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Invited review

Diagnostic strategies to investigate cerebrospinal fluid involvement in haematological malignancies

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ABSTRACT

Central nervous system (CNS) involvement is a fatal complication of certain haematological malignancies with an incidence as high as 25% in specific leukaemia/lymphoma subtypes. It is often accompanied by 'occult' cerebrospinal fluid (CSF) involvement at diagnosis, which is frequently missed by conventional cytology examination. Unfortunately, a diagnostic gold standard is yet unavailable since CSF morphology may be negative for malignant cells in up to 45% of patients with suspected meningeal involvement. New technologies such as flow cytometry, molecular genetics and newer biomarkers may improve sensitivity and specificity facilitating the diagnosis of CNS involvement as well as effective prophylaxis and successful treatment.

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Central nervous system (CNS) involvement is a frequent complication in haematological malignancies, developing in 5–15% of patients with leukaemia and lymphoma [1,2]. Many factors influence the reported prevalence (up to 25% in certain subtypes of leukaemia and lymphomas) leading to poor prognosis [3–5]. In sharp contrast with leukaemia and high-grade lymphoma, CNS involvement in multiple myeloma is uncommon and is only observed in approximately 1% of cases. It may manifest itself as dural or intraparenchymal myeloma, while the involvement of the

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cerebrospinal fluid (CSF) and the leptomeninges is exceedingly rare [6].

Leptomeningeal metastases are the most common form of CNS involvement in acute lymphoblastic leukaemia (ALL) and aggressive non-Hodgkin's lymphoma (NHL) [5]. In children with ALL, the risk of CNS involvement is 75% among patients who are not administered appropriate prophylaxis, whereas in adult without prophylaxis recurrence of CNS involvement has been observed in approximately 30% of cases [7]. Identified risk factors for CNS relapse in ALL include elevated lactate dehydrogenase levels, S phase fraction, T-cell phenotype in paediatric ALL, mature B cell phenotype in adult ALL, high white blood cell (WBC) counts and CNS leukaemia at diagnosis [8].

While prophylactic interventions and therapeutic approaches for CNS involvement are well-defined in ALL, there is open debate regarding patients affected by aggressive NHL where the percentage of patients who are at risk for CNS involvement is significantly lower (4–5%) [3–5]. The frequency of CNS involvement is related to histological subtype, ranging from uncommon in indolent lymphomas to more frequent in aggressive lymphomas such as diffuse large B cell lymphoma (DLBCL), lymphoblastic lymphoma, blastoid variant of mantle cell lymphoma and Burkitt's lymphoma (BL) [8,9]. Clinical criteria, such as involvement of the paranasal sinus, testes, orbital cavities or bone marrow, advanced stage, high International Prognostic Index, elevated LDH levels and the involvement of multiple extranodal sites all help to better identify the risk factors in patients for whom the administration of prophylaxis is strongly recommended [8,9]. Prophylactic treatment is necessary to reduce the incidence of CNS relapse in aggressive NHL but also increases the toxicity of systemic chemotherapy; therefore, clinical risk paradigms lead to the identification of patients who may benefit from CNS prophylaxis [4]. Unfortunately, because the cohort of patients characterised by risk factors could be 4–5-fold larger than the subgroup that will actually develop CNS disease, more sensitive and specific laboratory methods are needed to detect occult CNS infiltration and to ensure optimal treatment while avoiding unnecessary therapies.

This review will focus on newer strategies for the diagnosis of CSF involvement in haematological malignancies. Several methods for detecting CNS haematological malignancies are available. Magnetic resonance imaging (MRI) with gadolinium is the preferred neuroimaging method to investigate patients with clinical findings that are suggestive of neoplastic meningitis [10,11], and is reported to be of high diagnostic accuracy in patients with solid tumours. However, several studies have demonstrated that MRI is of limited utility in detecting meningeal infiltration by haematological diseases [12–14]; therefore light microscopic examination of cytospin preparations is still considered the gold standard for detecting neoplastic cells in cerebrospinal fluid (CSF) in haematological malignancies.

