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Meningeal cryptococcosis and SARS-CoV-2 infection in people living with HIV

Criptococosis meningea e infeccción por SARS-Cov 2 en personas que viven con VIH

Cryptococosis and SARS Cov 2 coinfection

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Todos los autores participaron en la revisión del manuscrito.

Introduction: Fungal infections in patients with COVID-19 was one of the most debated topics during the pandemic.

Objectives: To analyze the clinical characteristics and evolution of people living with Aids (PLWHA) who presented the association of meningeal cryptococcosis and COVID-19 (group A) and compare them with those PLWHA who suffered from meningeal cryptococcosis without COVID-19 infection (group B).

Materials and methods: An analytical and retrospective study was carried out in which the medical records.

Results: Sixty five PLWHA with cryptococcosis diagnosed from April 2020 to May 2021 were studied. Out of these, a total of 15 PLWHA suffered from cryptococcosis and COVID-19, 14 presented meningitis (group A). Twenty-eight patients suffered from meningeal cryptococcosis and didn't have covid19 (group B).

Conclusions: No significant differences were observed in relationship to intracranial hypertension, initial antigenorrachia, sensorium deterioration or mortality between both groups.

The detection of *Cryptococcus* antigen (CrAg) in serum by lateral flow assay (LFA) was of great effectivity to rapidly diagnose PLWHA with COVID-19. It was also observed that patients in both groups were late in their consultation compared to pre-pandemic cases.

Keywords: COVID-19; SARS-Cov-2; *Cryptococcus*; meningitis, cryptococcal; coinfección; HIV infections

Introducción. Las infecciones fúngicas en pacientes con COVID-19 fue uno de los temas más debatidos durante la pandemia.

Objetivo. Analizar las características clínicas y evolución de las personas viviendo con sida (PVVS) que presentaron la asociación de criptococosis meníngea y COVID-19 (grupo A) y compararlas con aquellas PVVS que padecieron criptococosis meníngea sin infección por COVID-19 (grupo B).

Materiales y métodos. Se realizó un estudio analítico y retrospectivo en el que se revisaron las historias clínicas de pacientes que padecieron criptococosis meníngea entre abril de 2020 y mayo de 2021.

Resultados. Se estudiaron 65 PVVS con criptococosis diagnosticadas entre abril de 2020 y mayo de 2021 (63 eran PVVS y 2 eran HIV (-)). De estos, un total de 15 PVVS padecían criptococosis y COVID-19, 14 presentaban meningitis (grupo A). veintiocho enfermos padecieron criptococosis meningea y no tuvieron covid19 (grupo B)

Conclusiones. No se observaron diferencias significativas en relación a hipertensión intracraneal, antigenorraquia inicial, deterioro del sensorio o mortalidad entre ambos grupos.

La detección de antígeno de *Cryptococcus* (CrAg) en suero por LFA fue de gran efectividad para diagnosticar rápidamente a las PVVS con COVID-19. También se observó que los pacientes de ambos grupos llegaron tarde a su consulta en comparación con los casos prepandemia.

Palabras clave: COVID-19; SARS Cov-2; *Cryptococcus*; meningitis criptocócica; coinfection; infecciones por VIH.

In December 2019, a previously unknown new coronavirus, named SARS-CoV-2 was identified for the first time in Wuhan, China. Later it caused the epidemic of atypical pneumonia (COVID-19; CoronaVirus Disease 2019) (1,2). Due to its speed of expansion and severity, on March 11, 2020, the World Health Organization (WHO) declared the pandemic (3).

The start of the pandemic raised the question about the evolution of Sars-Cov2 infection in people living with HIV-AIDS (PLWHA), mainly in those without antiretroviral treatment (HAART) and with low CD4+ T lymphocyte counts. In relation to this, these patients were initially considered at risk (4,5) of suffering from severe clinical forms. With the course of the pandemic, the results of studies on this subject were largely contradictory (6).

The association between fungal infections and COVID-19 has been the subject of multiple publications, especially for cases of aspergillosis, candidiasis, and mucormycosis (7-9). However, although the association of COVID-19 and cryptococcosis was reported in HIV-negative patients treated with immunomodulators (10) and in patients with autoimmune diseases (11), the number in PLWHA was low and in general, they were excluded of the most recent reviews (12).

