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FACTORS ASSOCIATED TO THE POSITIVE CEREBROSPINAL FLUID CULTURE IN TUBERCULOUS MENINGITIS

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ABSTRACT - Central nervous system involvement is the most common neurological complication in the course of tuberculosis. The lack of rapid and sensitive tests delays the early diagnosis. Here, we retrospectively reviewed the cerebrospinal fluid (CSF) examination of 30 patients with tuberculous meningitis confirmed by bacteriological tests (culture and/or polymerase chain reaction). The purpose of the present study was to determine the CSF parameters associated to the positive CSF culture for *Mycobacterium tuberculosis* in tuberculous meningitis. We found higher frequency of positive CSF culture in patients infected with HIV as well in patients with high number of neutrophils and high protein content (characteristic in the early or acute-stage patients), which suggests that the positive culture found in these patients may be associated with the presence of high bacillary load in CSF occurring in these stages.

KEY WORDS: tuberculous meningitis, cerebrospinal fluid, HIV, *Mycobacterium tuberculosis,* culture.

Fatores associados à positividade da cultura do líquido cefalorraquidiano na meningite tuberculosa

RESUMO - A meningite tuberculosa é a complicação neurológica mais freqüente no curso da tuberculose. Entretanto, a carência de testes rápidos e sensíveis dificulta o diagnóstico precoce, contribuindo para o elevado índice de letalidade desta condição. Na presente análise, é feita revisão dos achados do líquido cefalorraquidiano (LCR) de 30 pacientes com o diagnóstico de meningite tuberculosa confirmado pelo exame bacteriológico. O objetivo do estudo consiste em caracterizar os parâmetros associados à positividade da cultura para *Mycobacterium tuberculosis* no LCR. Observamos maior freqüência de cultura positiva entre os pacientes infectados pelo HIV e naqueles que apresentam aumento de neutrófilos e da concentração de proteína no LCR. Nossos achados se justificam pelo fato de que na co-infecção com o HIV ocorre maior carga bacilífera em comparação aos pacientes não co-infectados. A presença de neutrofilorraquia e hiperproteinorraquia são marcadores de inflamação aguda, onde se supõe existir também maior concentração de bactérias no LCR.

PALAVRAS-CHAVE: meningite tuberculosa, líquido cefalorraquidiano, HIV, cultura, *Mycobacterium tuberculosis.*

Tuberculous (TB) meningitis is a challenge for public health authorities around the world. In countries with high levels of poverty, the situation is even more serious. Around 30 percent of cases with TB meningitis result in death[1]. Mortality is even greater in individuals also infected by HIV[2,3].

The high mortality rate associated to TB meningitis is related especially to its late diagnosis. It is a result of: the similarity between its clinical manifestations to those of chronic infections of the central nervous system (CNS), such as neurocysticercosis, neurobrucellosis and cryptococcal meningitis[4,5]; the lack of quick and sensitive diagnostic tests; and the resistance to antituberculosis agents. The neurological complications that occur in survivors are common and include: decreased intellectual capacity, psychiatric disorders, recurring seizures, visual and oculomotor disorders, deafness and hemiparesis[4].

The aim of this study is to determine the parameters of the cerebrospinal fluid (CSF) that may be associated to a positive culture for TB meningitis. We also conducted a comparison between the results of the CSF exam in patients that have been infected with HIV-1 and those not infected with the virus in order to evaluate whether the state of immunodepression could alter the profile of the CSF, which

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would make the laboratorial diagnosis of the disease even more difficult.

METHOD

Cases with a diagnosis defined as TB meningitis were identified by reviewing 13,000 medical records of patients who were seen at Neurolife CSF Laboratory in the city of Rio de Janeiro, between the period December 1998 to July 2002. The diagnosis of TB meningitis was based on the presence of a medical history compatible with meningitis (headache, nausea, vomiting and signs of irritation of the meninges) and/or focal neurological signs (seizures, motor deficit, paralysis of cranial nerves) associated with the demonstration of the *Mycobacterium tuberculosis* agent[4,6] through bacterial culture exam (Lowenstein–Jensen medium) or polymerase chain reaction (PCR). The diagnosis of infection by HIV was confirmed with a seropositive demonstration for HIV (ELISA test confirmed by Western blot). The study was submitted to and approved by the Ethics and Research Committee.

All patients were submitted to a routine exam of the CSF, including: total and specific cytology with cytosedimentation using the Suta sedimentation chamber; determination of the protein and glucose concentrations by spectroscopy; microbiological study (direct test and exam of the culture for common germs, *Mycobacterium tuberculosis* (BK), fungi, latex for common germs and fungi); and immunological reactions to syphilis (VDRL) and HIV (ELISA). All patients with a negative culture for *Mycobacterium tuberculosis* were submitted to a PCR test for the bacterial genome.

