PANEL SESSION 4
CLINIC AND THERAPEUTICS

04-01

ACUTE FORM OF PARACOCCIDIOIDOMYCOSIS: ANALYSIS OF THIRTEEN AUTOPSIES WITH EMPHASIS ON THE PULMONARY INVOLVEMENT

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Acute paracoccidioidomycosis (A-PCM) is the aggressive form of PCM, characterized by severe involvement of lymph nodes, spleen, liver, and bone marrow. Dissemination of the fungus is very intense, but clinical and pathological data referred low involvement of the lung. In this study we re-evaluate the gross and microscopic aspects of 13 autopsies of A-PCM, with emphasis on the lung involvement. Gross pathology showed enlargement of lymph nodes of all territories, spleen and liver. In the lungs there were few alteration (emphysema, miliar lesions, discreet irregular fibrosis. Microscopic findings revealed that in these organs the lesions were characterized by large, confluent granulomas, with extensive necrosis, with many multiple budding fungi. The liver, bone marrow and adrenals were involved in 100%, 75% and 75% respectively. The adrenals showed in 3 cases, a large destructive, necrotic lesions. The lungs were involved in 100% of the cases, frequently with small, loose epithelioide granulomas, with few fungi, localized in the alveolar septa, which extended and "growing" into the alveolar space. In these cases, fungi were seen inside the alveolar capillary bed. Macrophagic alveolitis, with or without fungi, was seen all over the parenchyma in 85% of the cases. Granulomatous inflammation was also observed in the peribronchial interstitium. In all cases it was observed a difuse septal alveolar e peri-bronchiolar, non-granulomatous pneumonitis, made up of lymphocytes, plasma-cells and few eosinophils. All these cases were associated with involvement of hilar lymph nodes. **Conclusion**: In the A-PCM the lung is involved in 100% of the cases, in a form of miliar lesions, macrophagic alveolitis and interstitial non-specific pneumonitis. This is understandable, since all contaminated lymph drained, ultimately, to the lungs, where the fungi, entrapped in the septal capillary bed, proliferate and start the granulomatous inflammatory reaction.

04-02

CHRONIC FORM OF PARACOCCIDIOIDOMYCOSIS: ANALYSIS OF FORTY AUTOPSIES WITH EMPHASIS ON THE PULMONARY PATHOGENY

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Chronic paracoccidioidomycosis (C-PCM) is a slowly progressive and most commum presentation of PCM. Usually presents with involvement of only one organ or more than one organ or system. The frequency of lung involvement is reported to be hig h(up to 90%), but its pathogenesis is still unsolved, being explained by reverse lymph flow (from hilum to the periphery), as a consequence of severe hilar lymph nodes involvement. Considering the anatomy of the lymphatic system(rich anastomosis with valves), we realize that reflux of lymph flow is a misconception. Other mechanisms could explain the pulmonary involvement in C-PCM. To address this issue we reviewed the morphologic aspects of 40 autopsies of C-PCM, with emphasis on the lung. **Results:** 1)The lung was involved in 97.5% of the cases, followed by mucosas (70%), adrenals (63%), and hilar lung lymph nodes (50%); 2)There was no association with the presence and the intensity of involvement between lung-hilar lymph nodes and lung-other organs. The pattern/intensity of lung lesions were variable, localized most in the 1/3 peripheral parenchyma; 3) The pattern of the granulomas was variable, more often loose and confluent, with caseous necrosis, and frequently with fibrosis. These patterns could be seen in the same lung. Macrophagic alveolitis was observed in 52% of the cases. A lobar-type pneumonic involvement was seen, containing many fungi and a suppurative exsudate. 4) Old fibrosis was present in 70% of the cases, in the form of irregular nodules (fibrosis of granulomas) or forming fibrotic septa of variable length and width (post-collapse fibrosis); peribronchic fibrosis (15%) was minimal. **Conclusion:** The pleomorphism and intensity of the pulmonary lesion in C-PCM can be explained by a vicious cycle: pulmonary infection→lymphatic dissemination drained to the→Right heart→pulmonary artery→blocking fungi in the capillary→granulomatous reaction→fibrosis. Recurrent reinfections should be considered.

