is er sprake van een diffuse of multifocale uitzaaiing van kanker naar de leptomeningen, waarvan de diagnose veelal door het aantonen van tumorcellen in de liquor gesteld kan worden. De incidentie van leptomeningeale metastase wordt gesteld op 5-10%. Het lijkt erop dat de incidentie van leptomeningeale metastase stijgt. Aangezien de kwaliteit en de duur van overleving zal toenemen bij het vroeg stellen van de diagnose, is het van belang te kunnen beschikken over gevoelige tests voor de diagnostiek, die tevens kunnen beoordelen of de behandeling van een leptomeningeale metastase effect heeft gehad.

In hoofdstuk 1 wordt een literatuuroverzicht gegeven over de indeling en toepassing van tumor merkstoffen in de liquor. Het blijkt dat er nogal verwarrende gegevens zijn over de juiste beoordeling van de verschillende diagnostische tests. De tests zijn niet altijd op onafhankelijke wijze vergeleken met een gouden standaard voor de diagnose leptomeningeale metastase. Ook bevatte de onderzochte groep patienten niet altijd de ziekte, die vaak verward werd met de ziekte in kwestie. De factoren die van invloed kunnen zijn op de normaalwaarden, zoals leeftijd en geslacht, werden dikwijls niet geanalyseerd.

De conclusie uit dit literatuuroverzicht is, dat het bepalen van sommige tumor merkstoffen in de liquor duidelijk van waarde blijkt te zijn voor de diagnose en het meten van het effect van een behandeling. Echter voor een groot aantal tumor merkstoffen is de toepasbaarheid nog twijfelachtig. De potentiële diagnostische en prognostische waarde is afhankelijk van de specificiteit en de gevoeligheid (sensitiviteit) van de tumor merkstof van de betreffende tumor.

Hoofdstuk 2 beschrijft de resultaten van een onderzoek naar de sensitiviteit en specificiteit van beta-glucuronidase in de liquor. De relatie tussen hoogte van de plasmaconcentratie en liquorconcentratie

wordt nagegaan. Er blijkt geen verband te bestaan tussen de beide concentraties. Het blijkt dat B-glucuronidase sterk verhoogd is in de liquor bij leptomeningeale metastasen van soliede tumoren en bacteriële ontstekingen, en minder verhoogd is bij leptomeningeale metastasen van hematologische tumoren.

Hoofdstuk 3 vermeldt de resultaten van beta-2-microglobuline (B2-m) in de liquor als diagnostische test voor het aantonen van leptomeningeale metastasen. Het blijkt dat de concentratie van B2-m sterk beïnvloed wordt door de leeftijd. Deze leeftijdsafhankelijkheid van B2-m in de liquor is nog niet eerder beschreven.

De specificiteit en de sensitiviteit van B2-m als tumor merkstof in de liquor zijn bepaald. Voor het aantonen van leptomeningeale metastasen afkomstig van hematologische tumoren lijkt deze test het gevoeligst.

Hoofdstuk 4 beschrijft de bepaling van het carcinoembryonaal antigeen (CEA) in de liquor. Ook hier wordt geen duidelijk verband gevonden tussen de concentraties plasma CEA en liquor CEA. De gevoeligheid van het CEA als tumor merker is voor het aantonen van de diagnose leptomeningeale metastase, afkomstig van soliede tumoren, vrij laag. De specificiteit is daarentegen hoog. Er is meer onderzoek nodig naar de metastasen, afkomstig van die tumoren, die CEA produceren. Het is duidelijk dat de CEA bepaling in de liquor niet geschikt is voor de primaire diagnostiek, maar bij een onduidelijke diagnose bij een patient met een bekende maligniteit kan een CEA bepaling nuttig zijn.

Hoofdstuk 5 vermeldt de waarde van de tumor merkstof lactaat dehydrogenase (LDH) voor de diagnose en het controleren van het effect van een behandeling. Het LDH is gemeten in de liquor van een groot aantal patienten met verschillende neurologische ziekten. LDH in de liquor is weinig specifiek. Het kan verhoogd zijn bij primaire hersentumoren, bacteriële ontstekingen, trauma capitis en leptomeningeale metastasen. Echter als tumor merkstof is het toch belangrijk, omdat LDH goedkoop en in bijna ieder laboratorium gemakkelijk kan worden bepaald. Ook geeft de LDH concentratie in de liquor een aanwijzing voor de tumorlocalisatie. Bij vervolg bepalingen van LDH blijkt er een positief

verband tussen de concentratie van LDH en de ziekte-toestand op dat moment.

Hoofdstuk 6 beschrijft de kansen op het aanwezig zijn van leptomeningeale metastase, berekend aan de hand van het verleggen van de grenzen van de normaalwaarden en door het toepassen van een enkelvoudige test of een combinatie van tests. Door het verlagen van de grenzen voor normaal kan de sensitiviteit en de voorspellende waarde van de negatieve test uitslag 100% benaderen. Door het verhogen van de normaalwaarden kan de diagnostische test worden gebruikt om de ziekte uit te sluiten.

