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ARTICLE

Higher positive identification of malignant CSF cells using the cytocentrifuge than the Suta chamber

A identificação de células neoplásicas no LCR foi maior com o uso da citocentrifuga do que com câmara de Suta

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ABSTRACT

Objective: To define how to best handle cerebrospinal fluid (CSF) specimens to obtain the highest positivity rate for the diagnosis of malignancy, comparing two different methods of cell concentration, sedimentation and cytocentrifugation. **Methods:** A retrospective analysis of 411 CSF reports. **Results:** This is a descriptive comparative study. The positive identification of malignant CSF cells was higher using the centrifuge than that using the Suta chamber (27.8% vs. 19.0%, respectively; p = 0.038). Centrifuge positively identified higher numbers of malignant cells in samples with a normal concentration of white blood cells (WBCs) (< 5 cells/mm^3) and with more than 200 cells/mm^3, although this was not statistically significant. There was no lymphocyte loss using either method. **Conclusions:** Cytocentrifugation positively identified a greater number of malignant cells in the CSF than cytosedimentation with the Suta chamber. However, there was no difference between the methods when the WBC counts were within the normal range.

Keywords: cerebrospinal fluid; cytology, centrifugation, sedimentation.

RESUMO

Objetivo: Definir qual a melhor forma de concentrar amostras de LCR para obter maior porcentagem de positividade para o diagnóstico de infiltração neoplásica. comparando dois métodos diferentes de concentração de células, sedimentação e citocentrifugação. **Métodos:** Análise retrospectiva de 411 laudos de LCR. **Resultados:** Estudo comparativo descritivo. A identificação de células neoplásicas no LCR foi mais elevada quando usada a citocentrífuga do que a câmara de Suta (28% vs 19,0%, respectivamente; p = 0,038). Centrifugação identificou maior número de células neoplásicas em amostras com concentração de células < 5 células/mm^3 e superior a 200 células/mm^3, embora não significativo. Não houve perda de linfócitos usando qualquer um dos métodos. **Conclusões:** A citocentrifugação identificou um número maior de células malignas no LCR do que a sedimentação com a câmara de Suta. No entanto, não houve diferença entre os métodos quando as contagens de leucócitos estavam dentro do intervalo normal.

Palavras-chave: líquido cefalorraquidiano; citologia, centrifugação, sedimentação.

The most useful laboratory test for diagnosing neoplastic meningitis infiltration is cerebrospinal fluid (CSF) investigation. Accurate diagnosis is important for diagnostic, therapeutic, and prognostic consequences[1]. Cerebrospinal fluid cytology is mandatory in all cases of known or strongly suspected malignancy. This is particularly true in cases of leukemia and lymphoma, in which the results of CSF cell counts and cytology are important factors in determining and monitoring treatment[2,3,4].

The two main methods of CSF cell concentration are cytocentrifugation and the gravitational facility sedimentation chamber. The literature provides no consensus on the optimal technique[5,6], since both methods have advantages and disadvantages. The aim of this paper was to compare two different methods of cell concentration, cytosedimentation using a Suta chamber and cytocentrifugation.

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METHOD

This study was approved by the HC-UFPR review board. A retrospective, longitudinal study was conducted utilizing CSF laboratory results from the data files of the clinical pathology laboratory of the General Hospital – Universidade Federal do Paraná (UFPR). Samples of CSF were obtained from two populations of patients: adults and children who were referred to the laboratory with clinical suspicion of malignant CNS infiltration and patients who underwent prophylactic intrathecal chemotherapy. Samples were referred to the laboratory from hematology, bone marrow transplantation, neurology, and neurosurgery services. All CSF samples were obtained by lumbar puncture between March 1995 and December 2000. The CSF total cell count was assessed using a Fuchs Rosenthal chamber. The CSF was analyzed within a maximum of 30 minutes after arriving in the CSF section, and samples were maintained in room temperature.