1. Conventional CSF cytology

The identification of tumour cells in the CSF of patients who have leptomeningeal metastases has been referred to as the "strict but often unobtainable gold standard" [15]. Lymphoma patients frequently have clinical symptoms and neuroimaging features that suggest leptomeningeal involvement with a negative cytology result upon CSF analysis. Cerebrospinal fluid analysis includes the measurement of opening pressure, protein and glucose levels, cell count, and cytology. In aggressive NHL with leptomeningeal involvement, many of these indices are often abnormal, but only cytological demonstration of the presence of tumour cells is conceptually able to reach 100% specificity. Unfortunately, although conventional cytology has retained its status as the diagnostic gold standard, it has low sensitivity and specificity with reported false-positive or false-negative cases [16,17] due to the paucity of tumour cells in the CSF of patients with minimal disease and the presence of confounding reactive lymphocytes, respectively [15–18].

Glass et al. correlating malignant cells in CSF (positive cytology) and pathologic findings at autopsy in most of patients with solid tumour and in some patients with haematological malignancies, demonstrated in a post-mortem analysis that approximately 40% of patients with autopsy-proven leptomeningeal malignancies (LMs) had negative ante-mortem CSF cytology. Among patients with focal (limited extent) leptomeningeal disease, 50–60% of them had negative CSF cytology [17]. The multifocal nature of leptomeningeal metastasis both in solid tumours as in haematological malignancies, may partially explain why CSF obtained from a site distant from that of the pathologically involved meninges can yield negative cytology results [19].

To partially bypass the false-negative rates in CSF cytology, Glantz et al. performed a study on patients with 26 solid tumours and 13 lymphomas, suggesting that at least 10.5 mL of CSF should be withdrawn for cytologic analysis from a site of clinical or radiographic disease; this sample should be processed immediately, and the procedure repeated once if the initial cytology result is negative [18]. Additionally, staining for terminal deoxynucleotidyl transferase may help to distinguish normal lymphocytes from leukaemic cells in cases of precursor B-cell neoplasia with questionable morphology. To accomplish this, microscopic immunocytology has also been used to establish a diagnosis by detecting leukaemia-associated cell surface antigens [20,21]. Recently, Perske et al. suggested that no single parameter is sufficient to detect neoplastic lymphocytes, but rather a combination of cell size and irregular shape of the cell and nucleus may improve the diagnostic accuracy of CSF dissemination by aggressive haematological malignancies [22].

2. Flow cytometry

Flow cytometry is an objective and quantitative assay that can identify small populations of cells with aberrant phenotypes; it can identify a few neoplastic cells within a population of normal lymphocytes [23].

2.1. The feasibility of studying CSF samples with flow cytometry

One of the first reports suggesting flow cytometry as a useful means for detecting malignant cells in the CSF was published in 2000 [24]. Flow cytometry analysis (FCA) in conjunction with cytology substantially enhanced the detection of lymphoproliferative diseases (LPDs) involving the CSF. Interestingly, the 50% detection improvement observed in this study was similar to that in a previous report (43%) [25] where FCA alone allowed the detection of CSF infiltration in 3 cases of LPD, while 2 additional cases were diagnosed by cytology alone (due to insufficient sample quantity for FCA). The authors concluded that FCA markedly improved sensitivity when used in combination with cytology in the evaluation of lymphoid cells in the CSF, although some aspects of the state of the sample, such as size and cell viability, were critical for effective FCA [25]. At that moment flow cytometry on CSF samples was considered to have some *caveats* with regard to performing adequate analyses in cases of limited sample volumes and low CSF cell counts. Other limitations were its poor ability to provide definite diagnostic data when a limited number of antibodies are studied (likely due to paucity of cells or the volume of sample received) and when the exact phenotype of the neoplastic cells to be identified is unknown [26,27].