REVIEW ARTICLE

Paracoccidioidomycosis disease (Lutz--Splendore-Almeida disease): Additional workup, differential diagnosis, cure control

Paracoccidioidomicose (doença de Lutz-Splendore-Almeida): propedêutica complementar, diagnóstico diferencial, controle de cura

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ABSTRACT

The diagnosis of paracoccidioidomycosis requires epidemiological data to be available and for the presence of some more typical clinical manifestations.It requires complementary investigation with interventional methods, differential diagnosis of pathologies of great importance such as tuberculosis and lymphomas, and cure control. This update discusses the advances in these various areas, which include complementary investigation, differential diagnosis and cure control, pointing to development prospects that may help better define the best approach to this disease.

Key words: Paracoccidioidomycosis; Mycosis; Diagnosis, Differential; Diagnostic Techniques and Procedures.

RESUMO

O diagnóstico da paracoccidioidomicose requer a presença de dados epidemiológicos e de algumas manifestações clínicas mais típicas, entretanto, depende da propedêutica complementar que ainda requer métodos intervencionistas, o diagnóstico diferencial com patologias de grande relevância como tuberculose e linfomas, e o controle de cura. Nesta atualização são discutidos os avanços nessas várias áreas que inclui a propedêutica complementar, o diagnóstico diferencial e o controle de cura, apontando para as perspectivas de desenvolvimento que poderão ajudar a definir melhor a sua abordagem.

***Palavras-chave:** Paracoccidioidomicose; Micose; Diagnóstico Diferencial; Técnicas e Procedimentos Diagnósticos.*

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INTRODUCTION

The knowledge about paracoccidioidomycosis (PCM) still requires the unravelling of many relevant aspects in the biology of the fungus, its pathophysiology, less interventionist diagnostic methods, shorter therapy, and proper healing control .[1-7]

PCM is an endemic disease in many Brazilian regions, with prevalent nosology, requires early diagnosis, minimal easy, and available in basic health units interventions,

for which appropriate therapy can contribute to avoid premature death and prevent serious sequelae.[1-3]

It is important to consider on the initial diagnostic evaluation, in addition to the general state of the patient, the organs and systems most often affected, observing the clinical forms of the disease: acute-subacute and chronic.[1, 2, 5-7]

COMPLEMENTARY PROPEDEUTICS

The PCM diagnosis and its repercussions on organs and systems is based on direct and fresh identification in various clinical specimens; histopathological examination; or culture of the fungus, all considered gold standard methods; or in hematologic, serological, antigens detection, molecular biology, functional, and image exams.[1-7]

Direct examination

The mycological diagnosis of PCM is made from clinical specimen(s) obtained from lesion (s) suspected and examined directly on fresh samples between slides and coverslips and, preferably, after clarification and homogenizing with sodium hydroxide or potassium, with or without staining; or after cultivation. It is based on the identification of bi-refringent yeast cells or with double contour with single or multiple budding.

Sputum in exclusive pulmonary PCM is the most useful material for examination, even in cases of pulmonary lesions non-radiologically significant, collected from bronchial lavages or aspirate (bronchoalveolar lavage), transcutaneous pulmonary aspirate or biopsy. It is harder to identify P. brasiliensis in the sputum (between the slide and coverslip) than in scrapings of tegumentary lesions and lynphnodal secretions. The sputum, as in tuberculosis, must be examined through the daily collection of samples, in three consecutive days.[6-24]

The anatomopathological study of tissue fragments also allows the direct examination in histopathologic preparations through staining with hematoxylin-eosin or with the use of special dyes such as Gomori -Grocot or Schiff periodic acid (Graph 1). The open air lung biopsy should be considered when the diagnosis is impossible via other methods. The material obtained by transcutaneous pulmonary aspiration is subjected to microscopic examination in 10% potash and culture in routine media. The pulmonary biopsy obtained with lanceting needles or in the open air must be preserved in a sterile vial, over moistened gauze in sterile distilled water, and part of the material submitted to histopathological and mycological examination. Histological sections must be stained with hematoxylin-eosin and through the silver impregnation technique.[25] In PCM patients with pulmonary and cutaneous involvement, the fungus isolation is generally made from the cutaneous-mucous lesions of easy access. The histopathological analysis aims at recognizing yeast cell structures with bi-refringent walls and multiple budding, similar to a boat helm, considered pathognomonic. Still, the fungus is scarce in some specimens and can go unnoticed on the slide or be confused with other thermally dimorphic fungi such as Histoplasma capsulatum and Coccidioides immitis .[7, 8, 26-29] Obtaining material for histological analysis can be difficult and is not recommended, as in central nervous or pulmonary isolated involvement, which requires invasive procedures such as craniotomy and thoracotomy, which are often unavailable in many PCM endemic areas, in addition to their high costs.[10-25]