Statistical analysis – With the aim of verifying whether there were a significant difference in the cytology and biochemical measurements between patients who were seropositive for HIV and those who were negative as well as between those with a positive and negative culture, we conduced a Mann-Whitney test (a non-parametric test), since the variables did not show normal distribution (Gauss distribution). The criterion for determining significance that was used was a level of 5%, that is, if the value of *p* in the statistical test is smaller or equal to 0.05, it is statistically significant. The statistics software SAS System processed the statistical analysis.

Table 1. Correlation between HIV infection and positive BK culture in CSF of 30 patients with tuberculous meningitis.

Culture results	HIV+	HIV–	Total
Group A - positive culture for BK in CSF	16 (73%)	6 (27%)	22
Group B -negative culture for BK in CSF	0	8 (100%)	8

RESULTS

Of the total group, 30 (0.23%) fulfilled the established criteria for the diagnosis of tuberculous meningitis, of which 22 (73%) displayed a positive culture for *Mycobacterium tuberculosis* (group A) (Table 1). The remaining eight patients (group B), who had displayed a negative culture, turned out positive for BK in the PCR test. The direct exam (BAAR) was negative for all patients. There was no difference between the average and standard deviation of the age distribution between both groups: 47±15 years of age

Table 2. CSF findings in 30 patients with tuberculous meningitis, according the bacteriological positive (Pos)/negative (Neg) culture results.

	Culture	n	Median	Minim	Maximum	p value
Total leucocytes/mm^3	Pos	22	132	1	1070	0.17
	Neg	8	65,5	2	960	
Neutrophils (%)	Pos	22	72	0	93	**0.0007**
	Neg	8	5,5	0	33	
Lymphocytes (%)	Pos	22	17	4	90	**0.004**
	Neg	8	69	47	89	
Monocytes (%)	Pos	22	7	1	15	**0.0006**
	Neg	8	12	10	35	
Eosinophils (%)	Pos	22	0	0	1	*0.066*
	Neg	8	0,5	0	3	
Macrophages (%)	Pos	22	0	0	5	0.25
	Neg	8	1	0	12	
Protein (mg/dL)	Pos	22	150	24	840	**0.014**
	Neg	8	63,5	40	200	
Glucose (mg/dL)	Pos	22	32	5	136	0.17
	Neg	8	46,5	7	63	

Table 3. CSF findings according the presence or absence of HIV infection.

	HIV	n	Median	Minim	Maxim	p value
Total leucocytes/mm^3	Pos	16	132	1	1070	0.61
	Neg	14	106.5	2	960	
Neutrophils (%)	Pos	16	69.5	0	93	0.12
	Neg	14	27.5	0	86	
Lymphocytes (%)	Pos	16	22.5	6	90	0.26
	Neg	14	51	4	89	
Monocytes (%)	Pos	16	7.5	1	15	0.18
	Neg	14	10.5	4	35	
Eosinophils (%)	Pos	16	0	0	1	0.1
	Neg	14	0	0	3	
Macrophages (%)	Pos	16	0	0	5	*0.075*
	Neg	14	1	0	12	
Protein (mg/dL)	Pos	16	147	24	840	0.58
	Neg	14	115	40	580	
Glucose (mg/dL)	Pos	16	33.5	5	136	0.67
	Neg	14	39	7	63	

for group A and 42±27 years of age for group B (p>0.05). Whereas in group A, 16 patients (73%) showed positive serology for HIV, in group B, 100% were seronegative (Table 1). All the patients with positive serology for HIV showed a positive BK culture.

Of the total group, two patients presented normal cytological and biochemical CSF exams: one patient from group B (negative culture/positive PCR) had negative serology for HIV and, the other from group A (positive culture), presented positive serology for HIV. Most of the patients (28/30) presented neutrophils in the CSF. For the patients in group A, the median proportion of neutrophils in the CSF was 72% (0-93) and for group B, 5.5% (0-33) (p>0.05) (Table 2). The number of leukocytes and the percentage of monocytes in group A were significantly lower than those in group B (Table 2).

In relation to the comparison of the findings of the CSF in patients with or without HIV infection, there was no statistical difference in the cytological and biochemical values between both groups (Table 3). Of the total group, around half of the patients with positive serology (9/16) and negative serology (5/14) for HIV showed a proportion of neutrophils in the CSF above 50% in the differential cell count. However, if we consider the six patients who had negative serology for HIV and positive culture for BK in the CSF, five of these patients had more than 50% neutrophils in the CSF.