Hoofdstuk 7 vermeldt de resultaten van de concentratie van B-glucuronidase, B2-m, CEA en LDH, die tegelijk in de ventriculaire en lumbale liquor zijn bepaald. De factoren die een rol spelen in de concentratie gradient tussen ventrikel en lumbale liquor, zijn onderzocht. Er blijkt een verband te bestaan tussen de liquor cytologie en de LDH concentratie in de verschillende liquor ruimten. De mogelijkheid om met tumor merkstoffen het effect van de behandeling te meten, is nagegaan. Wij vonden dat de ventriculaire B2-m en LDH concentraties als parameters kunnen dienen om behandelingsresultaten te evalueren.

In hoofdstuk 8 wordt een literatuur overzicht gegeven van de verschillende tests, waarmee de diagnose leptomeningeale metastase kan worden gesteld.

Bij 41 patienten met de klinische verschijnselen, passend bij de diagnose leptomeningeale metastase, zijn een aantal van deze tests toegepast. Hieruit concluderen wij dat de tumor merkstoffen in de liquor een belangrijke rol kunnen vervullen in de diagnostiek van de leptomeningeale metastasen.

Conclusie

Uit de resultaten van de studie over B-glucuronidase, B2-m, CEA en LDH blijkt dat deze tumor merkstoffen een bijdrage kunnen leveren aan de diagnose leptomeningeale metastase. De toepassing van deze tumor merkstoffen om het effect van de behandeling te controleren is enigszins teleurstellend. Alleen de ventriculaire B2-m en LDH concentraties kunnen

als parameters gebruikt worden om het effect van de behandeling van de leptomeningeale metastasen te vervolgen.

Meer onderzoek is nodig naar de diagnostische en prognostische waarden van de tumor merkstoffen in de liquor.

Summary

The subject of this thesis is a clinical investigation of the value of biochemical tumour markers in cerebrospinal fluid (CSF) in the diagnosis and treatment of patients with leptomeningeal metastases from solid and hematological tumours.

Chapter 1 reviews the literature pertaining to the use of biochemical tumour markers in CSF and speculates on their role in the diagnosis and treatment of patients with primary and metastatic tumours of the central nervous system (CNS). Their classification and route of entry are also discussed.

From the data in the literature it can be concluded that the detection of tumour markers may be of value in the early diagnosis of CNS tumours, particularly metastases to the leptomeninges. In addition, they may assist in the evaluation of the efficacy of treatment. An attempt is made to define the principles of test selection and their use in the process of diagnosis. Most of the biochemical markers reviewed appear not to have been adequately studied. It is apparent that the problem of non-specificity is a major one in the development of reliable cerebrospinal fluid markers of malignancy.

Chapter 2 presents the results of a study on B-glucuronidase in the CSF of patients suffering from various neurological diseases. Reference values are established as 9-27 mU/l. A significant elevation of B-glucuronidase is observed with cytologically confirmed leptomeningeal metastases. Marked elevations are also observed in patients with bacterial meningitis. Moderate elevations are found in patients with leptomeningeal metastases from hematological malignancies.

In chapter 3 we study CSF beta-2-microglobulin (B2-m) in control subjects and in patients with a variety of neurological diseases to determine the usefulness of this marker test in the detection of leptomeningeal metastasis. In the literature, factors other than disease that can influence test results are not mentioned. We, however, find a highly significant correlation between B2-m in CSF and age ($p < 0.0001$). A

sensitivity of 70% for leptomeningeal metastases from solid tumours and of 90% for leptomeningeal metastases from hematological malignancies is calculated. The specificity of this test is determined to be 93%.

Chapter 4 deals with a study designed to evaluate the usefulness of the assay of carcinoembryonic antigen (CEA) in the diagnosis of CNS metastases. In this study, determinations are made of CEA levels in the CSF of controls and patients with various neurological diseases in order to establish the sensitivity and specificity of CEA as a test for the diagnosis of leptomeningeal metastases. The sensitivity of the CEA test for the presence of leptomeningeal metastasis from solid and hematological tumours is rather low (31%; 42% for solid tumours only); the specificity is 90%.

In Chapter 5 determinations are made of the lactate dehydrogenase (LDH) activities in the CSF of control subjects and patients with various neurological diseases in order to establish the sensitivity and specificity of LDH as a test for the diagnosis of leptomeningeal metastases. Marked elevations of LDH in the CSF are observed in patients with leptomeningeal metastases from solid tumours and with bacterial meningitis. A sensitivity of 79% and a specificity of 83% are calculated.