Culture and inoculum in animals

The diagnostic doubt can be resolved by the cultivation of examined material inoculating it in animals that are susceptible or through immunofluorescent reaction with hyper immune sera labelled with fluorescein. Fungus cultivation can be done in several media such as Mycosel®, Mycobiotic agar®, SaBHI®, sabouraud agar®, agar yeast extract; and subsequently identified. The culture is time-consuming (it requires three to four weeks) and requires biosafety facilities suitable for its handling, which is problematic, especially in non-endemic areas where the disease is rare and the diagnosis is usually difficult and delayed.[2-5,25]

Immunological tests

The presumptive diagnosis of PCM may be based on serological evidences, as indirect evidence of the presence of the fungus in the patient when its isolation is not possible. The detection of antibodies against antigens from Paracoccidioidis or the existence of these antigens in body fluids constitute indirect diagnosis criterion for PCM.[10-21]

These exams, although being very useful in PCM diagnosis and therapy monitoring they are not routinely used. Serological examinations based on the detection of circulating antibodies are not always conclusive and require more time to develop during the convalescence phase. Therefore, a commitment to standardization of techniques for efficient and fast diagnostic of PCM is highly justified.[30]

The serological diagnosis by specific antibodies anti-Paracoccidioidis research has limited value, being mainly used to monitor the response to treatment. The standardized serological reaction has the best specificity and sensitivity and is performed through agar gel double immunodiffusion using exoantigen extracts rich in the 43 kDaltons glycoprotein (gp43) obtained from samples of P. brasiliensis after seven days of cultivation. It is the simplest test and currently considered the main method for the serological diagnosis of PCM through serological tests as immunodiffusion, hemagglutination, ELISA, and western-blot, although the finding of specific serum antibodies has only a predictive value. The value of finding these serum specific antibodies is only predictive; they are not highly specific because of similarity between several antigens of P. brasiliensis with those from other fungi, especially the Histoplasma capsulatum, which often generates cross-reactivity. The immune response to gp43 involves Th1CD4+ lymphocytes, secreting gamma interferon, and interleukin 2. Cloning the 27 kDaltons (rPb27 recombinant protein) induces the production of high levels of IgG2a, TGF-beta, and interferon-gamma and low levels of interleukin 10 in mice. The quantification of serum levels of antibodies also correlates with the severity of the disease, being higher in severe forms, however, some PCM patients do not possess antibodies anti P. brasiliensis, especially those who are immunosuppressed.[1-3,10-21] The ELISA technique, applying two different antigens, Pb27 and Mexo, guarantees high diagnostic sensitivity to PCM, evidenced by its full compatibility with the histopathological analysis of specimen containing P. brasiliensis, however, its availability in the clinical practice is still very limited.[10-21]

The serology can be useful to define the criterion of cure and the duration of treatment. Antibody titers may decrease gradually with the clinical control of the disease and the serological cure criterion is based on negativity or stabilization in 1:2 dilution or less, but these data are not fully correct and exams with improved sensitivity and specificity need to be developed.[1-4, 19-21]

Other serological tests such as counterimmunoelectrophoresis (CIE), complement fixation (CF), indirect immunofluorescence (IIF), enzyme-linked immunosorbent assay (ELISA), and immunoblot (IB) are less used or are not part of the diagnostic routine for PCM.[1-4] The radial double immunodiffusion test is a method of high specificity (98%) and adequate sensitivity (84%), as well as low operating costs, requiring preparation of antigen gp43 by different laboratories to avoid conflicts when comparing results from different regions of PCM occurrence.

Each of these diagnostic methods has limitations. Serological tests indicate only that there was fungus infection – which occurs in about 50% of inhabitants of endemic areas –, not stating on disease activity, which must be inferred by the correlation with the current clinical manifestations, which resemble to other diseases such as histoplasmosis, coccidioidomycosis, and some neoplasia (lymphoma, adenocarcinoma, sarcoma) that constitute their differential diagnosis.

There are tests based on detecting fungal antigen in plasma and urine that are not standardized and are less effective than the previously cited.