DISCUSSION

TB meningitis is caused by the *Mycobacterium tuberculosis,* an acid-fast microorganism, and, in rare cases, by *Mycobacterium bovis.* The disease is a result of the bacterial dissemination originating in another location in the body[4]. According the Rich's description, in the initial phase of the disease the formation of meningeal tubercules which are secondary to the spread of the agent in the meninges and in the subpial regions of the brain. The bacterial is released in the subarachnoid space right after the break of one or more of these tubercules[4]. There is also a hypothesis that miliary bacterial dissemination occurs with a direct invasion of the bacillus in the choroid plexus or in the meningeal ducts[4].

The clinical manifestations set in slowly in the course of three weeks. Signs of meningeal irritation (stiffness of the neck, Kernig and Brudzinski signs), headache, low fever, anorexia and irritability appear already in the first week. After this initial period, signs of localization and high intracranial pressure appear, followed by paralysis of the III, VI and VII cranial nerves and seizures. After this, the patient evolves into a coma state with high fever. If the treatment is not initiated promptly, death occurs after five to eight weeks counting from the beginning of the symptoms[1,4].

An exam of the CSF in TB meningitis evidences an increase in pressure, a slightly turbid aspect with clot formation, pleocytosis of 10–500 leucocytes/mm^3,

with a predominance of lymphocytes. The CSF protein concentration is higher than normal (100-500 mg/dL) and increases even more if the flow of the CSF is obstructed[7,8]. Glucose levels are reduced, with levels below 40 mg/dL and the concentration of lactate is increased (>19 mg/dL). The lactate is not specific for tuberculous meningitis but is a good marker of cerebral metabolism in bacterial and fungal meningitis. It has no influence of blood concentration, as occurs with the glucose, and is a product of glucose metabolism in CSF[9]. Atypical findings in the CSF such as a concentration of neutrophils and normal exam results may contribute to making the diagnosis more difficult[7]. This is exacerbated due to the low sensitivity of the direct exam[10].

A concentration of neutrophils in the CSF or a normal biochemistry analysis occur in the initial and acute phases of the infection. In a previous study[7], a normal cytological exam of the CSF (<5 cells/mm^3 with an absence of neutrophils) was seen in 21.4%, a predomination of neutrophils in 39% and, 5.4% had a positive bacilloscopy. In our study, 6% of the patients had a CSF exam with normal cytology and biochemistry: one case was seropositive for HIV and, had positive BK culture in the CSF and, the other had negative serology for HIV and negative culture/positive PCR test for BK. On the other hand, in 94% of the cases, we found neutrophils in the CSF. The highest proportion of neutrophils was predominant in the CSF of patients with a positive culture for BK. There was no difference in the neutrophils count, as in other parameters of the CSF, except for the positivity of the culture, between patients with positive serology for HIV and those with negative serology for HIV. All cases with HIV infection had positive BK culture in CSF. From the seronegative HIV patients, six had positive CSF culture and, eight were negative. This finding showed that, in this study, the highest neutrophils count was associated to the positivity of the BK culture in the CSF, regardless of the presence or absence of infection by HIV. On the other hand, the differentiating factor between the patients with positive and negative BK cultures in the CSF was in the predominance in the total number of neutrophils in the CSF, not merely in its presence. It was suggested that pleocytosis with a predominance of neutrophils in the CSF could be associated to the increased probability of positivity in the bacteriological diagnosis[10].

The *Mycobacterium tuberculosis* may oftentimes lead to persistent neutrophilic meningitis in patients that are HIV positive. This is characterized by the initial and persistent presence (with a minimum interval of seven days) of neutrophilic pleocytosis (50%), high spinal fluid protein concentration (>40 mg/dL) and low spinal fluid glucose concentration (<2/3 glycemia). Hypothetically, in the case of HIV infection, the fundamental immunocellular response to control tuberculosis is not at full capacity and this could allow for the release of chemotaxic factors for neutrophils and a persistence of the response in the acute phase[11]. The type of cell response can have a prognostic effect. A higher percentage of neutrophils was associated to higher survival rates[12]. It has been suggested that neutrophils may have a protective role against *Mycobacterium tuberculosis.* As ours is a retrospective study, it was not possible to evaluate the survival rates of our patients. The slow course of TB meningitis in patients infected with HIV may also be associated to the use of other drugs that interfere with the levels of tuberculostatic agents in circulation or the presence of strains that are resistant to the medication[11].

The direct exam, using the Ziehl-Neelsen stain (BAAR), should be conducted, despite the fact that the CSF is paucibacillary and that there is low levels of positivity in the test. It is simple to execute and allows for an immediate confirmation of the diagnosis. Most researchers mention a sensitivity range that varies between 10% and 40%[10,12]. However, when larger volumes of CSF (>5 mL) and/or serial samples are analyzed, the positivity may reach 87%[6,13-15]. On the other hand, the sensitivity of the direct exam drops from 52% to 2% after approximately 5-15 days from the beginning of treatment[10]. In our caseload, the patients showed negativity in the direct exam, which was probably related to the small volume of the sample that was analyzed and could also be related to the detailed exam above-mentioned was not performed.