On the other hand, meningeal cryptococcosis in PLWHA is still one of the most frequent and serious opportunistic diseases in South America (13,14) as well as in the rest of the world (15).

The objectives of this study were analyzing the clinical characteristics and evolution of PLWHA who presented the association of meningeal cryptococcosis and COVID-19 (group A) and to compare them with those

PLWHA who suffered from meningeal cryptococcosis without COVID-19 infection (group B).

Materials and methods

An analytical and retrospective study was carried out in which the medical records of 65 PLWHA with cryptococcosis diagnosed between April 2020 and May 2021 in the Mycology Unit of our hospital were analyzed.

Inclusion criteria*:*

- PLWHA with meningeal cryptococcosis.

Exclusion criteria:

- HIV-negative patients with cryptococcosis
- PLWHA with extra-meningeal cryptococcosis.

Data on age, sex, signs and symptoms, comorbidities, antiretroviral treatment, physicochemical study of cerebrospinal fluid (CSF), opening pressure, antigenic score, lymphocyte subpopulation count, and risk factors for HIV infection were recorded.

The HIV-positive patients who met the inclusion criteria were divided into 2 groups. Group A included the patients who suffered from COVID-19 and group B the patients who did not (figure 1).

Mycological evaluation

Blood culture by lysis centrifugation was practiced on every PLWHA patient. The samples were cultured in Sabouraud-honey agar medium at 28°C and brain-heart infusion (BHI) agar at 37°C for 3 weeks, which were controlled twice a week (16,17). In addition, *Cryptococcus* antigen detection in serum was

performed by lateral flow assay (CrAg LFA) (IMMY, Norman Kew Surrey, OK, USA) in patients with TCD4+ lymphocyte count <200 cells/µl (18-20).

Patients with respiratory symptoms or having images compatible with pulmonary cryptococcosis were asked to take a respiratory sample (sputum or bronchoalveolar lavage) for mycological examination. Likewise, patients with initial suspicion of central nervous system (CNS) involvement were evaluated by mycological examination of CSF.

All patients with positive CrAg LFA, cryptococcemia or pulmonary cryptococcosis underwent lumbar puncture to rule out or confirm meningeal involvement through mycological examination of CSF.

All CSF samples were microscopically tested with Indian ink and cultured on Sabouraud-honey agar, sunflower seed agar, and BHI agar, incubated at 28°C and 37°C for 15 days. Cultures were observed daily seeking for colonies compatible with *Cryptococcus* spp.

Mycological control of patients with meningitis was performed with CSF cultures at 2-3 weeks after diagnosis and was repeated until a negative result was obtained.

Identification tests

The phenotypic typing of the isolates was carried out by studying their growth capacity at 37 °C, the presence of capsule, urease activity and phenol-oxidase production in sunflower seeds agar. Differentiation between *Cryptococcus neoformans and Cryptococcus gattii* was performed by the growth in canavanine -glycine-bromothymol blue (CGB) and Salkin 's medium (cycloheximide -glycine-phenol red) (16). Typification was confirmed by mass

spectrometry (Vitek® MS). Strains were also genotypically studied by amplification of the Ura 5 gene by PCR and subsequent RFLP of Ura 5 with Sau96I and HhaI restriction enzymes.

Semi-quantitative detection of Cryptococcus capsular polysaccharide antigen (CrAg)

Blood samples were taken from all patients at the time of diagnosis. Serum was separated and kept at -20°C until the time of determination.

The semiquantitative determination of CrAg in both serum and CSF was carried out using latex agglutination (LA) technique (Crypto Latex ®, IMMY, Norman Kew Surrey, OK, USA) according to the manufacturer's instructions. Undiluted serum and 1:10; 1:100; 1:1000; 1:5,000 and 1:10,000 dilutions were used to determine titer.

Coinfection Cryptococcosis meningitis-COVID-19

COVID-19 coinfection was considered in patients who presented a positive qRT-PCR for SARS-CoV-2 in nasopharyngeal swab within 30 days of meningeal cryptococcosis having been diagnosed. Patients who persisted with development of *Cryptococcus* in CSF culture and intercurred with COVID-19 diagnosed by qRT-PCR positive for SARS-CoV-2 using nasopharyngeal swab were also included.