Methods of molecular biology

Molecular biology methods such as in situ hybridization, traditional polymerase chain reaction, nested-polymerase chain reaction, and polymerase chain reaction in real time have been proposed as more sensitive and specific diagnostic methods for PCM, however, they are not yet available in the clinical practice.[31-34]

Multiple targets are used for the detection of P. brasiliensis and P. lutzii DNA such as markers for the coding regions of ribosomal RNA (rDNA) and glycoproteins gp43 and pb27.[32,35-37] PCR performed with primers based on gp43 gene sequences is an excellent instrument and highly sensitive method.[32] Microsatellites can be important markers for the detection P. brasiliensis DNA.[38]

PCR is an excellent alternative in PCM diagnosis compared to the conventional methods because it can detect low fungal load, such as picograms of DNA/mL in clinical specimens and can be used in small amounts of samples.[39,40] Genotypic profiles generated by molecular techniques need to be always combined with morphological characters for a complete identification of the species involved and to reach an established the diagnosis of PCM.

The technique of real time PCR or quantitative PCR (qPCR) has become an important method because from of the use of a species-specific probe it is possible to standardize a rapid and accurate diagnostic test for infectious parasitic diseases. Just as the conventional PCR, qPCR consists in the exponential doubling of specific parts of the genome of an organism in vitro. The qPCR uses the first amplification detected and not the product accumulated at the end of all cycles as it is the case in conventional PCR. The qPCR detection is performed by means of fluorescence, which requires, in addition to the reagents necessary for any PCR, a fluorescent probe to anneal in specific regions in the species' genomes. The qPCR equipment is a thermal cycler with a set of light beams and a mechanism that captures the fluorescence emitted during the reaction, converting it into numeric values, encoded in graphics by the program (Figure 1).[41-43] The qPCR allows quantifying the initial genetic material, since the higher the initial number of copies of DNA, the smaller the cycle in which occurs the first amplification. It can also be used as a qualitative test when the product is evaluated at the end of the reaction. Presence/absence testing with genetic discrimination constitutes endpoints .[42]

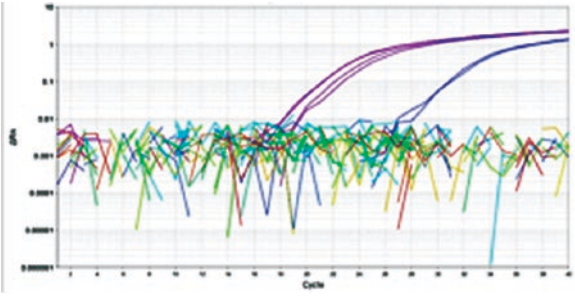

Figure 1 - Amplification curve from the qPCR technique. The two curves in purple are standard samples of the fungus P. brasiliensis at the concentration of 1 ng; in blue, DNA from cultured P. brasiliensis, isolated from a patient at the concentration of 0.01 ng. The other samples are from other fungal species, represented by the colored lines; these were not recognized by the probe because they do not present the species-specific region, and therefore, did not present the amplification peak. Mycology Laboratory, Graduate Program, Santa Casa in Belo Horizonte, Minas Gerais.

Maintaining the quality of tests using qPCR demanded the creation, by the scientific community, of guidelines for standardization and validation, entitled Minimum information for publication of quantitative real-time PCR experiments (MIQE), which normalizes the nomenclature and patterns of analysis. This manual allows the safe use of qPCR in the diagnosis of diseases.[31,42,44]

The qPCR using fluorescent probes derived from the gene that encodes the gp43 protein is able to detect the minimum of 10 copies of the gp43coding sequence, being efficient in the diagnosis of PCM.[42, 45, 46]The use of GP43 as the target gene allows 100% sensitivity and specificity with the ability to detect 10 copies of the GP43 gene in culture with 100% specificity and sensitivity of 61% in biological samples. The use of ITS1 rDNA region as the qPCR target shows 100% sensitivity and specificity in DNA from cultures and biological samples, such as from biopsies and bronchial alveolar lavage.[8, 40, 42, 46,47]

Molecular biology methods have also been increasingly used in the study of taxonomic relationships in fungi. [34,48-55]The molecular analysis, therefore, presents itself as an important tool in the identification of fungal species and aid in the diagnosis of mycoses. It is important to note that the genotypic profiles generated by molecular techniques need to be added to morphological characters and clinical aspects for complete identification and diagnosis of PCM.

