Paracoccidioides sp infection impairs central nervous system cell junctional complexes

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Research Article

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Abstract

Paracoccidioidomycosis (PCM), a systemic mycosis caused by the fungus *Paracoccidioides* sp. is the most prevalent fungal infection among immunocompetent patients in Latin America. The estimated frequency of central nervous system (CNS) involvement among the human immunodeficiency virus (HIV)/PCM-positive population is 2.5%. We aimed to address the impact of neuroparacoccidioidomycosis (NPCM) and HIV/NPCM co-infection on the tight junctions (TJ) and adherens junction (AJ) proteins of the CNS.

Four CNS formalin-fixed paraffin-embedded (FFPE) tissue specimens were studied: NPCM, NPCM/HIV co-infection, HIV-positive without opportunistic CNS infection, and normal brain autopsy (negative control). Immunohistochemistry was used to analyze the endothelial cells and astrocytes expressions of TJ markers: claudins (CLDN)-1, -3, -5 and occludin; AJ markers: β-catenin and E-cadherin; and pericyte marker: alpha-smooth muscle actin in formalin-fixed paraffin-embedded CNS tissue specimens were analyzed using the immunoperoxidase assay.

CLDN-5 expression in the capillaries of the HIV/NPCM coinfectected tissues (acute/subacute clinical form of PCM) was lower than that in the capillaries of the HIV or NPCM monoinfected (chronic clinical form of PCM) tissues.

A marked decrease in CLDN-5 expression and a compensatory increase in CLDN-1 expression in the NPCM/HIV co-infection tissue samples was observed. The authors suggest that *Paracoccidioides* sp. crosses the blood–brain barrier through paracellular pathway, owing to the alteration in the CLDN expression, or inside the macrophages (Trojan horse).

Introduction

Paracoccidioidomycosis (PCM) is an autochthonous disease distributed from southern Mexico to northern Argentina [1]. It is caused by the thermally dimorphic fungus *Paracoccidioides* sp. Co-infection of human immunodeficiency virus (HIV) with *Paracoccidioides* sp. has rarely been reported, with an estimate of 0.09% [2]. Typically, HIV-positive patients develop severe acute/subacute disseminated PCM disease [1]. The involvement of the central nervous system (CNS) in cases of HIV/PCM co-infection is rare, and its frequency in the HIV/PCM population is estimated at 2.5% [3].

Brain capillaries are the anatomical structure responsible for maintaining the blood–brain barrier (BBB). The BBB is an essential part of the neurovascular unit and far from being a simple physical barrier. It is a heterogeneous, complex, and dynamic tissue with an important role in maintaining a precisely regulated microenvironment. BBB protects the brain from foreign (infectious and neurotoxic) substances in the blood that might damage the brain [4, 5].

The adherens (AJ) and tight junctions (TJ) provide adhesive contact between neighboring epithelial cells. TJs among adjacent cells form the basic structure of BBB to limit paracellular permeability [6]. The TJ transmembrane proteins include the integral membrane proteins, i.e., occludin (OCLN), claudin (CLDN-5, -3, -11, -12, -1), and junctional adhesion molecule (JAM-A, -B, -C, -4, -L), which are important for paracellular space occlusion [6–10]. AJ is defined as a cell junction whose cytoplasmic face is linked to the actin cytoskeleton. They can appear as bands encircling the cell (zonula adherens) or as spots of attachment to the extracellular matrix (adhesion plaques). It comprises the transmembrane protein E-cadherin and intracellular components such as p120-catenin, β-catenin, and α-catenin [11, 12]. E-cadherin is the core transmembrane protein of AJ and is required for bonding and localization of some important cytoplasmic proteins termed catenin that connect the cadherin complex to the actin cytoskeleton [11]. Scaffolding proteins such as zonula occludens (Z0-1,-2,-3), vasodilator-stimulated phosphoprotein, and the actin cytoskeleton are vital for physical support as well as for maintaining AJ and TJ function [13–16].