Methods for cell concentration

For the differential cell count and detection of malignant cells, CSF samples were concentrated using either a Suta chamber (1995–1998; 220 CSF samples) or a cytocentrifuge (Cytopro 7620 Cytocentrifuge, Wescor) (1998–2000; 191 CSF samples).

Suta chamber

The CSF volume applied was adjusted according to the CSF total cell count, as described: 0.1 to 9 cells/mm^3- 2.0 mL; 10 to 50 cells/mm^3- 1.5 to 2.0 mL; 50–100 cells/mm^3- 1.2 to 1.8 mL; 100–200 cells/mm^3- 1.0 to 1.5 mL; 200–500 cells/mm^3- 0.8 to 1.0 mL; 500–1,000 cells/mm^3- 0.5 to 0.8 mL; > 2,000 cells/mm^3- 0.2 to 0.3 mL. The time of sedimentation was around 20 to 30 minutes[7,8].

Cytocentrifuge

One mL of CSF was centrifuged during two minutes in a regular centrifuge at 2,500 rpm; for CSF samples with total cell count ≥ 1,000 cells/mm^3, CSF was diluted 1/20 (in this study there were no samples with CSF total cell count ≥ 1,000 cells/mm^3). From the sediment 100 µL was transferred to the cytocentrifuge, centifugated for two minutes at 1,200 rpm.

In both methods the CSF samples were protein-enriched with albumin, and slides were stained by the May Grünwald-Giemsa technique and observed by two trained researchers.

Malignant cells characteristics

Malignant cells were defined by the presence of one or more of the following characteristics: large size and/or nuclei; an increase in the nucleus/cytoplasm size index in favor of the nucleus; multiple nuclei; great, prominent, or multiple nucleoli; variation in the size and format of the cells and nuclei; mitosis in groups of cells; frequent atypical mitosis; and irregular nuclear edges, hyperchromasia, and irregular grouping of the nuclear chromatin.

To calculate the positive rate of identification of malignant cells in the CSF, we considered the first sample (positive or negative) of each patient and subsequent positive samples for a total of 411 samples. The categorical variables were compared using either the Chi-square test (x^2) or Fisher's exact test, and the continuous variables were compared using the Student's t-test. A p-value ≤ 0.05 was considered significant. The results are presented as the mean ± standard deviation (SD).

Demographic and CSF characteristics of the groups studied

During the period of the study, 411 CSF samples were collected from 330 patients with possible malignant CNS infiltration. Of these patients, 180 (54.4%) were male and 150 (45.6%) were female. The mean ± SD age was 15.7 ± 15.8 years, and the median age was nine years. The participants in the group with the CSF samples prepared by the Suta sedimentation chamber (n = 220) and the group with the CSF samples prepared by the cytocentrifuge (n = 191) were well-matched with respect to age, gender, and main indication for CSF neoplastic cell search as well as basic CSF cell and biochemistry characteristics. The median interquartile range of age was 8 (4–23) years and 7 (4–23) years in the Suta chamber and cytocentrifuge groups, respectively (p = 0.82). A total of 57% (n = 125) and 47% (n = 90) of the CSF samples in the Suta chamber and cytocentrifuge groups, respectively, were from male patients (p = 0.08). The median interquartile range of white blood cell (WBC) count was 1.0 (0.3–3.0) and 1.0 (0.3–3.2) in the Suta chamber and cytocentrifuge groups, respectively (p = 0.52). The red blood cell count was 1.2 (0–16) and 1.3 (0–17) in the Suta chamber and cytocentrifuge groups, respectively (p = 0.06). The glucose level was 46 (46–67) and 59 (50–69) in the Suta chamber and cytocentrifuge groups, respectively (p = 0.22). The total protein was 23 (17–39) and 21 (14–38) in the Suta chamber and cytocentrifuge groups, respectively (p = 0.94).