Intradermal reaction

The intradermal reaction with paracoccidioidin (gp43 kDa) has a great value on PCM-infection. It is an immunological exam with applications in epidemiological surveys, prognosis, and cure control (anergia reactions). It suggests a lack of resistance by depletion of cellular immunity when it is negative in isolated cases of the disease in evolution, which translates into poor prognosis.[2-4, 56-58]

Hematology

The CBC may reveal normocytic and normochromic anemia, discrete leukocytosis with neutrophilia, sometimes deviated to the left in serious chronic forms. Eosinophilia is more frequent in the juvenile form (acute-subacute form) than in the chronic form. The erythrocyte sedimentation rate is in general elevated above 40 mm in the first hour.[2-4, 19-25]

Pulmonary function

Pulmonary function tests in PCM patients show variable default. PCM can cause diffuse lesions in all lung compartments (bronchial, alveolar, interstitial, and vascular) with important repercussions on pulmonary function. The fibrotic sequelae also contribute to changes in respiratory function. Spirometry often demonstrates obstructive ventilatory pattern, however, due to the fact that almost all patients are chronic smokers, this finding cannot be assigned solely to PCM. There is a predominance, in general, of the obstructive ventilatory disorder, followed by combined patterns (obstructive-restrictive) and restrictive pure. Spirometry suggests bronchial lesions, especially bronchial or in the conjunctive peri-bronchial set. Changes in the ventilation/perfusion (V/Q) ratio and diffusion of gases result from pulmonary destruction by fibrosis, compromising the bronchial tree, alveoli, and interstitial. Vascular alterations also lead to diffusion disorders that can be evidenced by the carbon monoxide diffusion test. The occurrence of hypoxemia is associated, in general, to the increased difference in alveolar –arterial oxygen, which expresses the predominance of perfusion alterations over the ventilatory ones. Changes in pulmonary perfusion and hypoxemia may result in pulmonary arterial hypertension. The six-minute walk test is useful to demonstrate a decrease in oxygen saturation by hemoglobin and the distance walked in six minutes. The reduction of the appearance of radiological lesions, in general, does not follow pulmonary function recovery. [22, 59-63]

Hormones and metabolism

Hormonal examinations are important on the suspicion of adrenal insufficiency from the identification of elevated urine levels of 17-hydroxysteroid and low cortisol in the plasma, before and after stimulation with ACTH and, in some patients, decreased plasma aldosterone.[23,24,64-66]

Hyperkalemia, hypercalcemia, hypochloremia, hyponatremia, and uremia are still observed.

The most complex exams are conditioned to clinical suspicion or alterations in the initial laboratory tests, indicating other pathophysiological involvements.[2-4]

Ionogram

Hypernatremia, hypercalcemia, hyponatremia, hypochloremia and uremia can be found in adrenal insufficiency.[2-4]

Imaging exams

Imaging exams are fundamental to establish the involvement of various organs through its patterns as in the study of sequelae after treatment.

The study of pulmonary manifestations images in the PCM-disease begins with the conventional radiography (teleradiography) followed by thorax computed tomography of high resolution. The chest teleradiography reveals bilateral lung involvement in most cases (90.5%). Pulmonary lesions occupy more than one-third of the lung fields (86%) with predominantly diffuse distribution (apices, medium fields, and bases) and apices and medium fields in 47.6 and 28.5% of the cases, respectively (Figures 2, 3, 4). The most common radiological patterns of lesions are: predominant nodular (23 to 48.2%), exclusive miliary (22.2%), predominant miliary (14.8%), predominant reticular (11 to 26%), and bronchopneumonia (3.7%). The cavitary form is unusual (4.8%).[67-71] CT scan reveals the same alterations such as alveolar opacities (24%), architectural distortion (30%), irregular enlargement of the air space (30%), nodules (38%), bronchiectasis (41%), bubbles (59%), pleural thickening (65%), opacity in frosted glass (67%), diffuse emphysema (70%), bronchial wall thickening (89%), septal thickening (100%), and rarely, honeycombing lesions or cystic bronchiectasis. In about 10% of cases the inverted halo sign can be seen, characterized by a growing or consolidating ring, with opacity in central frosted glass. The pulmonary sequelae after treatment are fibrosis and diffuse emphysema, with irregular enlargement of the air space, sometimes characteristic of pulmonary hypertension.[25,67-72]