04-03

BONE SCINTIGRAPHIC EVALUATION IN PATIENTS WITH PARACOCCIDIOIDOMYCOSIS

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Aim: To evaluate the bone involvement in patients with confirmed paracoccidioidomycosis (PBM) using the skeleton scintigraphy. **Method**: Forty-three patients were evaluated, 33 male and 10 female, with median age of 42 years (18 to 88). Three of them had presented the acute form (AF) with confirmed bone involvement and were on apparent cure at the moment of the scintigrapic evaluation. Forty patients presented active disease, 11 had the AF and the other 29 had the choric form (CF). All of them were submitted to bone scintigraphy after two hours of the injection of 740 MBq of 99mTc-MDP. Magnetic Resonance Imaging (MRI) was performed when indicated. **Results**: Twelve patients (30%) presented scintigraphic abnormalities. Eight had the AF and 4 the CF and 10 of them had no symptoms associated. The ribs (8 patients), scapula (2), femur (2), fibula (2), clavicle (1), humerus (1), pelvis (1), tibia (1), face (1) and skull (1) were the abnormal bones. All but one patient had focal abnormalities. This patient presented a linear lesion on ribs. Another patient had diffuse hyper-concentration of the 99mTc-MDP on the left distal femur and proximal tibia. The MRI showed a solid lesion and the biopsy demonstrated a granulomatous process. The three patients with apparent cure revealed normal scintigraphic studies. **Conclusion**: The bone scintigraphy with 99mTc-MDP was useful to evaluate the osteoarticular system in patients with PBM. The early detection of abnormalities, the whole skeleton evaluation, the definition of the region to look for the infectious agent, its no invasiveness and its normalization after treatment were the most prominent features. The bone scintigraphy was also useful to evaluate the intensity of the disease and its prognosis because the majority of the detected lesions were free of symptoms.

04-04

PARACOCCIDIOIDOMYCOSIS IN A TRANSPLANTED KIDNEY PATIENT – REPORT ON THE SECOND CASE OF THE LITERATURE

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Paracoccidioidomycosis (PCM) is one of the most prevalent systemic mycosis and its etiological agent is *Paracoccidioides brasiliensis* (Pb) a dimorphic fungi. The clinical manifestations vary from patient to patient. The long period of latency and the lack of epidemic data, as once the disease does not require notification of its occurrence, make difficult to have real data about the epidemiology in our country. The susceptibility to the fungus probably has a genetic background, which results in a depressed cellular immune response in susceptible patients. The immune suppression of the transplanted recipients has opened the door to certain opportunistic and non opportunistic fungal infections. We present a case of a 64 year-old male, etiology of ESRD unknown, admitted for kidney transplantation after 68 months of hemodialysis treatment. His PRA was 15% at transplantation. He received a 44 year-old cadaver kidney and was initially treated with Tacrolimus, Prednisone and Mycophenolate mofetil. On the 5th day after surgery he was treated with methilprednisolone pulse for acute rejection episode (Banff IA). He was discharged from hospital on the day 12th with a Creatinine of 2.5mg/dl. Three months after transplantation he developed an ulceration lesion on the right leg and biopsy of the lesion disclose PCM. He was treated with itraconazol for three months. His epidemiology was positive for this disease. He is 6 months after treatment with no signs of recurrence. The biopsy showed presence of chronic epithelioid granulomatous inflamation with necrosis and numerous Pb, most of them as viable forms, which characterized an active process. As the patient had movied to an urban area, the present case should be interpreted as an endogenous reactivation of the infection.