Indication for neoplastic cell search in the CSF

The most frequent indication for neoplastic cell search in the CSF was the diagnosis or follow-up of acute lymphocytic leukemia (ALL) (62% of participants in both groups, p = 1.0). Other indications for neoplastic cell search in the Suta chamber and cytocentrifuge groups by participants included lymphomas (Burkitt, Hodgkin, and non-Hodgkin) [18 (8%) vs. 23 (12%), p = 0.25]; chronic lymphocytic leukemia [1 (0.6%) vs. 0 (0%)]; acute myeloid leukemia [43 (20%) vs. 20 (10%), p = 0.013]; and chronic myeloid leukemia [9 (4.1%) vs. 7 (3.7%), p = 1.0]. Isolated cases included metastasis of lung, gastric, breast, melanoma, or prostate cancer; Ewing's sarcoma; and primary CNS neoplasm (rhabdomyosarcoma, glioblastoma, astrocytoma, and Schwannoma). All were grouped into an "other" category [13 (5.9%) vs. 23 (12%) in the Suta chamber and cytocentrifuge groups, respectively, p = 0.035].

RESULTS

Impact of CSF concentration methods on the WBC differential count

The differential characteristics of the WBCs in the CSF of both groups are indicated in Table 1. There was no statistical difference in the percentage of lymphocytes and neutrophils, suggesting no small cell loss in the Suta chamber. The percentage of monocytes was higher in Suta chamber preparations.

Rate of neoplastic cell detection by each concentration method

The cytocentrifuge detected malignancy in 27.8% (53/191) of the available samples. The Suta chamber detected malignancy in 19.0% (42/220) of the available samples. The difference in detection rate was statistically significant (X^2 p = 0.038; OR = 1.6, 95%CI 1.0–2.6). There was 9% increase in the positive identification of neoplastic cells in the CSF using the cytocentrifuge (Figure 1). Although it was not statistically significant, there was an increased likelihood (1.6%) of identifying malignant CSF cells using the cytocentrifuge compared to the Suta chamber. There was no relationship between the positive identification rate for a given method and the number of WBCs in the sample (Table 2).

Impact of the CSF WBC count on the rate of neoplastic cell detection in each cell concentration method

The majority of samples (74% and 76% in the Suta chamber and cytocentrifuge groups, respectively) had a normal number of WBCs in the CSF (p = 0.64 or = 1.12, 95%CI = 0.71–1.75) because the majority of samples (62%) in both groups were from patients with ALL. Patients with ALL receive prophylactic intrathecal chemotherapy; therefore, these samples were not necessarily from patients with CNS neoplastic involvement. There was no statistical difference between the two methods when analyzing CSF samples with a normal number of CSF WBCs (p = 0.17 or =1.5, 95%CI = 0.82–2.9; Table 2). The number of malignant CSF cells that were positively identified was higher in the samples with either a normal range of WBCs (< 5 cells/mm^3) or WBC count greater than 200 cells/mm^3, with a statistical trend observed in the normal range (Figure 2, Table 2). If the WBC count was greater than 200 cells/mm^3, then the percentage of CSF neoplastic cells identified by the cytocentrifuge was 78% (7/9), whereas 50% (3/6) were identified by the Suta chamber. Thus, the probability of a clinical diagnosis of malignant CSF cells was 3.5-fold higher using the cytocentrifuge than that using the Suta chamber, although this difference was not significant (Table 2).

Table 1. Cerebrospinal fluid (CSF) white blood cells (WBCs) characteristics by cell concentration method.

Variable	Cytocentrifuge		Suta chamber		Student's t-test*	P
	n	Mean + SD	n	Mean + SD		
Lymphocytes (%)	44	63 + 27	42	56 + 30	1.05(84) = 1.98	> 0.05
Monocytes (%)	29	10 + 8.2	30	35 + 26	4.81(57) = 2.00	< 0.05
Neutrophils (%)	17	20 + 25	17	26 + 31	0.62(32) = 2.04	> 0.05

n: the number of CSF samples in which the WBC type was identified. The differential cell count was not performed in all samples. *$T_{calculated}$ (df) = $T_{critical}$

Table 2. Percentage of CSF malignant cells by CSF WBCs level.