Osteoarticular lesions can be demonstrated by simple radiological examination or ultrasound of the locomotor system, being especially determined by scintillography with MDP-99mTc and MRI (Figure 5).[3-9]

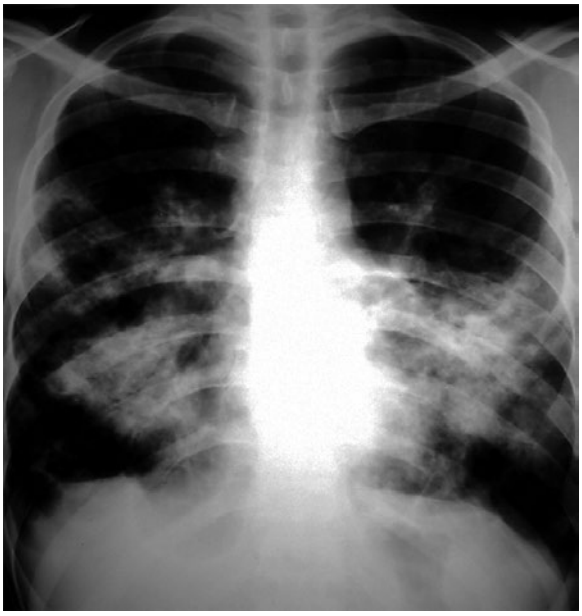

Figure 2 - Thorax teleradiography showing bilateral pulmonary involvement, especially of the medium lobes, with average bronchopneumonia infiltrate with the aspect of a butterfly wing in a PCM patient. Patient assisted at the PCM Reference Center at the Internal Medicine Hospital from UFMG.

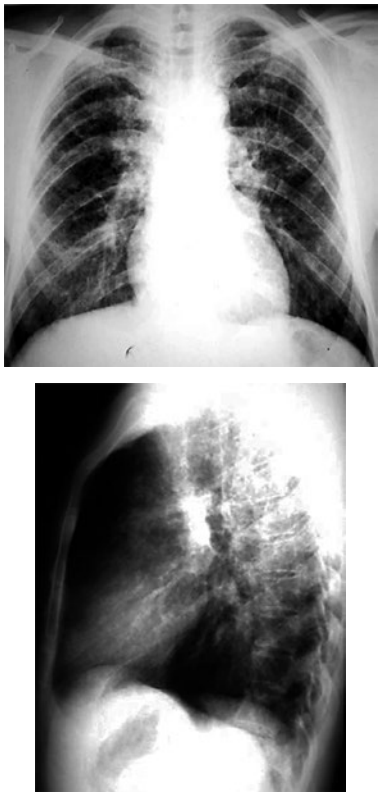

Figure 3 - Thorax teleradiography showing bilateral pulmonary involvement with nodular pattern. Patient assisted at the PCM Reference Center at the Internal Medicine Hospital from UFMG.

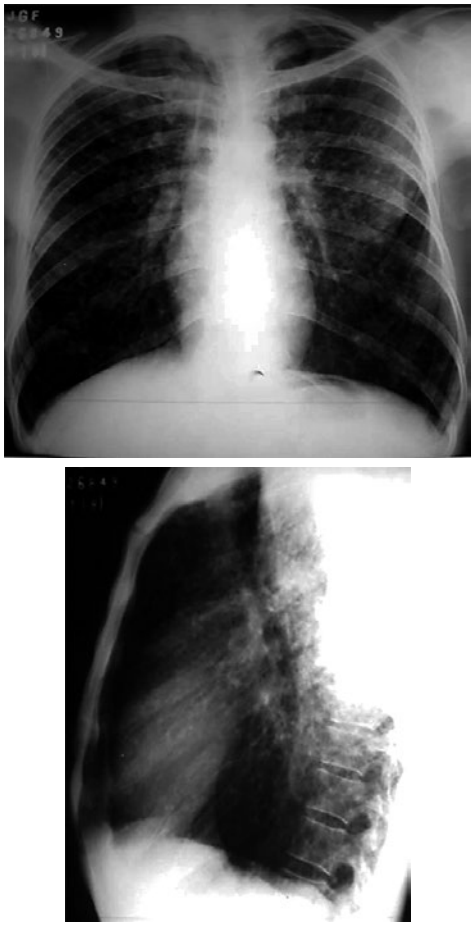

Figure 4 - Thorax teleradiography showing bilateral pulmonary involvement with nodular pattern-micro-nodular in PCM patients. Patient assisted at the PCM Reference Center at the Internal Medicine Hospital from UFMG.