LDH levels in CSF may help to define histologic type of tumours. In this study the LDH in ventricular and lumbar CSF from patients with leptomeningeal metastases is measured simultaneously during their treatment with chemotherapy. The LDH in CSF may also help to differentiate the site of the leptomeningeal involvement of the neuraxis. Serial measurements of LDH in ventricular CSF show a positive correlation between LDH level changes and responses to therapy and progression.

In chapter 6 the clinical efficacy of B-glucuronidase, B2-m, CEA and LDH in detecting leptomeningeal metastases from breast carcinoma is studied. Through the application of multiple biochemical marker tests, simultaneously measured in the CSF, we assess the diagnostic potential of combined testing. The sensitivities and specificities of these tests in the CSF are then evaluated.

As a single test, B-glucuronidase is the most sensitive (93%) and specific (93%) in discriminating between leptomeningeal metastases and other CNS metastases from breast carcinoma. More specific results are achieved by combining B-glucuronidase and LDH.

Chapter 7 describes the serial measurements of B-glucuronidase, B2-m, CEA, and LDH in lumbar and ventricular CSF. It is observed that the CSF tumour markers obtained from prophylactically treated patients with hematological malignancies are not influenced by chemotherapy. Response rates are not associated with the pretreatment levels. A gradient is found between the four markers in ventricular and lumbar CSF. For most of the markers, this gradient cannot be explained by a difference in the site of leptomeningeal metastases. Only LDH in CSF exhibits a significant correlation with the metastatic area of the neuraxis ($p < 0.03$). The pretreatment levels of CEA in CSF correlates with the histology of the primary tumour (non-small cell lung cancer; $p < 0.01$). The LDH in ventricular CSF correlates with a positive CSF cytology. The B2-m and LDH in ventricular CSF appear to change with changes in disease status.

Chapter 8 reviews the diagnostic possibilities of establishing the diagnosis leptomeningeal metastasis. The clinical findings of leptomeningeal metastases from solid and hematological malignancies are analyzed. The role of CSF cytology and measurement of biochemical markers including B-gluc., B2-m, CEA, and LDH are evaluated. Computerized tomography and myelography also prove helpful in establishing the diagnosis of leptomeningeal metastases.

However, since no one of these tests is both highly sensitive and highly specific, tests are combined and evaluated for their combined diagnostic potential. Identification of B-gluc., B2-m, and LDH within the CSF appear to offer promise as an adjunct to the cytological analysis of CSF.

Conclusions

The results of the biochemical tumour marker studies described in this thesis demonstrate that measurements of B-gluc., B2-m, CEA, and LDH can assist in the diagnosis of leptomeningeal metastasis. The markers

B-glucuronidase and LDH appear to be the most sensitive and specific in leptomeningeal metastasis from solid tumours. More specific results are achieved by combining the two. B2-microglobulin is found to be the most sensitive and specific marker in leptomeningeal metastases from hematological malignancies.

It appears that the serial measurements of changes in the levels of B2-m and LDH in ventricular CSF are valuable in the follow-up of patients with leptomeningeal metastases. Moreover, our findings indicate that routine measurements of B2-m in ventricular CSF may be reliable in monitoring the efficacy of chemotherapy. There are also indications that these measurements are independent of the most abnormal site of the neuraxis, histology of the primary tumour and CSF cytology. However, the role of the serial measurements of B-glucuronidase and CEA in monitoring therapy is smaller than what was anticipated and, therefore, somewhat disappointing.

The potential use of these biochemical markers in assessing early disease activity remains open for further investigation.

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Curriculum vitae.

Albert Twijnstra was born on June 27, 1944 in Meppel, The Netherlands. He attended high school in Zwolle and later studied at the Medical Faculty of the University of Groningen. During these years he was involved in clinical and research work at the Department of Neurology, under the supervision of Prof.Dr. J.M. Minderhoud.

After graduating in 1972, he worked as a general physician throughout the country. After obstetrical and urological residencies at the Department of Obstetrics and Gynaecology, headed by Prof.Dr. Joosse, University Hospital of Groningen, and at the Department of Urology, headed by Dr. F.J. Felderhof, at the Leyenburg Hospital, The Hague, respectively, he worked from 1973 - 1975 as a resident in internal medicine at the Department of Internal Medicine, headed by Dr. L.W. Statius van Eps, at the St.Elisabeth Hospital in Willemstad, Curacao, Netherlands Antilles.

In September of 1975 he returned to the Department of Neurology at the University of Groningen (chairman Prof.Dr. J.M. Minderhoud), to start his neurological training. In September of 1980 he completed his training, and in November of the same year, he became "chef de clinique" in the Department of Neurology, chaired by Prof.Dr. P.J.M. van der Lugt, at the St.Annadal Hospital in Maastricht, University of Limburg.

In November of 1981 he jointed the staff of the Department of Neurology at the St.Annadal Hospital, where he continues to work as a neurologist.