Laboratory parameters

Erythrocyte sedimentation rate (ESR) was evaluated on VES Matic Cube 300 (Diesse); D-dimer (DD) was determined in a VIDAS autoanalyzer (ELFA method: enzyme-linked fluorescence assay); C-reactive protein (CRP), Ferritin

and lactic dehydrogenase enzyme (LDH) were analyzed on the Cobas C311 analytical platform (immunoturbidimetry and UV kinetic method for LDH). The leukocyte count was performed in the Sysmex XN-1000 hematology counter (optical/fluorescence impedance method). This can be used as an immune response severity index. Also, certain types of leukocytes such as neutrophils (N), lymphocytes (L) and the ratio between the two (RNL) are useful markers of systemic inflammation.

Severity criteria in meningeal cryptococcosis

As severity criteria, the following were evaluated in both groups to establish whether the two populations were homogeneous and comparable: intracranial hypertension, antigenorrachia ≥ 1/5000, impaired sensorium and TCD4+ lymphocyte count < 100 cells/mm3.

Statistical analysis

Continuous variables were expressed as mean or median as appropriate. Student's t-test or the Mann-Whitney-Wilcoxon test were used to evaluate the statistical differences between groups A and B. Categorical variables were expressed as percentages; and Fisher's exact test was used. Differences were considered significant when the p value was <0.05 and Statistix® 8.0 software was used for analysis.

Ethical considerations

The study was conducted in accordance with the protocol and the standards of good clinical practice established by the research ethics committee of the FJ Muñiz Hospital.

Results

A total of 15 PLWHA suffered from cryptococcosis and COVID-19, 14 had meningitis and only 1 had positive antigenemia by LFA with no other finding. Regarding gender, 9 were men and 6 women. The median age was 26.5 years (range: 23-60).

None of the patients showed good adherence to HAART. Two of them were aware of their immunocompromise at the time of hospitalization and the remaining 12 despite knowing their disease were not under HAART.

Four patients had pulmonary compromise compatible with COVID-19 (figure 2), out of whom three required mechanical ventilation which ended in decease.

The 14 PLWHA with meningitis showed a brain tomography without signs of lesions in the parenchyma. None presented comorbidities related to severe COVID-19 (obesity, hypertension, heart failure, or diabetes). However, 13 of them had less than 100 LTCD4+ cells/µL.

Four patients had positive blood cultures for *C. neoformans.* Pulmonary commitment by *C. neoformans* was not confirmed in any patient, although pathological images suspicious for this mycosis were obtained in 3 of them.

The 3 patients who died had positive CSF cultures for *C. neoformans* at the time of death. Nevertheless, it was impossible to establish whether they died from COVID-19 and/or cryptococcosis.

All isolates corresponded to *C. neoformans* VNI, according to phenotypic and genotypic identification.

Increased lactodehydrogenase (LDH), dimer D and lymphopenia were the most frequent laboratory parameters in patients with covid and cryptococcosis (table

1). On the other hand, in no Covid patients the lymphocytes count was not registered. Clinical and microbiological parameters related to severe CM were similar in both groups. Only more patients with dyspnea were observed in the group with covid and CM (group A) (table 2), probably because the lung is the main organ involved in patients with covid19. Although the latter did not modify mortality.

The main signs and symptoms of patients with meningeal cryptococcosis and COVID-19 in both groups are shown in table 2.

Discussion

SARS-CoV-2 infection can cause asymptomatic clinical pictures as well as highly variable presentations. From myalgia, arthralgia and anosmia, to severe bilateral pneumonia (21). As it occurs in cryptococcosis, in PLWHA with COVID-19 fever and headache were the most frequently observed symptoms (9). For this reason, in the pandemic context, in many patients the symptomatology could have been attributed to COVID-19 and thus the fungal picture overlooked.