04-05

PARACOCCIDIOIDOMICOSIS AS OPPORTUNISTIC DISEASE

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Introduction and objectives: The Mycological infections have increased considerably, prevailing the criterion of Opportunistic Disease, by diverse factors that can take to the Inmuno depression. In the First Symposium of Paracoccidioidomicosis (Medellin) 1971, we commented our first observation of the association of Tuberculosis with a later exteriorization of PCM, pos administration of anti tuberculosis treatment. Until February of 2002 was informed 12 new cases, 10 of them presented the scattered form. Our objective is to relate a case of PCM as opportunistic disease in a person with tuberculosis. **Case Report.**Patient of 26 years, tractor driver in culture lands (Milagro-Ecuador), with antecedents of being a smoker in excess, without Epidemiology for infection by HIV and that displayed in 1971 etiological diagnosis of and advanced pulmonary tuberculosis. The surprising thing was the appearance of cutaneous PCM, 25 days after being administered the conventional anti tuberculosis treatment, observing in the cutaneous vesicles forms of crypto sporulation with Positive Inmuno diffusion. After 60 days of evolution displayed a cirrhotic syndrome confirmed by hepatic biopsy, nevertheless we used Anphotericin B with remission of the ascites and health improvement. We wrote down five incomes to the hospital that by familiar, and economic problems settles the opportunism of these pathology. After 6 years the patient passed away, demonstrating the serious visceral ganglionary and lymphatic compromise. **Discussion and Conclusion**: With criteria emitted in Argentina and Brazil that the Isoniazida acted like Inmuno suppressor drug, we indicated to the head physician of these patient to suspend the administration of the drug, without paying attention of our observation the clinical picture got worse with the cirrhotic syndrome so the patient was called for a new evaluation. We administered Anphotericin B, a drug that is described highly hepatic and nephrotoxic and we observed results of enormous improvement. Our patient has the characteristics of Opportunistic Disease presenting recidivates of the Tuberculosis and PCM.

04-06

PARACOCCIDIOIDOMYCOSIS IN PATIENTS INFECTED WITH THE HUMAN IMMUNODEFICIENCY VIRUS: REPORT OF THREE CASES

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We present three cases of coinfection HIV/paracoccidioidomycosis. The first patient, male, was in hospital with diagnosis of resistant pulmonary tuberculosis; he had a history of irregular treatment for tuberculosis and the diagnosis of HIV infection was done during his stay in hospital. The second patient, female, was in hospital with a diagnosis of HIV infection and pulmonary tuberculosis with history of irregular treatment. In both cases, with a detailed history and physical examination associated with laboratory tests, we were able to do the diagnosis of paracoccidioidomycosis. The third patient, male, was sent to the Ambulatory of Infectology, after diagnosis of paracoccidioidomycosis in hips (biopsy), using cotrimoxazol irregularly; patient belonged to risk group for sexually transmitted diseases and laboratory tests confirmed HIV infection. All patients were investigated and, after clinical and laboratorial examination, the diagnosis of coinfection HIV/paracoccidioidomycosis was done. We discuss the clinical form, therapeutic and evolution of these three cases.

04-07

PARACOCCIDIOIDOMYCOSIS AT NECROPSIES IN UBERABA, MG 1990-2000.

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INTRODUCTION: Paracoccidioidomycosis, as other mycosis, is not notified in the local health systems where it is endemic. The estimations regarding its prevalence are based on a great series of cases and universitary research centers where many of these patients are diagnosed and treated. The features of necropsy have allowed to determine the extension of the systemic attack of this pathology. This report describes the post mortem diagnosis of paracoccidioidomycosis in five individuals hospitalized for other causes. METHODS: By reviewing the records of paracoccidioidomycosis diagnosis, five reports of necropsy with this diagnosis were found, and the medical records corresponding to these patients in the Hospital Escola de Uberaba were reviewed. RESULTS: From 1990 up to 2000, 1,216 necropsies were performed in this Hospital. Among 5 (0.4%) of these necropsies, paracoccidioidomycosis was diagnosed as coadjuvant factor to death in three cases and as its direct cause in two. Four individuals were male, aged between 12 and 90 years. The hospitalization time was < 5 days for three individuals. Only two cases presented clinical and radiological signs of pneumopathy. However, in none of them the diagnosis of this mycosis was considered when living. Pulmonary lesions were found in all of them and two others shown dissemination to other organs and systems. COMMENTS: The five cases reported here shown paracoccidioidomycosis reactivation due to or associated with other medical conditions. Besides reinforcing the significance of necropsy performing in the Hospital Escola, this report shows that paracoccidioidomycosis can be asymptomatic or oligosymptomatic, thus making difficult its early diagnosis.