CSF WBC (cells/mm^3)	Cytocentrifuge				Suta chamber				p*	OR	95%CI
	Positive		Negative		Positive		Negative				
	n	%	n	%	n	%	n	%			
0–4	26	18	120	82	20	12	143	88	0.17	1.5	0.82–02.9
05-10	7	50	7	50	8	30	19	70	0.20	2.4	0.63–09.0
10-50	9	56	7	44	8	45	10	55	0.49	1.6	0.41–06.2
50–200	4	67	2	33	3	50	3	50	0.56	2.0	0.19–20.6
> 200	7	78	2	22	3	50	3	50	0.26	3.5	0.49–56.8
Total	53	28	138	72	42	19	178	81	0.04	1.6	1.00–02.6

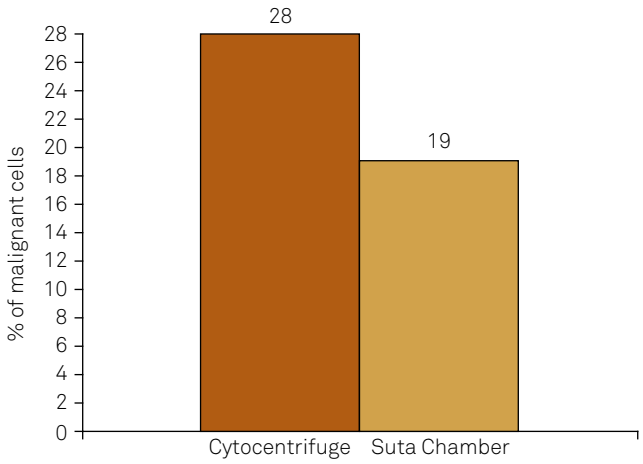
CSF: cerebrospinal fluid; WBCs: white blood cells; *Fisher's exact test.

Rate of positive identification of neoplastic cells in different neoplasm types by CSF concentration method

The main indication for neoplastic cell search in the CSF was ALL. Among these cases, the number of cells that were positively identified as malignant was higher when the CSF samples were prepared using cytocentrifuge (31%, n= 37) compared with when the samples were prepared using the Suta chamber (19%, n= 26). This difference was statistically significant (p = 0.03).

There was no difference in the number of cells that were positively identified as malignant by both methods in lymphoma cases. The detection of neoplastic cells in acute myeloid leukemia and chronic myeloid leukemia cases was higher when the CSF samples were prepared by the Suta sedimentation chamber compared with the cytocentrifuge, but this difference was not significant (Table 3).

These results suggest that the use of the cytocentrifuge increases the identification of specific types of malignant cells, such as those found in ALL. Among ALL cases, the probability of a clinical diagnosis of malignant CSF cells was 0.5 times higher using the cytocentrifuge compared with the Suta chamber to prepare the CSF samples.



X^2 p = 0.04, OR = 1.6 (95%CI 1.0 to 2.6)

Figure 1. The percentage of positively identified malignant cells with respect to the cell concentration method used.

DISCUSSION

The CSF cells must be concentrated before microscopic examination for differential WBC count or the identification of