Studies investigating the BBB in patients with neuroparacoccidioidomycosis (NPCM) are scarce. A study on the cerebrospinal fluid (CSF) has described BBB disruption in 50% of NPCM cases [17]. Neuroimage studies on patients with chronic NPCM have shown ring-enhancing lesions with mass effect and surrounding vasogenic edema, representing focal BBB disruption [18, 19]. In contrast, studies on BBB and HIV are more frequent. Some studies provided evidence of BBB and blood-cerebrospinal fluid barrier dysfunction in HIV [20, 21, 22], which can occur early in HIV infection and persist in patients on highly active antiretroviral therapy (HAART) [23]. Elevated CSF markers of BBB damage, including CSF/serum albumin ratio [22, 24, 25], matrix metalloproteinase (MMP) [26–28], and nitric oxide metabolites [29] occur in HIV-1-infected patients. In addition to host components, viral proteins contribute to changes in BBB function during HIV-1 infection [30, 31]. The HIV-1 proteins implicated in BBB disruption are transcriptional activator (Tat), envelope glycoproteins (gp) 120 and 41, or the viral protein R (VPR). Negative regulatory factor (Nef) may also alter brain endothelial cell metabolism by disrupting TJ and the BBB integrity [32, 33].

Based on the aforementioned, this study hypothesized that there is an additive effect on BBB disruption with HIV/NPCM co-infection compared with HIV or NPCM mono-infection. To assess this, a descriptive study was conducted to evaluate the TJ and AJ proteins using immunohistochemistry (IHC) in HIV monoinfected, NPCM monoinfected, and HIV/NPCM coinfected CNS specimens.

This study is novel as it addressed the impact of NPCM and HIV/NPCM co-infection on TJ and AJ proteins in CNS tissue.

Methods
Brain tissue—human postmortem CNS tissue specimens from HIV infected, NPCM infected, HIV/NPCM coinfected, and normal brain autopsy (control) were selected from the formalin-fixed paraffin-embedded (FFPE) tissue specimen bank of the Anatomic Pathology Service, Hospital de Clínicas, Universidade Federal do Paraná (HC-UFPR). The study included CNS tissue specimens from patients with definite NPCM. Diagnosis of definite NPCM was done by visualization of multiple budding yeast-like cells known as the pilot wheel (typical image of Paracoccidioides sp) using the Grocott–Gomori stain on CNS autopsy or biopsy specimens [1]. HIV/AIDS was diagnosed according to the guidelines published by the Brazilian Ministry of Health [34].

The selection of NPCM positive, HIV positive, and normal CNS tissue specimens was made from the autopsy and biopsy reports from 1951 to 2015; there were 378,323 reports in this period. Specimens with FFPE not available or under inadequate conditions for immunohistochemical study (necrosis, scarce material, and poor fixation) or with medical records not available were excluded from the study. In this period, 10 reports of autopsy or biopsy with NPCM diagnosis were identified. Of these, it was localized FFPE of one NPCM/HIV coinfection case and one NPCM without HIV co-infection; the other eight FFPE were not localized. The typical image of Paracoccidioides sp was found to confirm the diagnosis on these cases.

The autopsy reports of HIV/AIDS from 1986 (the first autopsy on an AIDS patient on the service) to 2014 were studied. From the 38 autopsy reports in patients with HIV/AIDS diagnosis, one FFPE CNS sample without CNS opportunistic infections (positive control) was found. One case HIV negative with normal brain autopsy (negative control) was added (Fig. 1).

Immunohistochemistry

Immunohistochemistry (IHC) was used to analyze the mural cells of the microcirculation (pericytes and endothelial cells) and the glial cells (astrocytes and oligodendrocytes). Expression of TJ markers: CLDN-1, -3, -5, and OCLN; AJ markers: β-catenin and E-cadherin [11]; and alpha-smooth muscle actin (α-SMA) [35], in FFPE CNS tissue specimens [36] was analyzed using immunoperoxidase assay with modifications [37].

FFPE CNS tissue with 4-µm thick specimens were cut and placed on slides. The slides were subjected to deparaffinization, dehydration with successive alcohol baths, and rehydration with water. Methyl alcohol and H₂O₂ were used for the first blockade of endogenous peroxidase, whereas distilled water and H₂O₂ were used for the second blockade. The slides were incubated with the primary monoclonal antibody (Ab) for one hour in a humid chamber at room temperature. Secondary polymer (Reveal Polyvalent HRP-DAB Detection System, Spring Bioscience, CA, USA) was incubated with the material for 30 minutes, also at room temperature. DAB complex and substrate (DAB + substrate-chromogen system) were added to the slides and counterstained with Mayer's hematoxylin, followed by dehydration with 100% ethyl alcohol baths and clarification with xylol. Concomitant reactions were performed with positive (human oral mucosa and colon cancer samples) [36, 38] and negative external controls, the latter obtained by omitting the primary antibody [37]. The Abs used during IHC were CLDN-1, -3, -5, and OCLN (rabbit polyclonal Abs, Thermo Fisher, Rockford, USA; dilutions 1:50, 1:50, 1:600, and 1:400 respectively); alpha-smooth muscle actin (α-SMA; rabbit polyclonal Ab, Abcam, Cambridge, UK; dilution 1:600); and β-catenin and E-cadherin (mouse monoclonal Abs; Novocastra, Newcastle Upon Tyne, UK; dilution 1:200).