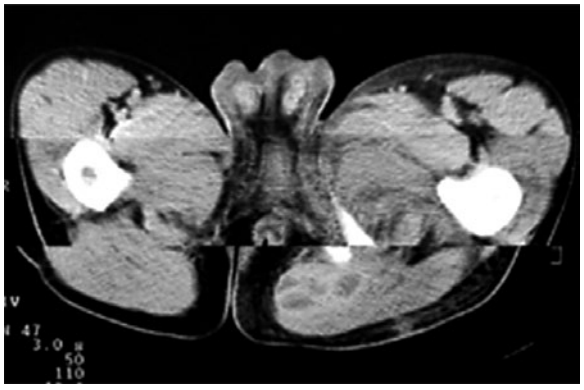

Figure 5 - Muscular abscesses. Patient assisted at the PCM Reference Center at the Internal Medicine Hospital from UFMG.

Upper gastrointestinal endoscopy or colonoscopy, abdominal ultrasound, computed axial tomography, and lymphoscintillography help defining the involvement of intra-abdominal structures – including intes-

tines, liver, spleen, kidney - and the deep lymphatic system (Figures 6 and 7).[73-84]

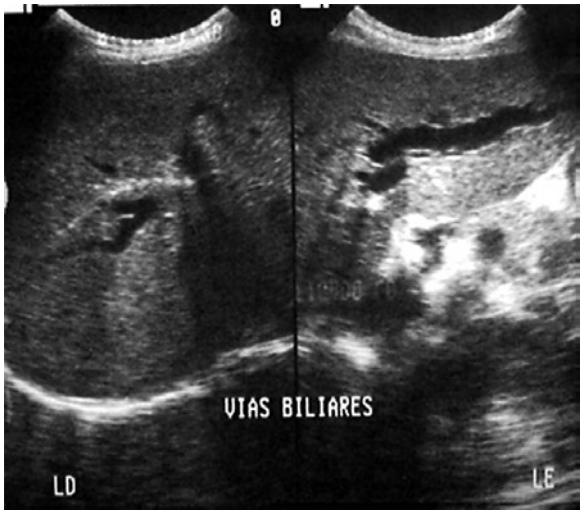

Figure 6 - Dilatation of biliary intra-hepatic pathways. Patient with lymphadenopathy in the hepatic hilum. Patient assisted at the PCM Reference Center at the Internal Medicine Hospital from UFMG.

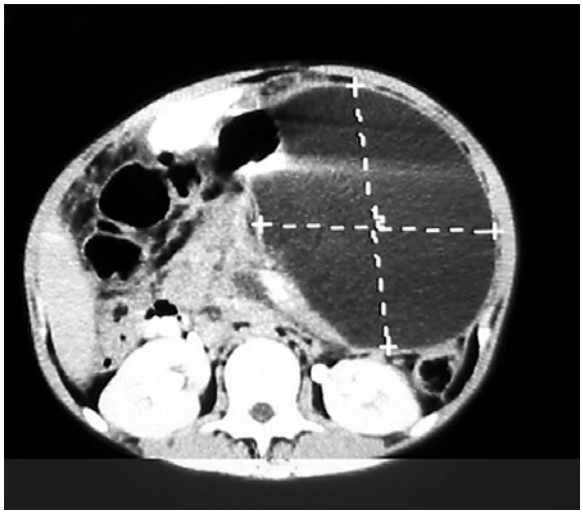

Figure 7 - Mesenteric cystic formations. Patient assisted at the PCM Reference Center at the Internal Medicine Hospital from UFMG.

Anatomical alterations in the adrenal can be defined by ultrasound or computed tomography (Figure 8).[64-66,75,76]

FO involvement of the central nervous system can be identified by computed tomography, being characterized by rounded injury, of variable location, without signs of neoformation or bone destruction, with small amount of perifocal edema, discreet and compressive effect, and contrast buildup (circinate lesion) in a ring shape (Figure 9).[85,86]