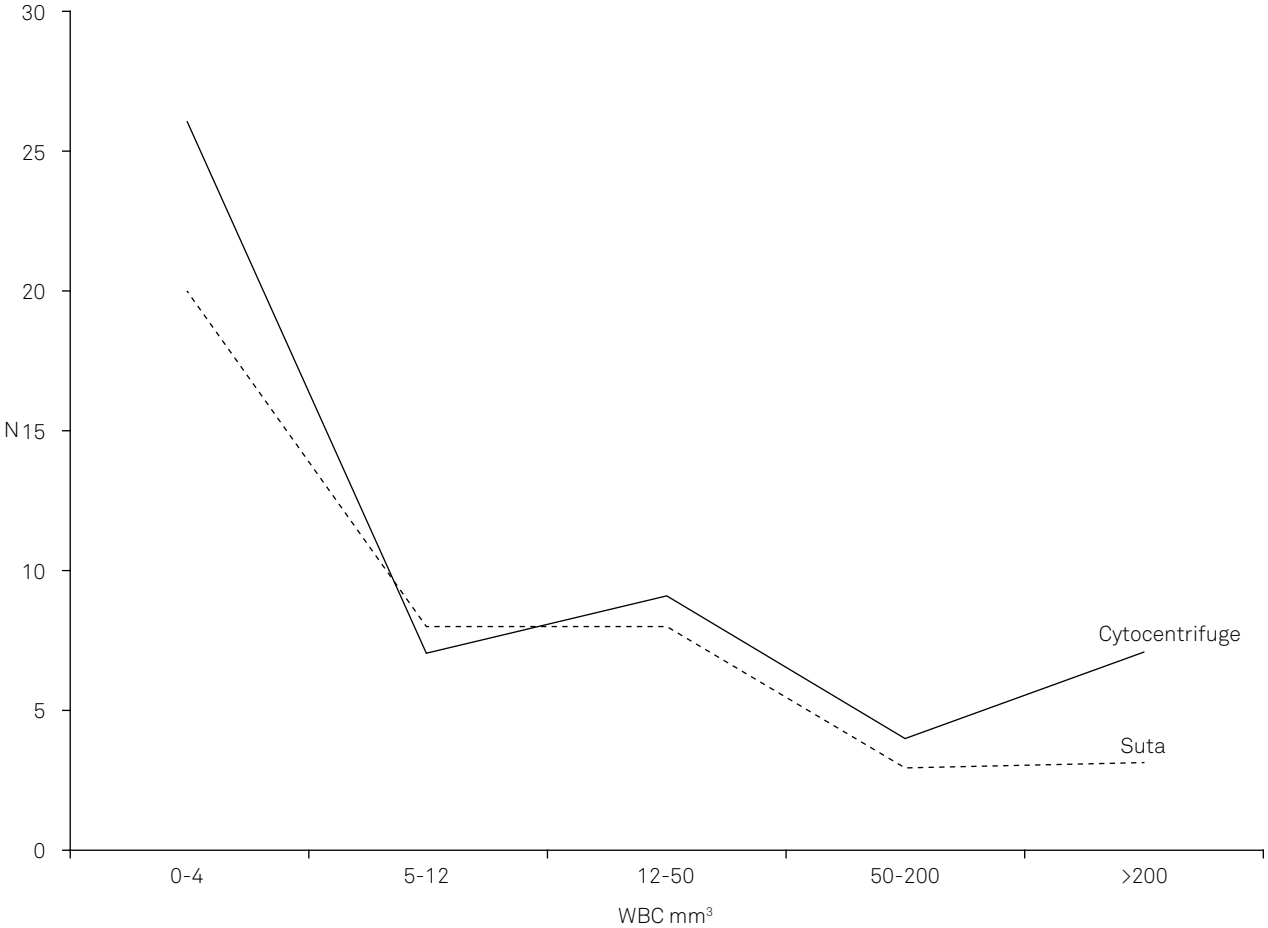


Figure 2. The number of positively identified malignant cells in the cerebrospinal fluid (N) plotted against the number of white blood cells (WBCs) in the CSF for each cell concentration method.

Table 3. Number of malignant cells detected in different neoplasm types by concentration method.

Variable	Cytocentrifuge (n=191)		Suta chamber (n= 220)		p	OR	95%CI
	Positive	Negative	Positive	Negative			
Lymphoma	5 (22%)	18 (78%)	4 (22%)	14 (78%)	1.0	0,97	0.22–4.31
ALL	37 (31%)	81 (69%)	26 (19%)	110 (81%)	0.03	0.52	0.29–0.92
AML	3 (15%)	17 (85%)	10 (23%)	33 (77%)	0.52	1.72	0.42–7.08
CML	0	7 (100%)	2 (22%)	7 (78%)	0.48	5.00	0.20–123
CLL	0	0	0	1 (100%)	-	-	-
Other	8 (35%)	15 (65%)	0	13 (100%)	0.03	0.07	0.004–1.28

ALL: acute lymphocytic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphocytic leukemia.

malignant cells owing to their small number. Cellular components in the CSF can be concentrated by sedimentation, membrane filtration, or centrifugation in a standard laboratory centrifuge or a cytocentrifuge. Although several studies have investigated[9,10,11] which concentration method (sedimentation vs. cytocentrifugation) is better for preparing CSF specimens for cytology diagnosis mainly in suspected malignancy cases, two recent publications stated that the best method is not yet well established[7,8].