The immunexpression of the microcirculation mural and glial cells was evaluated using semi-quantitative analysis with the Allred score modified. The Allred score was obtained by adding the scores of intensity and proportion, ranging from 0 to 8. The proportion score represents the percentage of stained cells: score 0, no stained cells; score 1, < 1%; score 2, 1–10%; score 3, 11–33%; score 4, 34–66%; and score 5, > 66%. The intensity of positivity was scored as follows: negative, score 0; weak, score 1; moderate, score 2; and strong, score 3 [39]. The quantification was done in a blinded manner.

Results

The demographic and clinical characteristics of the cases included in the study are presented in Table 1.
Table 1
Epidemiological, clinical characteristics of the participants studied

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Exam</th>
<th>Date</th>
<th>Cause of death</th>
<th>CNS site</th>
<th>HIV</th>
<th>ARV</th>
<th>CD4 Cells/mm³</th>
<th>HIV RNA Log₁₀</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPCM</td>
<td>Male</td>
<td>30</td>
<td>Autopsy</td>
<td>23/05/2011</td>
<td>AIDS, Multiple opportunistic infections</td>
<td>Parietal cortex</td>
<td>Yes</td>
<td>No</td>
<td>&lt; 48</td>
<td>2.3³</td>
<td></td>
</tr>
<tr>
<td>/HIV¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>Male</td>
<td>49</td>
<td>Autopsy</td>
<td>27/03/2004</td>
<td>AIDS, Infectious pneumonia</td>
<td>Parietal cortex</td>
<td>yes</td>
<td></td>
<td>89</td>
<td>Blood &lt; 1.7</td>
<td></td>
</tr>
<tr>
<td>NPCM²</td>
<td>Female</td>
<td>42</td>
<td>Biopsy</td>
<td>05/08/1998</td>
<td>Alive, Ectopic pregnancy</td>
<td>Spinal cord</td>
<td>No</td>
<td>No</td>
<td>No⁵</td>
<td>WBC-0.3 cell/mm³; glucose-45 mg/dL; Protein-45 mg/dL</td>
<td></td>
</tr>
<tr>
<td>NML³</td>
<td>Female</td>
<td>20</td>
<td>Autopsy</td>
<td>15/03/2018</td>
<td>Ectopic pregnancy, Pancreatitis</td>
<td>Parietal cortex</td>
<td>No</td>
<td>No</td>
<td>No⁵</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Antiretroviral central nervous system penetration effectiveness (CPE).

[1] The HIV/AIDS diagnosis was established post-mortem with positive HIV tests (ELISA and immunoblot). PCM clinical form was classified as acute/subacute (juvenile) multifocal form. PCM with systemic hematological dissemination, involving the lungs, heart, liver, spleen, lymph nodes, thyroid, kidneys, adrenal glands, skin, intestinal wall, meninges, and brain. Post-mortem brain sample cultures were positive for yeasts compatible with *Candida* spp; the autopsy was described previously ³.

[2] Case with CNS PCM, chronic form of PCM in spinal cord (T12 level) and lungs, no immunosuppression. Computed myelotomography of the thoracic spine (24/06/1998); tomographic sections were performed in the axial plane. After the intra-tecal injection of iodinated, non-ionic contrast, with interest in the t11-t12 segment, were observed: vertebral canal with normal morphology; increase in the diameter of the medullary cord with distal limit at the level of t12; there was gradual and symmetrical reduction of the contrast column that surrounds the spinal cord. Suggestive of intra-dural and intra-medullary tumor.

[3] Case with normal brain autopsy, autopsy with multiple pregnancy (twin) of approximately 12 weeks, one of the fetuses was ectopic in perisplenic location; pulmonary amniotic embolism; massive liver necrosis, renal tubular necrosis and pulmonary edema; disseminated intravascular coagulation.

