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USE OF CEREBROSPINAL FLUID SIALIC ACID TO DIAGNOSE AND MONITOR CENTRAL NERVOUS SYSTEM LEUKEMIA

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INTRODUCTION

Despite the improved survival of patients with acute lymphoblastic leukemia (ALL) in the past decade,[1] infiltration of the leukemic cells to the central nervous system (CNS involvement) is still an important factor influencing hematological remission and survival.[2] Although this concept has resently been challenged by Nesbit et al.,[3] prophylactic or therapeutic treatment of CNS leukemia seems to effectively lengthen survival.[4] Mavligit et al.[5] reported that an elevation of β2-microglubulin (β2m) concentrathion in the cerebrospinal fluid (CSF) could be used to detect CNS involvement early in children with ALL and lymphoma. More recently, CSF neuron-specific enolase was reported to rise in nearly all patients with CNS involvement and thus was useful in monitoring the therapy.[6]

Sialic acids are residues of various glycoproteins or sphingolipids, and are negatively charged as surface polyanions on various cell membranes.[7,8,9] Malignant cells have been reported to have sialic acid more abundantly in the cell membranes than normal cells.[10,11] Elevated serum sialic acid levels have been reported in skin melanoma,[12] breast cancer,[13,14] or hepatoma,[15] and are now considered as sensitive markers of malignant cells. However, little is known about the clinical significance of CSF sialic acid in various diseases, including those with leukemic CNS involvement.

We have studied the changes in CSF sialic acid concentrations in various diseases, and report on the usefulness of CSF sialic acid determination in diagnosing and monitoring leukemic CNS involvement.

MATERIALS AND METHODS

1. SUBJECTS

One hundred and three children aged one to fourteen years (58 males and 45 females) were included in this study. CSF samples were collected on routine lumbar punctures for the cytological analysis and prophylactic or therapeutic intrathecal administration of methotrexate (MTX) and hydrocortisone combined with cranical radiation (hereafter abbreviated as "intrathecal therapy"). The diagnosis of CNS involvement was based on increased cell counts of over 20/mm^3 including the presence of leukemic cells in CSF with or without compatible neurologic symptoms and signs such as headache, vomiting, or neck stiffness.

Fifty two patients with leukemia (29 males and 23 females) were classified into three groups as follows. ("n" in following parentheses represent the number of CSF samples which were taken from one to six times in each patient.)

ALL WITH CNS INVOLVEMENT (8 patients, n=39): Three of eight patients developed headache or neckstiffness as clinical manifestations of CNS involvement.

ALL WITHOUT CNS INVOLVEMENT (39 patients, n=59)

ANLL (Acute Non-Lymphocytic Leukemia) WITHOUT CNS INVOLVEMENT (5 patients, n=6): Included in this group are three patients with Acute Myelogenous Leukemia (AML) and two patients with Acute Myelomonocytic Leukemia (AMMoL).

The following fifty one patients (29 males and 22 females) with diseases other than leukemia were also studied as control.

NON-HEMOPOIETIC AND NON-NEUROLOGIC DISEASES (23 patients, n=23): These patients were admitted because of fever or vomiting, and lumbar puncture revealed no abnormal CSF findings.

NON-SUPPURATIVE MENINGITIS (5 patients, n=29): The patients were diagnosed based on characteristic clinical course and an increased number of lymphocytes in CSF.

EPILEPSY (9 patients, n=9).

OTHER NEUROLOGIC DISEASES (14 patients, n=14): Included in this group are hydrancephaly(2), tuberous sclerosis(3), polyneuritis(1), rubella encephalitis(2), intracranial hemorrhage(2), brain tumor(2), and cerebral vascular obstruction(2).

Lumbar punctures were performed with informed consent obtained from at least one parent.