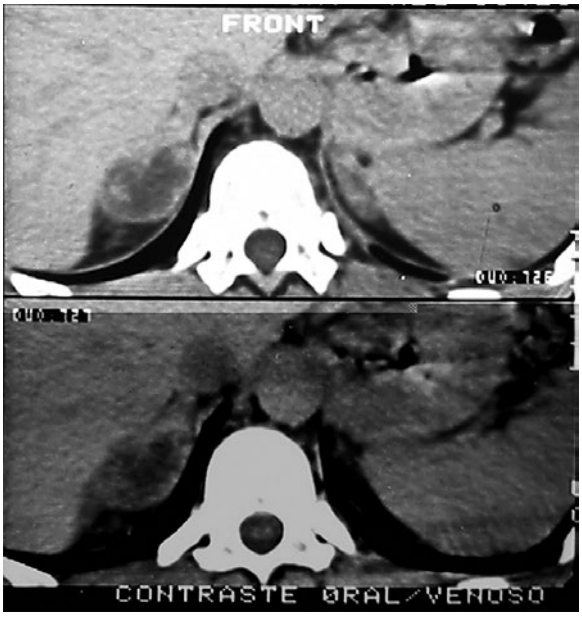

Figure 8 - Adrenal involvement, hypoechogenic nodule, central necrosis, and calcification.

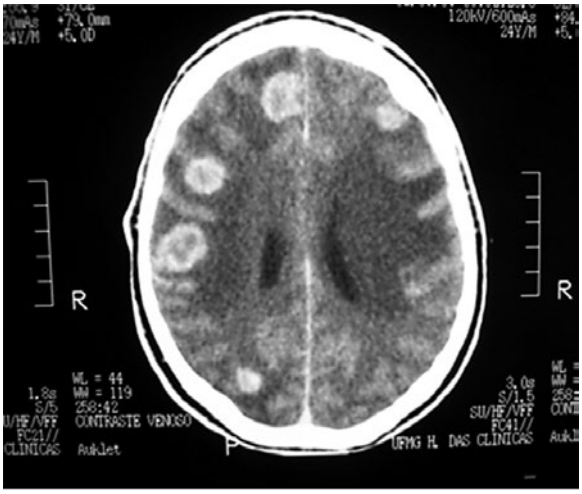

Figure 9 - Contrasted CT in patient from Figure 1 who presented left hemiparesis besides skin lesions and lymphadenomegaly. The image shows multiple parenchymal lesions with contrast uptake in anelar enhancement. Patient assisted at the PCM Reference Center at the Internal Medicine Hospital from UFMG.

Liquor examination

Alterations in the liquor observed when there is involvement of the central nervous system are characterized by variable pleocytosis, usually, discreet, with a predominance of lymphocytes, proteinorachia (predominance of gamma globulin), and hypoglicorachia.[1-7,18]

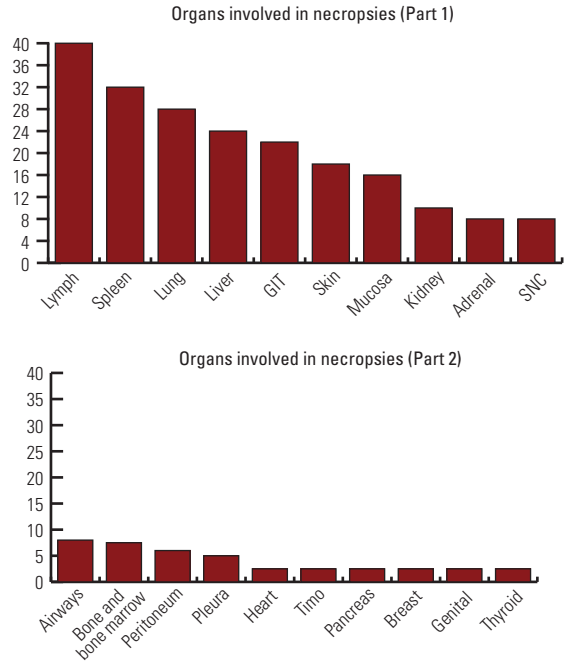

Figure 10 (Parts 1 and 2) - Distribution of 41 patients with necropsied paracoccidiodomycosis, between 1944 and 1999, in the Service of Pathological Anatomy at the HC/UFMG, in relation to affected organs.

Evaluation of diagnostic methods

All methods have some limitation, being necessary to gather clinical evidence with laboratory results for a complete diagnosis.

The visualization of fresh yeast forms in the tissues has low sensitivity.

Culture is time-consuming, requiring three to four weeks for the determination of the etiological agent and biosafety facilities suitable for its handling, which is difficult to obtain, especially in regions where PCM is not endemic, where the disease is rare, and the diagnosis is usually difficult and delayed.