[4] CD4 estimated from blood white blood cell (WBC) count

[5] not quantified, no history of immunosuppression

[6] post-mortem CSF HIV RNA; HIV RNA was not assessed in blood.

**Tight junctions**

Regarding the expression of TJ markers in endothelial cells, there was severe decrease of CLDN-5 expression in the capillaries of the HIV/NPCM coinfected; and of CLDN-3 in the NPCM monoinfected tissues, than in those of the control tissues. The expression of CLDN-1 was higher in HIV/NPCM tissues; probably reational and compensatory to the decrease of CLDN-5. There was a marked increase of CLDN-1 in NPCM monoinfected tissues, and comparable levels of CLDN-5 in this case with the normal control tissues. There was a moderate decrease in the expression of CLDN-1, 3, 5 and OCLN in HIV monoinfected tissues. In NPCM and NPCM/HIV coinfected tissues, the OCLN expression was similar to that of the normal control tissues (Fig. 2).

The expression of CLDN-1, 3, 5 and OCLN in the astrocytes was comparable in all cases (Fig. 2).

**Adherens junction**
Regarding the expression of AJ markers in endothelial cells, β-catenin and E-cadherin expressions were similar in the four cases with mild expression (Fig. 3).

α-SMA expression (BBB pericyte marker) was slightly lower in NPCM monoinfected tissues than in normal control tissues and in the other tissues (Fig. 3).

Discussion

This study was the first to describe the IHC findings of BBB in CNS tissue specimens of NPCM and HIV/NPCM participants. This study observed a marked decrease in CLDN-5 expression in the capillaries of the HIV/NPCM coinfected tissues (acute/subacute clinical form of PCM) compared with that in the HIV or NPCM monoinfected (chronic clinical form of PCM) tissues. This is in accordance with the stated hypothesis that there would be an additive effect on BBB disruption with HIV/NPCM co-infection compared with monoinfection. However, additional studies with more CNS specimens are warranted to prove this hypothesis.

A higher expression of CLDN-1 in the NPCM and NPCM/HIV coinfected tissues, but not in the HIV monoinfected tissues, was observed compared with that in the normal control tissues. This is in accordance with the observation that CLDN-1 is rarely expressed at the normal BBB, although it is expressed in pathological conditions. The CLDN-1 expression, post stroke, results in limited brain endothelial barrier recovery, partially owing to restricted CLDN-5 expression [40]. The increased expression of CLDN-1 in the CNS vasculature of the NPCM/HIV tissues described in the current study is probably compensatory to the decrease of CLDN-5 in the same patient. Some studies question if CLDN-5 deficiency is compensated with other TJ proteins. For others, an interplay between TJ proteins is considered a pre-requisite for the regulation of barrier permeability and for compensatory mechanisms, but little about this is known [41]. At the brain endothelium, CLDN-5 is the major claudin, but there are conditions (e.g., vasculo genesis, tumors, and traumatic brain injury) in which other claudins such as CLDN-1 are present and might participate in paracellular space occlusion [42, 43]. It was found that there was significant upregulation of CLDN-1 mRNA protein and a nonspecific CLDN in blood vessels and downregulation in CLDN-5 expression [40].

Redistribution of transmembrane junction proteins, particularly CLDN-5 and occludin as well as Ve-cadherin, is closely associated with loosening of adhesion at the TJ complex, which ultimately leads to paracellular gap formation [14].

According to the present study, in the normal control brain tissue specimens, the expression of CLDN-1 was lower than that of CLDN-3 or -5. This is in accordance with the hypothesis that CLDN-1 is not essential for BBB TJ complex function under physiological conditions and its induction is rather associated with an altered TJ function. The cause and effects of CLDN-1 expression are still unknown. Some studies suggested an equal and beneficial role in BBB TJ complex organization, helping to seal the paracellular route, or via astrocytic expression, helping to form secondary astrocyte/gliai barriers to protect the brain from an endothelial breakdown [42, 44]. In this study, CLDN-1 expression was highly expressed in NPCM monoinfected tissues, which indicates a chronic form of PCM in the spinal cord. This is explained by the fact that the chronic form of PCM presented with a longer clinical duration than the acute/subacute form in HIV/NPCM coinfected cases. The appearance of CLDN-1, chronically in stroke, raises the possibility that it may support and facilitate BBB recovery, reestablishing integrity, as indicated in the study on the beneficial effects of overexpressing CLDN-1 in experimental autoimmune encephalomyelitis [44].

