3. Detection of central nervous system involvement in patients with leukemia or non-Hodgkin’s lymphoma by immunological marker analysis of cerebrospinal fluid cells

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Introduction

Central nervous system (CNS) involvement is observed at diagnosis in up to 20% of all patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), or non-Hodgkin’s lymphoma (NHL) [1–12]. Follow-up of these patients revealed that without prophylactic CNS therapy, up to 70% of the ALL, up to 25% of the AML, and, depending on the histological subtype, up to 40% of the NHL patients will develop the serious complication of CNS involvement [1, 5, 7, 12–26]. This increase in CNS involvement has been explained by the application of improved systemic therapy leading to longer survival of patients, which allows the growth of tumor cells present in relatively therapy-resistant sites such as the CNS. In addition, primary NHL can be located in the CNS, and they constitute about 1% of all brain tumors and about 2% of all NHL [27–30]. These primary NHL located in the CNS are increasingly diagnosed, especially in immunodeficient individuals, probably as a result of the increased use of radiotherapy and immunosuppressive drugs; furthermore, improved diagnostic procedures might be responsible for this increase [31–36].

Prevention and — if not possible — treatment of preferably early diagnosed CNS involvement are important goals of therapy. Prophylactic treatments, such as cranial irradiation and intrathecal chemotherapy, have greatly reduced the occurrence of CNS involvement in ALL, AML, and some types of NHL; still, in 2%–15% of patients, primary meningeal relapse occurs [1, 6, 8–10, 13, 15, 19, 37–44]. This CNS involvement is especially a major concern in childhood ALL, where the majority of patients now have become long-term survivors [45]. Although the detection of malignant cells in the CSF is often the first sign of CNS involvement, in most cases the disease process has started in an earlier phase by leukemic infiltration of the superficial leptomeninges. Such a leukemic infiltration will eventually lead to destruction of the trabeculae with release of cells in the CSF [46]. These infiltration processes may differ depending on the type of malignancy [47]. It has been pointed out that the malignant cells appear less often in the CSF when they are locally seeded and almost never when the tumor is
limited to the brain and the pial surface has not been breached [48]. Thus, failure to find malignant cells in the CSF does not exclude the possibility of CNS involvement when clinically suspected. Therefore, multiple CSF sampling is preferred, especially in cases with little leptomeningeal involvement [49, 50]. In cases of NHL in the CNS, it has been recommended to perform at least three consecutive examinations; then a positive cytology is obtained in 60%–70% of patients [12, 51]. Cytological examination of the CSF at regular intervals has been integrated in many protocols for the management of patients with leukemia or NHL. It is obvious that reliable methods for the detection of low numbers of malignant cells in the CSF will allow an earlier diagnosis of CNS involvement, which might be beneficial for the patient, as therapy can be started earlier, before massive infiltration and destruction of the meninges occur.

**Methods for the detection of malignant cells in cerebrospinal fluid**

CSF samples often contain few (tumor) cells, and this determines to a large extent which methods are suitable for the detection of malignant cells in the CSF [52]. The standard methods for the (early) detection of CNS leukemia or NHL are cell counting and cytomorphological examination of the CSF [50, 52]. This detection can be improved by immunological marker analysis of the CSF cells with monoclonal antibodies or conventional antisera, specific for cell surface membrane antigens [52–73] or for the nuclear enzyme terminal deoxynucleotidyl transferase (TdT) [65, 72, 74–79]. In addition, cytogenetic analysis of CSF cells [80–84], electron microscopy [56, 85, 86], flow cytometric analysis of DNA and RNA content of cells in the CSF [87, 88], or measurements of CSF β-2-microglobulin (β2m) [89, 90] have been proposed for the (early) detection of meningeal involvement in acute leukemia or NHL. Recently, gene rearrangement studies have been added as a diagnostic tool for proving monoclonality of CNS lymphomas [91]. The application of the aforementioned techniques, and especially the immunological marker analysis, in the diagnosis of CNS involvement will be reviewed and discussed.

**Cytomorphology**

For the morphological analysis of CSF cells, cytocentrifugation of the CSF is widely performed [92–98], although some prefer membrane filter preparation [99–101] or gravity sedimentation of CSF on slides [102, 103]. Which method is best is still controversial [104–107]. Cell loss, recovery rate, and preservation of cell morphology are matters of debate, although it has been stated by Bigner and Johnston in their extensive review [52] that ‘excellent cellular recovery and preservation can be obtained using any of these techniques, if careful attention to detail in cytopreservation is observed.’ However, diagnostic problems can arise when the CSF cell count is low,