2. Determination of CSF sialic acid concentration.

An enzymatic assay[16] was done using the enzymes, N-acetylneuraminic-acid-aldolase, neuraminidase, and 4-aminoantipyrin which are containted in a commercially available kit (Kyokuto Sialic Acid Test, Kyokuto Chemical, Japan).

3. Determination of CSF β2m concentration.

To compare the data of CSF sialic acid with those of CSF β2m, we simultaneously

measured CSF β2m by enzyme-immunoassay using commercially available kits (Phadezyme β2m Test, Shionogi Chemical, Japan).

RESULTS

1. CSF sialic acid concentration (Figure 1).

CSF sialic acid concentration (mean±SD) in patients who had leukemia with CNS involvement was significantly higher (2.92±1.32mg/dl) than that in patients who had ALL without CNS involvement (1.26±0.72mg/dl) or ANLL without CNS involvement (0.94±0.41mg/dl). In children with the diseases other than leukemia, the CSF sialic acid concentrations were as follows: non-hemopoietic diseases (1.49±0.90mg/dl, [this range will be used later as the control range for CSF sialic acid concentration.]), non-suppurative meningitis (1.76±1.27mg/dl), epilepsy (1.91±0.87mg/dl), and other neurologic diseases (1.36±0.89mg/dl). These values were significantly lower than that in the patients who had ALL with CNS involvement. Differences in CSF sialic acid concentrations among patients with other diseases were not statistically significant.

2. CSF β2m concentration (Figure 1).

The CSF β2m concentration in patients who had leukemia with CNS involvement was significantly higher (1.95±0.57mg/l) than that in the patients who had ALL without CNS involvement (0.94±0.59mg/l), but not significantly different from that in the patients who had ANLL without CNS involvement (1.50±1.46mg/l, $0.1<p<0.2$) or nonsuppurative meningitis (2.51±1.53mg/l, $0.05<p<0.10$).

The CSF β2m concentration in the patients who had leukemia with CNS involvement was significantly higer than that in the patients with non-hemopoietic disease (0.92±

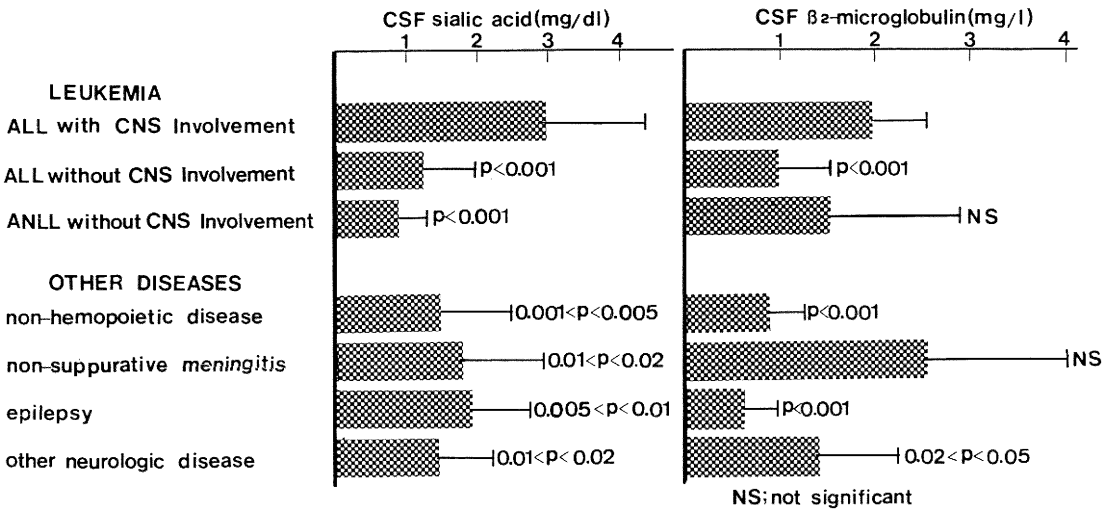

Fig. 1. CSF sialic acid and β2-microglobulin concentration in children with various diseases. The data are expressed as the mean ±SD (p values as compared with ALL with CNS involvement).