Disruption of TJ is commonly observed in HIV-1-infected patients [45]. The HIV-1 and its proteins may act alone or in cooperation with host cytokines and chemokines to affect the integrity of the BBB [46].

Tat can affect protein expression and distribution of TJ; such changes may lead to disturbances in BBB integrity and allow viral flow to the brain [47]. In the present study, the expression of CLDN-1 was lower in HIV monoinfected tissues than in NPCM/HIV coinfected tissues. It was described that the Tat protein caused a reduction of CLDN-1 immunoreactivity and loss of CLDN-5 expression, resulting in marked cellular infiltration [47]. Changes in CLDN-5 immunoreactivity were associated with increased cells in the brain tissue, suggesting that monocytes may migrate to the brain through tight juncure disruption caused by CLDN loss [47]. Tat also disrupts the integrity of BBB by diminishing OCLN production and cleaving OCLN through matrix MMP-9 [16, 48]. HIV-1 Tat also affects AJ proteins; it suppresses β-catenin activity in astrocytes by inhibiting microRNAs [49]. It was reported that gp120 induced disruption of the BBB via brain microvessels injury, MMP activation, and degradation of the vascular basement membrane and vascular TJ [50]. Gp120 proteins caused disruption and downregulation of the TJ proteins ZO-1, ZO-2, and OCLN in these cells. Other junctional proteins such as CLDN-1 and CLDN-5 were unaffected by gp120 treatment. These data demonstrate that the gp120 proteins alter both the functional and molecular properties of the BBB, which could increase trafficking of HIV, infected cells, and toxic humoral factors into the CNS and contribute to the pathogenesis of HIV-associated dementia (HAD) [31]. Moreover, gp-120 proteins increased the expression of MMP-2 and −9, and subsequently that of CLDN-5 and laminin, which are key targets of these MMPs [51]. A role for gp120 in BBB disruption is also supported by in vitro evidence that gp120 added to cultured human brain endothelial cells enhances monocyte migration, increases permeability, decreases TEER, and disrupts expression of TJ proteins ZO-1, ZO-2, and OCLN (but not CLDNs or actin) [31, 52]. The VPR is a 96-amino acid 14-kDa protein. It has a regulatory function of viral transcription, plays an important role in regulating nuclear import of the HIV-1 pre-integration complex, and is required for viral replication in non-dividing cells such as macrophages. High levels of VPR in the CSF are found in patients with HAD. It induces apoptosis in neurons via caspase activation [53]. Other HIV proteins, such as nef and regulator of expression of virion (rev) proteins, also
play a role in the rupture of BBB, inducing neurotoxicity [54]. A segment in Nef protein contains identical amino acids at key positions and structurally mimics the β-catenin binding sites on endogenous β-catenin ligands. The interaction between Nef and β-catenin was confirmed with in vitro studies [55].

The main limitations of this study were its descriptive design and the few numbers of brain tissue specimens studied. The scarce number of CNS specimens from autopsy or biopsy could explain this paucity of information. In the present study, it was possible to localize only two FFPE CNS tissue specimens out of 10 identified with NPCM, in the Anatomic Pathology Service of a University Hospital, localized in an endemic area, in a period of 64 years [56]. To date, all five reported cases of NPCM in HIV-positive patients are from Brazil [3, 57–60]. This study was performed with FFPE, for which, the brain tissue was stored in 30% formaldehyde during the 30 days before the brain macroscopic study. This hindered antigen recuperation, which takes 60 minutes, resulting in the development of a strong background on IHC. Besides this, there were marked differences between the NPCM monoinfected and NPCM/HIV coinfected tissue specimens. In NPCM, the clinical form was chronic and the second was acute/subacute, which implies differences in disease duration. The NPCM monoinfected tissue specimen was from a previously immunocompetent patient, whereas that of NPCM/HIV was from a severely immunosuppressed patient, with multiple CNS opportunistic infections, such as toxoplasmosis, histoplasmosis and candidiasis [3]. The NPCM/HIV specimen was that of an autopsy case, and the NPCM monoinfected specimen was obtained following spinal cord biopsy (alive). These differences can cause an impressive impact on the BBB. NPCM/HIV, besides the NPCM, presented at autopsy, multiple opportunistic infections. These differences could be responsible for the differences in the level of the CLDN studied.