04-08

PALPEBRAL OCCURRENCE IN PARACOCCIDIOIDOMYCOSIS: REPORT OF FOUR CASES

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Paracoccidiodomycosis, the most common fungal respiratory infections in the South America, is caused by the dimorphic fungus *Paracoccidioides brasiliensis*. The primary infection is pulmonary, and may present as a self-limited pneumonia. The lymph nodes are commonly involved, extension to cutaneous tissue can occur, and systemic involvement of multiple organ systems is common. Ocular lesions in this fungal infection are rare being blefaritis and nodular uveitis the lesions more frequently described. However, chorioretinitis, conjunctive and lesions affecting the cornea have also been associated to some cases of paracoccidioidomycosis. We describe four cases of paracoccidiodomycosis with palpebral involvement among the 830 patients followed at Instituto de Pesquisa Clínica Evandro Chagas during 1960 to 2000. All of them showed ulcerated lesions on palpebra. Clinical, diagnostic, and treatment issues are discussed.

04-09

GASTRIC LESIONS OF PARACOCCIDIOIDOMYCOSIS (PCM)

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Gastrointestinal involvement of in PCM has been reported. The most affected segments are ileal and cecal regionsand lesions of proximal digestive tube are rare. With the aim of remembering PCM as a cause of gastric ulcers, we report a case of a patient who presented gastric lesions and adenopathy. SRF, a 29 years old white male presented an eight month history of generalized adenopathy. Histopathology of a ganglion specimen revealed PCM and he was treated with sulfametozaxol/trimetoprim for 8 months, without regularity, with clinical improve although residual inguinal adenopathy remained. Three years after, he presented abdominal pain and digestive endoscopy showed severe enanthematous exudative pangastritis and ulcerated lesion suggestive of gastric-duodenal neoplasm. Histopatology revealed "blastomicotic gastritis". Ultrasound and blood exams demonstrated splenomegaly and eosinophilia respectively. He used itraconazol for 6 months and remained asymptomatic for 3 months, when neck, gastric and back pain reappeared. By this time, clinical examination showed only painful cervical adenopathy. Laboratorial exams revealed only reactive IID, low adrenal reserve in response to ACTH rapid test and gastro-duodenal histopathology with eosinophilic duodenitis. He was treated with itraconazol, prednisone and antacids for more 6 months regularly, became asymptomatic and is still under regular follow up showing negative serology, pyloric stenosis (sequel) and low adrenal reserve. Paracoccidioidomycosis is a sistemic disease that may present as multiform lesions of skin and different mucosae, involves multiple organs and systems and patients who live in endemic areas must be submitted to full investigation for this mycosis.