Fortunately, CrAg LFA detection has been performed routinely for some years in patients with a LTCD4+ count ≤ 200 cells/µL prior to starting antiretroviral treatment to avoid syndrome immune reconstitution (20). In the same way, it has been used in patients with a low TCD4+ lymphocyte count when admitted with suspected opportunistic infection (13,15,18). This provides a rapid diagnosis and the possibility of detecting meningitis that otherwise, due to its scarce CNS-focused symptoms, could arrive late. As it can be seen in figure 3 most patients had a diagnosis of *Cryptococcus* meningitis close to the

nasopharyngeal admission swab was performed. Only in a few cases of Covid infection occurred during hospitalization (figure 3). Only one patient was diagnosed with meningeal cryptococcosis after 3 weeks since he was referred from a less complex center that did not have CrAg LFA test available.

Other cases undergoing meningeal symptoms with positive CSF culture, presented coinfection due to an intranosocomial outbreak of COVID-19.

We detected an increase in the percentage of patients with HTE [28/42 (66%)] and antigenorrachia ≥ 1/5000 [25/42 (59.5%)] in both groups in correlation with other pre-pandemic periods [HTE: 22/62 (35.5%)] [antigenorrachia ≥ 5000: 23/127 (18.1%)] (22) This could be due to late consultation. However, considering pre-pandemic meningeal cryptococcosis mortality (20.31%) and the one observed during the pandemic (21.4%), no significant differences were found (22).

Numerous authors support that the hyperinflammatory immune response induced by the virus plays a central role in the pathogenesis of the disease, in this work we analyze the prognostic value of certain laboratory biomarkers (BM) in hospitalized patients with COVID-19. The recommendations suggest evaluating inflammatory markers such as: leukocyte differential count, erythrocyte sedimentation rate, D-dimer, Ferritin, C-reactive protein, Interleukin-6 (IL-6), Lactate Dehydrogenase, Procalcitonin (PCT), Troponins and natriuretic peptides (23).

Most severe cases of COVID-19 presented low absolute count of lymphocytes (lymphopenia <1000/mm^3), increased leukocytes, high absolute count of neutrophils and increased RNL. It is important to mention that in this

retrospective study IL-6, PCT and cardiac BM (troponins and natriuretic peptides) were not analyzed.

On the other hand, lymphopenia in patients with COVID-19 was associated with poor prognosis in different publications (24).

Throughout the pandemic, cut-off values for RNL between 3.0 and 6.0 have been reported, however Sayah et.al (25) have proposed RNL values ≥ 5.9 to assess severity and RNL ≥ 7.4 for mortality.

In our work, lymphopenia was observed in the majority of patients. However, it does not seem to have been decisive, probably because the number of patients studied was too small to reach conclusions. Furthermore, we clarify that we could not obtain the lymphocyte counts in patients with CM without covid, being a deficiency of this publication.

A recent meta-analysis of patients with COVID-19 shows that the evolution of PLWHA with antiretroviral treatment (HAART) was similar to that of those with HIV and without HAART (9). In turn, in this study the PLWHA did not have a worse evolution than the HIV-negative patients, despite their degree of immunocompromise. But the evidence regarding the relationship between PLWHA and the increased risk of contracting or presenting severe forms of COVID-19 is still controversial. A recent study reflects those cases of unfavorable evolution were related to comorbidities related to HIV.

A recent review on COVID-19 and cryptococcosis suggests that the use of corticosteroids may be a risk factor for developing this fungal disease. Unlike our cases, the patients mentioned in this review were mostly non-HIV patients

with cardiovascular and metabolic comorbidities. Most of these patients suffered from pulmonary cryptococcosis (26).

However, large prospective cohorts may be needed to differentiate between HIV-positive and HIV-negative patients and thus arrive at definitive recommendations.

Considering those predominant symptoms of both cryptococcosis and COVID-19 may overlap, the detection of CrAg by LFA in serum was of great relevance to rapidly diagnose the former in our patients.

The pandemic generated a late consultation in most of the PLWHA. Despite this, mortality in this period was similar to the one observed in the pre-pandemic.