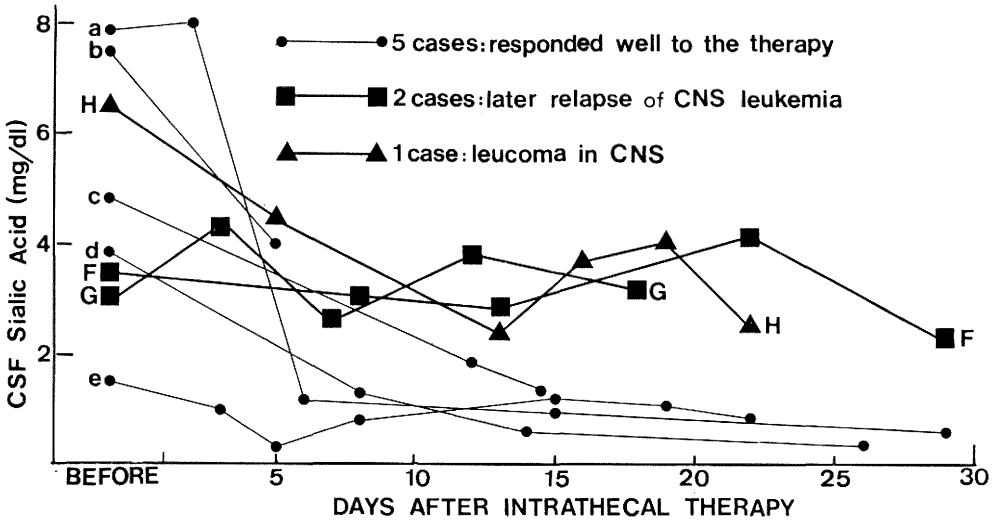

Fig. 2. Sequential changes of CSF sialic acid concentration before and after the intrathecal therapy in patients with CNS leukemia. For further details, see text.

0.37mg/l), epilepsy (0.67±0.22mg/l), or other neurologic diseases (1.48±0.76mg/l).

3. Changes in CSF sialic acid and β2m after intrathecal therapy (Figure 2).

Eight patients with CNS involvement were serially measured for CSF sialic acid and β2m (in five patients) concentration before and after the intrathecal therapy including intrathecal methotrexate injection and cranial radiation. Three (cases a, c, and H) of the patients presented signs and symptoms of CNS leukemia, which disappeared within five days after the intrathecal therapy except for case H. Seven (cases a, b, c, d, e, F, and G) of the patients responded well to the therapy with normalization of CSF findings except for sialic acid, although complete remission of leukemic CNS involvement was not achieved in the other patient (case H). Elevated CSF sialic acid concentrations were rapidly lowered and returned to the control range in five patients (cases a, b, c, d, and e), who have still been in complete remission of CNS leukemia.

The remaining two patients (cases F and G), who had showed sustained elevations in CSF sialic acid concentrations, developed leukemic CNS relapse six to twelve months after the therapy. These facts retrospectively suggested that the intrathecal therapy had not been satisfactory and complete remission of CNS involvement had not been achieved despite the normalization of CSF findings other than sialic acid. Furthermore, cases F and G later developed depression, mental deterioration, or tremor which seemed to be side effects of the intrathecal therapy. In case F, computed tomography demonstrated areas of hypodensity in the central white matter (leukoencephalopathy) ten months after intrathecal therapy combined with cranial radiation.