In this study, we compared two different CSF preparation methods that are widely used in routine CSF laboratories to diagnose malignant CNS infiltration. Although the methods were used at two separate periods in time, the groups were well-matched with respect to age, gender, neoplastic cell search indication, and basic CSF cytology and biochemistry characteristics.

The rate of diagnosed malignant cells was higher when the CSF sample was concentrated by the cytocentrifuge than by the Suta chamber. In this study, the main indication for neoplastic cell search in the CSF was ALL, and the second most common indication was lymphoma. The other types of malignant diseases occurred at a low frequency, which limited a definitive conclusion. In contrast to acute leukemias, the involvement of the CNS in chronic leukemias such as chronic lymphocytic leukemia and chronic myeloid leukemia is not common. Therefore, we cannot conclude which method is better for the identification of specific types of malignancy.

No difference in the lymphocytes and neutrophils was found between the two methods, which suggests that the CSF concentration method does not impact the WBC differential count. The percentage of monocytes was lower among the CSF samples concentrated by the cytocentrifuge, which suggests a potential loss of these cells by the cytocentrifuge method. As an alternative interpretation, the lower number of monocytes detected using the cytocentrifuge may reflect the relative enrichment of monocytes using the sedimentation method due to the loss of other cell types. Bots et al. concluded that sedimentation yielded a greater proportion of monocytes and eosinophils[12]. Although monocytes are larger cells than lymphocytes (a small lymphocyte is 8 to 10 μm and monocytes are 12 to 20 μm in diameter), the difference is negligible[13]. However, the percentage of small cells that were lost was not significantly different between the methods, confirming Dyken's finding[14].

Concerning the impact of CSF WBC count on the rates of neoplastic cells detection by cell concentration methods, there was no difference in the rate of neoplastic cell detection between the two methods with respect to the CSF WBC count. However, the percentage of positively identified malignant cells was higher by the cytocentrifuge method when the WBC count was normal and when it was greater than 200 cells/mm^3. The majority of samples in this series had a CSF WBC count within the normal range (WBC < 5 cells mm^3). Cerebrospinal fluid samples with a normal WBC count range are regularly seen in cases with a suspected malignant CNS infiltration, particularly in cases of leukemias or lymphomas.

The most useful laboratory test and gold standard for diagnosing neoplastic meningitis infiltration is CSF investigation[1]. However, CSF cytology is highly disease specific, with a diagnostic sensitivity up to 45% when the patient presents with negative cytology on initial examination[1,15]. Sensitivity increases to 80% with a second CSF examination, but cannot be enhanced significantly by further lumbar punctures[16].

The preparation of the cell sediment is one of the more difficult technical components of the cell enrichment process, because it is subject to a number of potential disturbances. The literature is controversial regarding which technique is optimal for cellular identification[5]. The cell sedimentation method is considered the best method for the preservation of cellular structure and is superior, in this respect, to all methods involving filtration or centrifugation[7,9,17]. Sedimentation also permits the best cytological differentiation of the CSF cells[11].

Both sedimentation and cytocentrifugation have advantages and disadvantages. Sedimentation methods can result in a 50% to 80% cell loss[12,18], and it is not known whether one cell type is affected more than another in this loss. While some studies have shown that lymphocytes are disproportionately lost with the sedimentation method[17], the morphology of the cells is better preserved with this method. However, the centrifugation method has deleterious effects on more fragile cells[14].