No differences were observed in the mortality rate of patients with meningeal cryptococcosis with COVID-19 or without coinfection.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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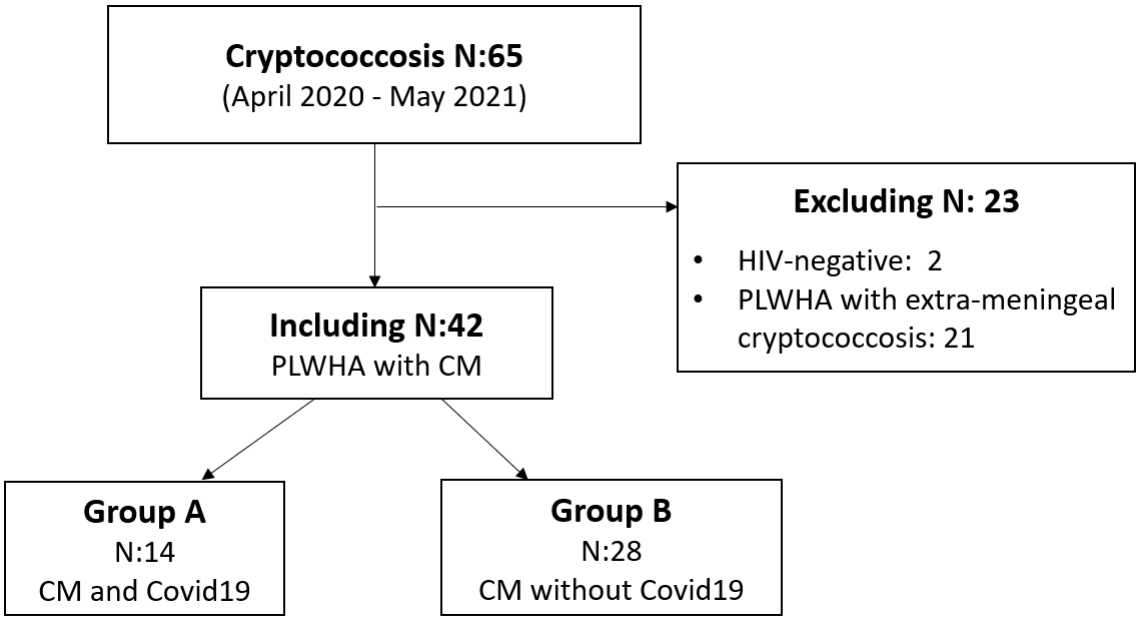
Figure 1. Flow chart with all patients with cryptococcosis (April 2020 – May 2021)



PLWHA: people living with Aids; CM: meningeal cryptococcosis.

Figure 2. Chest tomography without contrast with ground glass lesions (compatible with COVID-19) and a cavitating nodule (compatible with pulmonary cryptococcosis)

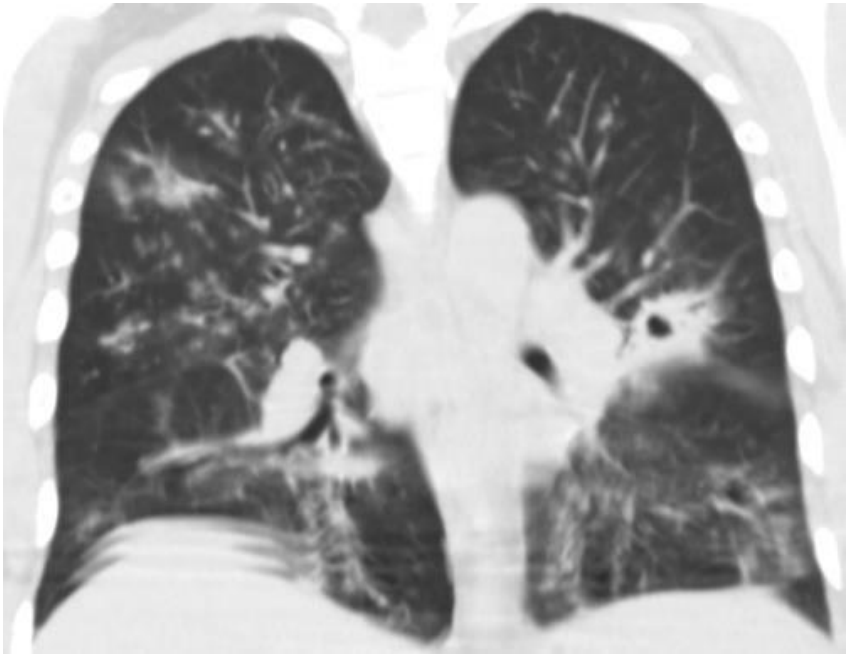


Figure 3. Relationship between the time of diagnosis of cryptococcosis and COVID-19.