04-10

JUVENILE TYPE PARACOCCIDIOIDOMYCOSIS IN OLIGOSYMPTOMATIC PATIENT

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Paracoccidioidomycosis (PCM) has recently been classified into juvenile (JT) and adult chronic (ACF) forms. Juvenile form affects the reticuloendothelial system of children and adults, and is usually a more agressive disease with multiple complications. With the aim of remembering PCM as an important differential diagnosis of lymphadenopathy in children and young adults, we present this case. TCM, a 13 years old, white, female, from Barra Mansa (RJ), who used to fish and play around a river next home, was referred to this institute with a 4 month history of productive cough, with chest pain and one cervical lymphonode enlargement of 2 cm Ø. Chest X-ray at that time was normal and cervical ganglion biopsy specimen histopathology revealed PCM. She was treated with sulfamethoxazole/trimethoprim for one week. Physical examination here, showed only a small cervical palpable ganglion (0.7 cm) with a neighbor cicatrix; the respiratory, digestive and cardiovascular physical examination was normal. Abdominal ultrasonography, chest RX, hemogram, urine sedimental and stool examination, ACTH rapid test and serology for mononucleosis, toxoplasma, CMV and rubeola were normal. IDD showed 1:16 reaction and cervical limphonode histological cuts showed abnormal configuration, granulomatous chronic inflamatory process, multinuclear giant cells. Grocott stain showed spherical fungal elements of many sizes, exhibitting multiple gemulating pictures. She is being trated with itraconazol for 3 months and remains asymptomatic. We would like to remember that this clinical picture is rarely seen at medical school's hospitals or reference centers, where JT PCM presents as severe disseminated disease, and also call attention to the importance of precocious suspicion and investigation of PCM in patients with epidemiological history associated with lymphadenopathy.

04-11

SEROLOGICAL FOLLOW-UP OF PARACOCCIDIOIDOMYCOSIS (PBM) PATIENTS

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The aim of this study was to compare serological tests in the follow-up of PBM patients. The course of specific serological tests was studied in 528 serum samples from 50 patients with PBM confirmed by the identification of the etiological agent. Double immunodiffusion test, with culture's filtrate (ID/F) or a mixed sonicated and culture's (ID/SF) antigen, indirect immunofluorescence assay (IIFA) and the complement fixation test (CFT) were studied before treatment and during the follow-up. To compare the results observed at different tests, the following ranges of levels were considered as inversion of the dilution: a) mild: positive results up to 8 in ID/F and ID/SF, up to 128 in test IIFA and up to 16 in CFT; b) moderate: 16 and 32 in ID/F and ID/SF, 256 and 512 in IIFA and 32 and 64 in CFT; c) intense: over 32 in ID/F and ID/SF, over 512 in IIFA and over 64 in CFT. Sensitivity of ID/F, ID/SF and IIFA was respectively 77%, 83% and 94% ($p>0.05$). Considering the sequence of dilutions and differences with more than one dilution, the concurrence among serological tests ranged from 54 to 80%.The correlation among ranges of serological levels at different tests showed concurrence from 88% to 99%. The time necessary for regression to negative results was 15 months (range: 3 to 66) for ID/F and 19 months (range: 2 to 63) for ID/SF, and for regression to doubtful (1:64) or negative results was 23 months (range: 1-67) for IIFA. The comparisons among serological levels suggest the use of ranges of intensity as to the proposed limits. The different profiles of these serological tests must be considered in monitoring the treatment. The differences in the time necessary for regression to negative results, the low sensitivity of the ID tests and the low specificity of the IIFA confirm that the suppressive treatment must be maintained for one year after achievement of a negative serology.