The histopathological analysis aims at the recognition of yeast structures with bi-refringent cell walls and multiple budding with the aspect of a boat helm, considered pathognomonic. Still, the fungus is scarce in some specimens and can go unnoticed on the slide, or be confused with other thermally dimorphic fungi. Obtaining material for histological analysis is occasionally difficult and inadvisable, as in isolated involvement of the central nervous system or pulmonary disease requiring invasive procedures, it is time consuming, of high complexity, and has high costs and risks. Although presenting low sensitivity, histopathology, is very important because it confirms the presence of the agent in injured tissues and in various situations it is quicker than culture. However, it only presents pathological characters with the disadvantage of not identifying the species.

Serological methods are not highly specific (showing cross reaction with other fungi such as Histoplasma capsulatum) and featuring variable sensitivity, being low, especially in immunosuppressed patients, in which there is scarce production of antibodies; and indicate only that there was a fungus infection, which occurs in about 50% of the inhabitants of endemic areas, not revealing about disease activity, which must be inferred by the correlation with current clinical manifestations that resemble other fungal diseases (histoplasmosis, coccidioidomycosis) and some neoplasms, which constitute its differential diagnosis.

Tests that are based on detecting fungal antigens in plasma and urine are not standardized and are less effective than the previously mentioned tests.

Molecular biology methods (in situ hybridization, PCR, nested PCR, and real-time PCR) have been proposed for more sensitive and specific diagnostics of PCM, but are not yet available in the clinical practice.[3,6-8,20,48-55]

DIFFERENTIAL DIAGNOSIS

PCM constitutes, in its different ways, differential diagnosis with tuberculosis, histoplasmosis, coccidioidomycosis, cryptococcosis, neoplasias (lung, larynx, mouth, adrenal, skin), kala-azar, cytomegalovirus, chromomykosis, sporotrichosis, syphilis, Churg-Strauss disease, Wegener's granulomatosis, Hansen's disease, lymphoma, adenocarcinoma, sarcoma, infectious mononucleosis, sarcoidosis, toxoplasmosis, cat scratch disease, and mononucleosis simile syndrome (cytomegalovirus, toxoplasmosis, acute retroviral syndrome).

Ocular involvement can be confused with sporotrichosis, leishmaniasis, systemic lupus erythematosus, secondary syphilis, trachoma, tuberculosis, and toxoplasmosis.

Bowel alterations can simulate colon cancer, inflammatory bowel disease, lymphoma, tuberculosis, and toxoplasmosis.

Bone involvement may require differentiation with leishmaniasis, Hansen's disease, with bone metastases of breast, prostate, kidney, and thyroid cancer, or cartilaginous bony tumors, multiple myeloma, and tuberculosis. Bone tuberculosis is one of the major differential diagnoses of PCM. On tuber-

culosis, lesions tend to be asymmetrical and preferentially affect the lumbar spine and hip and knee joints, while PCM tend to be symmetrical and affect preferably shoulder girdle, upper arms, ribs and the acromioclavicular joint.[3.6 -8.20]

CURE CONTROL

The appropriate time for treatment interruption remains controversial. It must endure until the observation of criteria for cure is determined by parameters:

- **clinical:** characterized by the regression of signs and symptoms, healing of lesions, and involution of lymphadenopathies. Improvement can be quick, generally in four to five months, infusing the patient with the feeling that he no longer needs medication and the wish to stop it, even without medical authorization. This behavior in patients should be prevented to avoid any discontinuity in therapy and recurrent installation of PCM. The careful clinical surveillance in outpatient consultations constitutes an essential measure for treatment adherence until complete suspension of treatment is medically decided;
- **radiological:** stabilization of radiological images, maintenance of the same scarring lesions in five x-rays performed year-round;
- **immunological:** negativity of double immunodiffusion or stabilization of titers with values up to 1:2, observed in three serum samples at two months intervals; in general, 17 months is required. It is important to consider the relationship between the levels of IgG and chemokines, with clinical improvement, which is not always direct, which prevents the conclusion if serological methods available are of value to indicate the cure of PCM, and therefore, should not be followed without clinical judgment;
- **mycological:** negative research of the fungus in exams of secretions in which it has been previously identified;
- **apparent:** refers to the clinical, mycological, radiological, and immunological cure for two years, without receiving maintenance treatment.[7-9,86-89]

The observation of patients with recurrent PCM is frequent when treatment is interrupted. Thus, there is a need for the definition of parameters or laboratory tests with increased confidence for the decision-making regarding the duration of therapy.

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