The isolation of mycobacterias in the Lowenstein-Jensen culture in the CSF represents the gold standard for the diagnosis of TB meningitis. Nevertheless, due to the slow growth (30 to 60 days) this exam is useful only from an epidemiological point of view, not a clinical one. The radiometric method (BACTEC) detects the growth of mycobacteria by measuring the levels of $14CO_2$ released. This method is more sensitive and the bacterial growth can be verified in approximately 14 days[8].

The PCR test has variable sensitivity and specificity. In some studies, the sensitivity is low (56-70%), while specificity is high (98-100%)[10,12]. It is extremely important to conduct a PCR test in situations where

bacilloscopy of the samples come out negative, as it contributes to the early diagnosis and swift application of the appropriate treatment.

Previous reports of patients with tuberculous meningitis did not show a significant change in the cytological and biochemical parameters in terms of co-infection with HIV[2,3]. However, differences have been reported in bacteriological tests of patients co-infected with HIV in relation to those not infected. In the case study by Dubè et al.[2], despite not having statistical value, the direct exam for BK was positive in 27% of patients co-infected and only 6% of those not co-infected with HIV[2]. Some authors suggest that the patients co-infected with HIV present more intense bacterial proliferation and greater concentrations of bacilli in the meninges than do patients that are not co-infected[10,16]. In our caseload, we observed a direct correlation between positivity for *Mycobacterium tuberculosis* in the bacterial culture and co-infection with HIV.

Chandramuki et al., certified that anti-*M. tuberculosis* antibodies were found more frequently in patients clinically diagnosed with a negative culture when compared to those who tested positive to the culture[14]. Based on these data, the authors suggested that there might be in inverse relation between the presence of antibodies in the CSF and an increase in the bacillary load for BK. In this same case study, patients who were co-infected with HIV showed a weaker antibody response in the CSF than those who were not co-infected[14].

Twaites et al., made a list of some of the factors involved in a greater accuracy of the bacteriological confirmation of the CSF for TB meningitis: at least 6 mL of CSF, direct exam of the smear for at least 30 minutes, the presence of neutrophils, and an increase in lactate in the CSF[6].

The adenosine deaminase (ADA) activity in the CSF may be used as a screening test. It is increased in the CSF of TB meningitis. But it is not specific because may be found in high levels in other bacterial meningitis, lymphoma, neurobrucellosis and cryptococal meningitis[17].

The changes in the CSF respond slowly to treatment for TB meningitis (6 to 12 months). Twaites et al, studied pro- and anti-inflammatory cytokines in serial samples of CSF and peripheral blood of 21 patients diagnosed with TB meningitis[13]. They found that high concentrations of lactate, o interleukin-8 and gamma-interferon steadily decreased in amount after the beginning of the specific treatment. On the other hand, the dysfunction of the blood-CSF barrier and immunoactivation remained constant after 60 days of treatment. Pleocytosis (>5 cells/mm^3) was found in 62% of patients after nine months of treatment and high spinal fluid protein concentration (>40 mg/dL) was found in 38% of patients, while the decreased ratio between CSF glucose and that of the blood was found in 6% of cases. The levels of TNF-alpha were persistently high in the CSF in TB meningitis, which could represent the primary biological factor responsible for maintaining the inflammatory process. The high initial concentration (during the first seven days of treatment) of lactate in the CSF, the low leukocyte count and the glucose concentration were all associated to a higher death rate. Lactate production is secondary to the phenomenon of hypoxia, which results from obliterative vasculitis and ischemic infarction which occur in TB meningitis. The high concentration of lactate may represent a good prognostic marker for this disease, due to the evidence that it has a correlation with the seriousness of the process.

In the present study, we found that the factors associated to positivity in the CSF culture in TB meningitis consisted of: the presence of co-infection with HIV, high spinal fluid protein concentration and larger proportions of spinal fluid neutrophil in the CSF (markers of the acute phase), probably associated to a higher bacteremia. According to the literature, other factors associated to a greater positivity in the bacteriological diagnosis include a greater volume of CSF and a longer microscope analysis time. It is important to emphasize that the CSF exam in TB meningitis can present, in a small minority of cases, atypical findings such as normal cellular or biochemical results or persistent spinal fluid neutrophil concentrations. These parameters should not be used as exclusion criteria during diagnosis. On the other hand, the inflammatory alterations may persist for a period greater than nine months after the beginning of the treatment for complete normalization[10]. This study reinforces the need for exams with greater sensitivity and specificity and that can allow for an early diagnosis of TB meningitis, thereby avoiding the delay and lack of diagnosis and reducing lethal outcomes.

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