In case H, an initial response to the therapy was noted with a slight decline in CSF cell counts or amelioration of clinical signs and symptoms, but complete remission of

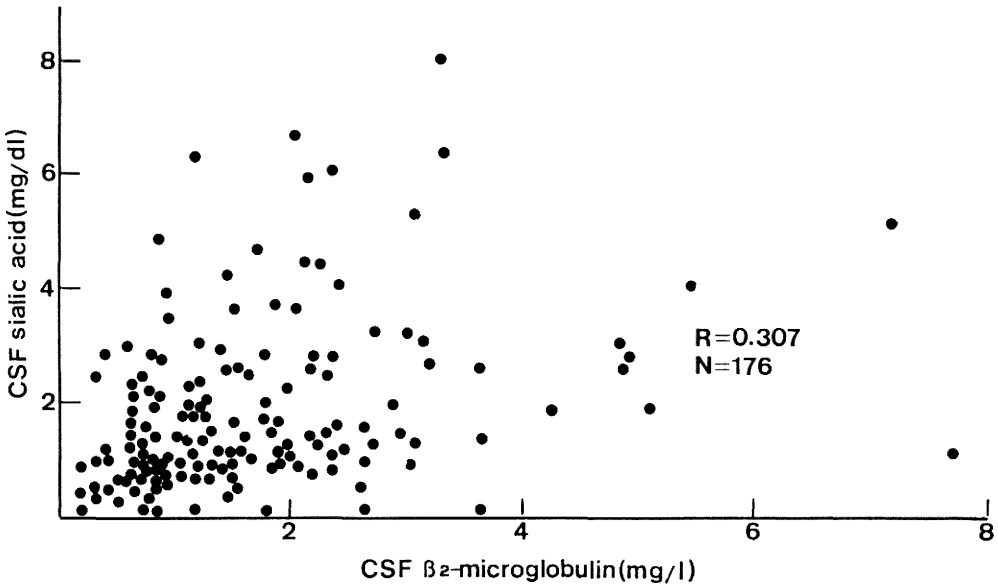

Fig. 3. Correlation between CSF sialic acid and β2-microgloubulin concentrations in children with various diseases shown in Fig 1.

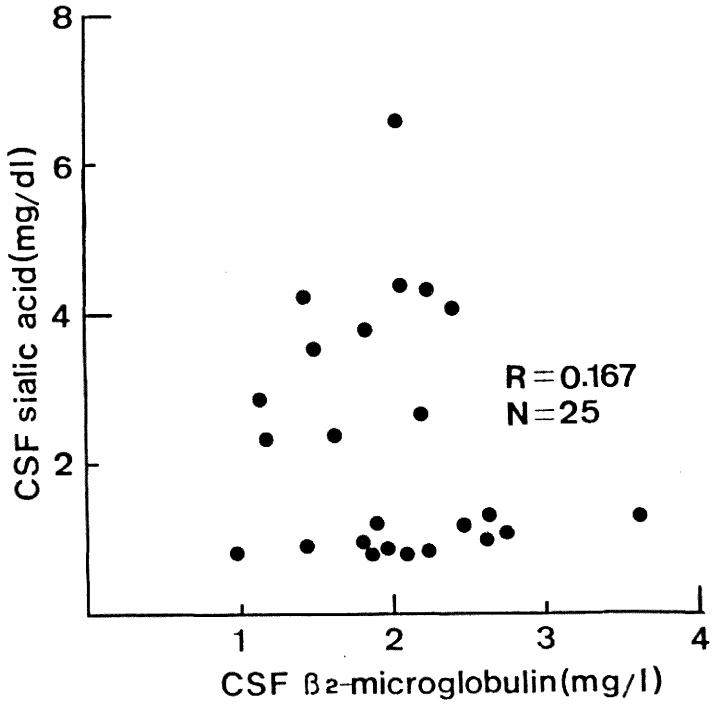

Fig. 4. Relation between CSF sialic acid and β2-microglobulin concentrations in children with CNS leukemia. No significant correlation was noted.

CNS involvement was not achieved and leukemic tumors (leukoma) of CNS were found in this patient.

Changes in CSF β2m concentration were also noted, but they were not as prominent as those of CSF silalic acid.