04-12

DYSPHONIA AND LARYNGEAL LESIONS IN TREATED PARACOCCIDIOIDOMYCOSIS PATIENTS

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Paracoccidioidomycosis (PBM) is a systemic disease caused by *Paracoccidioides brasiliensis*. The larynx is the third most frequent involved organ. In order to describe the persistent dysphonia and the laryngeal lesions we studied 15 normal male subjects and 30 post-treatment follow-up patients, 15 of them with pulmonary disease and 15 with larynx involvement. All the patients were submitted to perceptual and acoustic analysis of the voice; the 15 patients with larynx involvement were submitted to an endoscopic examination in order to describe the lesions of the larynx. The voice analysis showed more severe dysphonia in the group of patients with laryngeal lesions ($p<0.01$), characterized by roughness and breathness. The voices of all the groups of patients showed instability at perceptual analysis ($p<0.01$). 56% of the patients with PBM and laryngeal lesions had severe dysphonia, with a dysphonia index (ID) >7 . The program Dr. Speech, Tiger Electronics, did not accept the voices of five patients with laryngeal lesions for acoustic analysis, because of the severe dysphonia. Jitter was elevated in five patients with PBM and laryngeal lesions. 80% of the patients with PBM and laryngeal lesions showed two or more laryngeal structures involved at endoscopic examination. The vocal folds showed changes in all the patients with PBM and laryngeal lesions. The most freqüent lesion found was a fibrous thickenning of the vocal folds. The other structures affected were arytenoides, epiglottis and vestibular folds. We concluded that laryngeal involvement of paracoccidioidomycosis frequently causes severe dysphonia, and that otorhinolaryngologic examination is fundamental in all patients with PBM.

04-13

EVALUATION OF THE ACUTE OR SUBACUTE FORM OF PARACOCCIDIOIDOMYCOSIS

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The aim of this study was the evaluation of the acute or subacute form of paracoccidioidomycosis (PBM). The criteria of inclusion in this study were patients below 18 years old or patients with predominant involvement of the mononuclear phagocytic system, such as lymph nodes enlargement, hepatomegaly and/or splenomegaly. The 86 PBM patients were divided into 3 groups, according to the age distribution: A – 29 patients below 20 years old; B – 38 patients between 20 and 29 years old; C – 19 patients above 29 years old. Statistical evaluation was performed by non parametric test using a p value under 0.05 to characterize differences among groups. The male/female relationship, according to the age in years distribution, showed a male predominance only after 13 years old. The duration (days) of the symptomatology revealed no differences between groups (A=60; B=90; C=90). The groups were not different according to the frequency of involvement of the lungs, abdominal lymph nodes, mucous membranes of the airdigestive tract and bones. However, the skin was more frequently involved in Group B. Haemogram analysis revealed no differences among groups as to haemoglobin, erythrocyte sedimentation rate, leukocytes, band cells, segmented cells, lymphocytes and monocytes. On the contrary, the number of eosinophils was higher in patients below 20 years old. Albumin and γ-globulin presented no differences among groups. Group B showed the highest levels of antibodies by agar gel immunodiffusion test [B>(A=C)]. The differences observed among groups according to the incidence of the skin lesions, the number of eosinophils, and the titres of the serum antibodies allow for subdivision of the acute or subacute form into: a) childhood, adolescence and early adulthood form, and b) adulthood form.

04-14

RAPD AS A VALUABLE TOOL FOR IDENTIFYING A SUBGROUP OF Paracoccidioides brasiliensis PRESENTING CLINICAL TRIMETHOPRIM/SULPHAMETHOXAZOLE RESISTANCE

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Twenty and nine isolates of *Paracoccidioides brasiliensis* obtained from different geographic regions of Brazil (MT, MG, SP, RS states), Peru, Colombia, Venezuela and Argentina were investigated in relation to clinical and *in vitro* trimethoprim/sulphamethoxazole susceptibility. Furthermore DNA profiling of the same strains was done by 05 arbitrary primers (OPA-1, OPA-2, OPA-3, OPA-4 and OPG-14). The DNA amplification patterns obtained allowed the differentiation of all 29 analyzed isolates. The RAPD data were used in a phenetic approach constructed by UPGMA., which showed two major clusters named group I and group II. The first one encompassed only isolates from MT state of Brazil (07 from 11 analyzed) and another grouped the remainder strains including the other isolates from MT (04), the isolates from the other geographic areas of Brazil (13) as well the strains obtained from the distinct South Americas countries (05). These last strains were subgrouped in a specific branch within group II. No correlation between the RAPD patterns and pathological features of the disease, geographical regions or period of isolation of strain was observed. However we detected a interesting association between the strains belonging to group I, wich were isolated from chronic clinical cases never treated before and a high susceptibility to trimethoprim/sulphametoxazole verified *in vitro* and *in vivo*. These results seem to indicate the existence of genetically more related groups of *Paracoccidioides brasiliensis* more susceptible to the sulphas.