This study has several strengths as well. TJ and AJ proteins were studied for the first time in NPCM, contributing to the pathophysiology of CNS PCM infection and HIV co-infection. In conclusion, there was a marked decrease in CLDN-5 expression in the NPCM/HIV coinfected tissue specimen, with compensatory increase of CLDN-1, probably different from NPCM or HIV monoinfected cases.

The mechanism by which Paracoccidioides sp. invades the CNS is unknown. Probably Paracoccidioides sp. invades the CNS using any of the two mechanisms (Fig. 4): (a) by using macrophages (Trojan horse), as Paracoccidioides sp. was identified inside macrophages [61] or (b) by using the paracellular pathway, mainly owing to the alteration of CLDN expression, which could be enhanced by HIV infection. However, additional studies are necessary to better explore this subject.

**Declarations**

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**Competing interests**: The authors declare no conflict of interest.

The HC-UFPR, Brazil, and institutional review board approved ethics approval - This study.

**Consent to participate**: Not applicable

**Consent for publication**: Not applicable

**Availability of data and material**: data and material are available after approval of the institutional review board and request to the corresponding author.

**Authors’ contributions**:  
Sergio Monteiro de Almeida: conceived and designed the study, performed research, analyzed data, contributed new methods or models, wrote the paper.
Amanda Kulik: performed research
Mineia Malaquias: performed research
Seigo Nagashima: performed research
Caroline de Paula: performed research
Marisol Muro: conceived the study
Lucia de Noronha Noronha: conceived and designed the study, performed research, analyzed data, contributed new methods or models, wrote the paper.

**References**

Figures

Flowchart describing the selection of neuroparacoccidioidomycosis (NPCM) cases from the formalin-fixed paraffin-embedded (FFPE) archives of the Medical Pathology Unit, HC-UFPR, Brazil, between 1951 and 2015.

Definitive NPCM defined and central nervous system (CNS) autopsy or biopsy shows the typical image of the fungus Paracoccidioides sp. using light microscopy.

To compare with the HIV/PCM coinfected case, the autopsy reports of HIV/AIDS were studied over the period from 1986 (the first autopsy on an AIDS patient on the service) to 2014.
Central nervous system (CNS) tissue expressions of tight junctions (TJ) proteins (claudin-1, -3, -5, and occludin) using immunohistochemistry (IHC) in all the cases included in the study

The graphs represent the Allred score of claudin-1, -3, -5, and occludin on CNS specimens stained with IHC, at endothelium (dark column) and at glial cells (light grey column). Below each graph is the respective IHC photomicrographs by CNS specimens. Claudin-1 (CLDN-1) is highly expressed in the brain tissue specimen of the patient with neuroparacoccidioidomycosis (NPCM) monoinfection compared with that in the other cases (NPCM/HIV, HIV, and normal control), IHC photomicrographs on top left. Regarding CLDN-3, a higher CNS tissue expression in the case of NPCM and HIV (NPCM/HIV) co-infection and in the control cases is observed compared with that in the NPCM and HIV monoinfected cases, IHC photomicrographs on top right. CLDN-5 is the major claudin at the brain endothelium, and a higher expression of this protein in patients with NPCM monoinfection and in normal control cases is observed, IHC photomicrographs on bottom left. Occludin (OCLN) analysis shows that only the case infected with HIV has a lower tissue expression than the other cases, IHC photomicrographs on bottom right.

CLDN-1, -3, -5, and OCLN expressed in glial cells were comparable in each cases (respective photomicrographs).

Figure 3

Central nervous system (CNS) tissue expression of alpha-smooth muscle actinin (α-SMA) and the adherent junctions (AJ) proteins (β-catenin and E-cadherin) analyzed using immunohistochemistry (IHC) in all the cases included in the study

The graph represents the Allred score of the expression of α-SMA and a pericyte marker on CNS specimens analyzed using immunohistochemistry (IHC). The respective photomicrographs of each case are shown (upper and centerline). The case with neuroparacoccidioidomycosis (NPCM) monoinfection has a slightly lower expression than the other three cases (NPCM/HIV, HIV, and normal control).

β-catenin and E-cadherin tissue expression are mild in all cases of the study. β-catenin (bottom left) and E-cadherin (center) photomicrographs in the NPCM/HIV coinfected CNS specimens. Histological section of the brain of the NPCM/HIV coinfected, stained with Gomori-Grocott, showing large yeast cells bearing multiple buds, known as pilot wheels, which are the specific optic microscopic diagnostic criterion for *Paracoccidioides* sp., *i.e.* definite PCM (bottom right).