4. Relation between CSF sialic acid and β2m concentration in leukemia with CNS involvement (Figure 3, 4).

A significant, but weak, correlation was noted between CSF sialic acid and β2m concentrations ($n=175$, $r=0.30$, $0.02<p<0.05$) in children who had diseases other than leukemia (meningitis, epilepsy, or other neurologic diseases as shwon in Figure 1). In CNS leukemia there was no significant correlation between them ($n=25$, $r=-0.17$), which suggested a possible derivation of CSF sialic acid from infiltrating leukemic cells to CNS.

Discussion

Mavligit et al.[5] reported that the β2m concentration is elevated in CSF and that it would be a useful marker for early diagnosis of CNS involvement and for monitoring intrathecal therapy in patients with acute leukemia or lymphoma. However, the CSF β2m concentration did not seem to reflect CNS involvement specifically, because the elevations were also noted in non-leukemic diseases such as meningitis and ANLL without CNS involvement as shown in this study. Serial measurement of neuron-specific enolase isozymes repoted by Royds et al.[6] seems useful in diagnosing and monitoring the therapy for leukemia including CNS involvement. However, the specificity of the elevation of CSF enolase and isoenzymes for leukemic CNS involvement and for toxicity of glial or neuronal cells remains uncertain, because non-hemopietic, neurological, or non-neurological diseases other than CNS involvement were not examined in their study, and their method of using radioimmunoassay seems rather complicated.

In our study, a significant elevation of CSF sialic acid concentration was observed only in the cases of leukemia with CNS involvement, whereas β2m levels were elevated not only in the cases with CNS involvement, but also in the cases of ANLL without CNS involvement and in those of non-suppurative meningitis in which the mean CSF β2m level excceded that in ALL with CNS involvement. Although CSF lymphocyte counts were markedly increased in non-suppurative meningitis, the sialic acid concentration did not show a significant elevation. Furthermore, the significant correlation found between CSF sialic acid and β2m concentrations in the diseases other than leukemia with CNS involvement was not observed in the cases of leukemia with CNS involvement.

These results suggest that the elevation of CSF sialic acid concentration reflects CNS leukemia specifically. A more rapid and more prominent decline in elevated sialic acid concentration than in β2m after the intrathecal therapy, may also support the idea that sialic acid is a more sensitive and more specific marker for leukemic CNS involvement than β2m. Five cases without clinical signs and symptoms of leukemic CNS involvement also showed sequential declines in CSF sialic acid concentrations after the intrath-

ecal therapy. The sustained elevations of CSF sialic acid observed in three cases, retrospectively, predicted the later development of CNS leukoma and CNS relapse six to twelve months after the therapy, followed by clinical manifestations of the side effects of the intrathecal therapy.

In Mavligit's study,[5] the CSF β2m concentration exceeded the serum β2m level in CNS involvement. Serum sialic acid concentration measured in normal children was 47.6$\pm$6.3mg/dl, and values exceeding the CSF sialic acid concentrations were found in various diseases including ALL with a range 40 to 140mg/dl, which is ten to fifteen-fold higher than the CSF sialic acid level. The comparison of the CSF sialic acid level to the serum sialic acid level thus does not seem to be significant in assessing leukemic CNS involvement.

Sialic acids are residues of glycoproteins or sphingolipids of cell components, and are negatively charged as surface polyanions on various cells.[7,8,9] There has been much evidence that malignant cells have more sialic acid on the cell membranes than normal cells,[10,11] and the serum sialic acid level has been reported to be elevated in cases of skin melanoma,[12] breast cancer,[13,14] or hepatoma.[15] Therefore the elevated sialic acid in CSF may have been derived from leukemic cells infiltrating the CNS. The measurement of CSF sialic acid using enzyme assay is very easy and requires only 0.05ml of CSF sample, and only twenty minutes for reaction.

In conclusion, CSF sialic acid may serve as a useful marker for the diagnosis of leukemic CNS involvement and for monitoring intrathecal therapy.

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