Centrifugation methods are subject to error due to the distortion and fragmentation of cells. Large numbers of leukocytes are destroyed by cytocentrifugation, limiting the ability to accurately identify malignant cells, because cellular morphology is altered extensively. In this study, the main indication for neoplastic cell search was ALL. The results of our study suggest that the cytocentrifuge was a better concentration method for CSF samples with this indication. The cytocentrifuge also is preferred in studying meningeal leukemia because this method allows for a better correlation between the cells found in the CSF and peripheral blood[19,20]. It should be noted that in the present study, the preservation of cells was measured by the observation of cellular detail but was not reported herein.

The CSF volume needed for analysis in the Suta sedimentation chamber is between 0.5 and 1.5 mL and the preparation time is 30 to 40 minutes. In contrast, cytocentrifugation requires only 200 μL of CSF for analysis, and the preparation time is four minutes. The volume of cells concentrated by cytocentrifugation is smaller than the volume concentrated by the Suta chamber, which decreases the time required to analyze the slide for malignant cells. In this study, we did not evaluate the time required to analyze the slides. With the increased number of WBCs, a smaller volume of CSF is necessary. An advantage of both methods is that each allows for hematological staining after concentrating the cells.

In addition to the concentration method, several pre-analytical steps are important to enhance the chances of tumor cell identification. For example, large volumes of CSF samples should be used, and samples should be carried to the laboratory immediately after the lumbar puncture in order to minimize cell distortion or lysis[17]. It is preferable for slides to be prepared within 30 minutes of CSF collection, as 1/3 of the cells, mainly neutrophils as well as malignant cells that have altered stability, disintegrate within 24 hours.

The rate of positive identification of malignant cells in the CSF varies in the literature and is assumed to depend on several factors, including the method used to concentrate the CSF[21]. Other factors that influence the ability to detect malignant CSF cells include the type of neoplasm, anatomic location of the neoplasm, presence of meningeal involvement and meningeal extension, and number of malignant cells in the CSF[21,22]. Primary cerebral tumors that exfoliate cells to the CSF were all located adjacent to the ventricle. In contrast, cells from tumors deeply localized in cerebral parenchyma are more difficult to detect in the CSF[23,24].

Currently, three methodologies prevail to generate data for counting and differentiating cells in body fluids. These methods are manual microscopy, automated flow cytometry, and automated impedance technology. Traditional manual microscopy is the gold standard. Automated cell analyzers have been able to generate automated counts of cells present in CSF samples in recent years, although most cannot provide reliable counts of the low cell levels usually present in the CSF, including the normal WBC counts that are frequent in CNS malignant infiltration[8,25]. The disadvantages of this method include high imprecision in low ranges (depending on the method) and interfering factors, which reinforces the importance of traditional methods and the necessity of establishing the best concentration method[3].

Flow cytometry used in combination with conventional cytology can lead to a significant increase in the detection rate of leptomeningeal infiltration of malignant cells and is therefore of value in detecting these diseases[4,7,26,27]. The potential application of flow cytometry to the CSF study is more limited, however, because cell concentration is low in normal CSF, and the WBC concentration in particular is generally no more than 1/1000 that of blood. Ancillary techniques such as flow cytometry are of increased importance but their use is restricted to specific or large laboratories. Concentration methods remain important, and to define how to best handle CSF specimens to obtain the highest sensitivity and specificity for the diagnosis of malignancy remains an important issue.

The strength of this study is the substantial number of cases that were analyzed. The main limitation of this study is the lack of an optimal gold standard. Although only one method was used for each sample, the groups were comparable over time in several characteristics already reported. This is a descriptive comparative study, due to the small volume of CSF samples sent to the laboratory and because of this, there was no split of the samples for analysis. This could be a design bias. The sample size for non-hematologic cases is small. Furthermore, the preservation of cellular morphology was not reported in this study, and this is an important feature when identifying malignant cells. Other issues not studied were the analysis of differences in the objective assessment of intra and inter-observer slides.

In conclusion, the positive rate of identification of malignant cells in CSF was slightly higher when the CSF sample was concentrated by cytocentrifuge than Suta chamber. If the number of CSF WBCs was within the normal range, there was no difference between the methods.

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