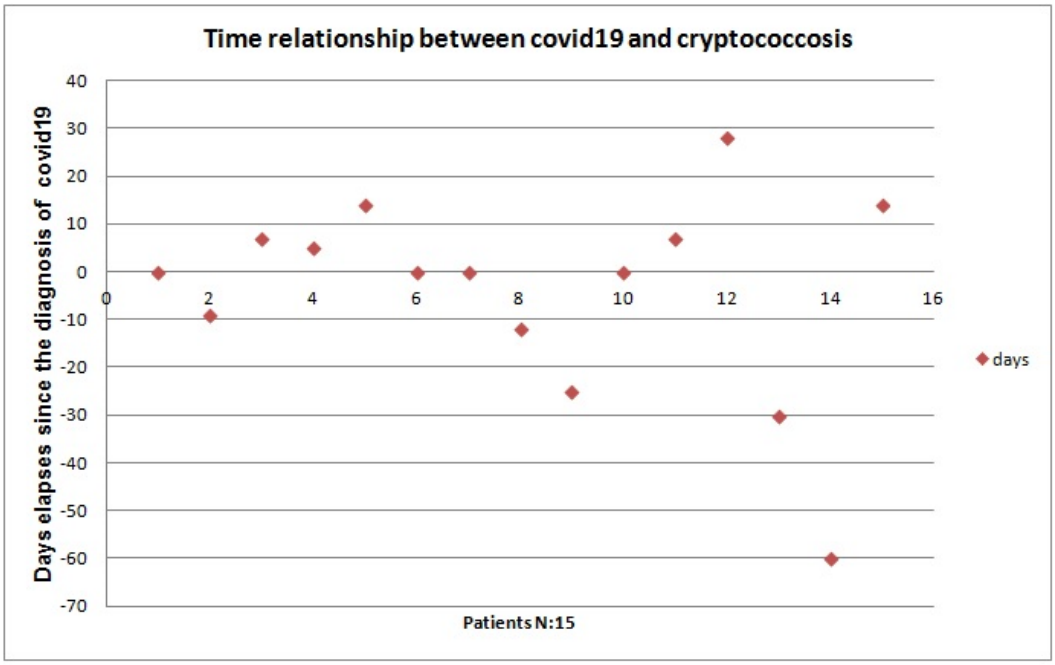


Table 1. Laboratory parameters considered for severe COVID-19 and prevalence found in group A.

	Group A **N: 14**
Neutropenia	1/14
lymphopenia	12/14
NLR ≥ 5.9	7/14
LDH > 280 U/L	11/14
LDH > 600 U/L	2/14
Ferritin >400ng/ml	5/8
D-dimer > 500 ng/mL	8/9
C-reactive protein >5 mg/L	4/5
ERS > 25 mm/h	10/12

NLR: relationship between neutrophils and lymphocytes**; LDH:** lactic dehydrogenase

ERS: Erythrocyte sedimentation rate;

Table 2: clinical and microbiological characteristics of patients with meningeal cryptococcosis (group A patients with COVID-19 and group B patients without COVID-19).

	Group A N: 14 (%)	**Group B N: 28 (%)**	**p**
Fever	**13 (92.8)**	**24 (85.7)**	**0.2775**
headache	**12 (85.7)**	**24 (85.7)**	**0.6471**
vomiting	**11 (78.6)**	**17 (60.7)**	**0.1595**
Dyspnea	**7 (50)**	**2 (7.1)**	**0.0009***
Sensory impairment	**5 (35.7)**	**8 (28.6)**	**0.54**
Cough	**3 (21.4)**	**2 (7.1)**	**0.1507**
Elevated intracranial pressure	**10 (71.4)**	**18 (64.3)**	**0.4629**
CSF CrAg - LA ≥1/5000	**8 (57.1)**	**17 (60.7)**	**0.9763**
LTCD4+ < 100 cells/mm3	**13 (92.8)**	**25 (89.3)**	**0.3912**
Deceased	**3 (21.4)**	**6 (21.4)**	**0.9129**

CSF: cerebrospinal fluid**; CrAg:** cryptococcal capsular polysaccharide antigen; **LA**: latex agglutination test; *p<0.05