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04-15

PARACOCCIDIOIDOMYCOSIS: CLINIC CHARACTERISTICS IN 72 PATIENTS DIAGNOSED IN THE MICOLOGY'S LABORATORY AT THE UNIVERSITY OF CARABOBO. VENEZUELA. APRIL 1992-2002

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Background. Paracoccidioidomycosis (PCM), is a Deep Systemic Mycosis limited to Latin América. The etiologic agent is dimorphic fungus *Paracoccidioides brasiliensis*, which enters the body by inhalation, affecting initially the lungs from which it spreads causing variable clinical forms. Objetive. To present the clinic characteristics of 72 patients with PCM, to be diagnosed in the Mycology's Laboratory. Methods. Between April 1992-2002, 2.136 patients suspected of having got Deep Systemic Mycosis were summited to, Clinical, Radiological, Mycological (Direct and Culture tests of sputum, exudates and pus, specimens), Inmunological (by Inmunodiffusion and skin tests) tests. Results. The diagnose of PCM, was confirmed in 72 patients 72/ 2.136 (3.3%), males 62/72 (86%) and females 10/72 (14%), adults, mainly ages between 45-54, 20/72 (28%), farmers 58/72 (81%), from Carabobo state 60/72 (97%). Clinical manifestations: respiratory 72/72 (100%), asthenia 70/72 (97%), mucosal and skin lesions 30/72 (42%), cervical lymphadenopathy 27/72 (38%), dysphonia 10/72 (14%), dysphagia 10/72 (14%), Nervous Central Sistems lesions 1/72 (1%). Clinical forms: Pulmonary Unifocal PCM 42/72 (58%), Multifocal Chronic PCM 30/72 (42%). Laboratory diagnosis: Direct examination and Cultures in Sabouraud agar revealed P brasiliensis in 68/72 (94%), Serologic test, by Inmunodiffusion (positive) in 72/72 (100%), Paracoccidioidin skin test (positive) 50/72 (69%), Radiologic test: confluent nodular infiltrates bilateral, symmetrical basal portions of the lungs 60/72 (83%). Treatment: 20/72 (28 %) patients treated with Sulfamethoxazole and trimethoprim, Imidazole derivatives: Itraconazole 44/72 (61%), Ketoconazole 6/72 (9%), Fluconazole 1/72 (1%), during 12 months or more, with regular check-ups, depending on the patient's response and the results of mycologic, radiologic and serologic tests, Amphotericin B + Itraconazol 1/72 (1.%) Conclusión. The Paracoccidioidomycosis is endemic in Carabobo State, Venezuela. Variable clinical forms were diagnosed.

04-16

IN VITRO COMPARISON OF ACTIVITY OF TERBINAFINE AND ITRACONAZOLE AGAINST *Paraccoccidioides brasiliensis*

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In vitro, terbinafine is highly active against a broad spectrum of pathogenic fungi. We evaluated terbinafine and itraconazole against 31 isolates of *Paracoccidioides brasiliensis*. The tests were determined using a broth macrodilution procedure. Tha MICs, in micrograms per milliliter, were as follows: Terbinafine, 0.015 to 1.0 (geometric mean, 0.1188) and itraconazole 0.007 to 0.5 (geometric mean, 0.03165). The usual therapy for paracoccidioidomycosis is sulphonamides, amphotericin B and azole derivatives (ketoconazole, itraconazole, fluconazole). With respect to amphotericin B, the azole derivatives allow shorter treatment courses, can be administered orally, and are equally effective. Itraconazole has the high efficacy of ketoconazole, but with superior tolerance. It is the current drug of choice in the treatment of paracoccidioidomycosis. The data obtained in this study indicate that terbinafine is active against *P. brasiliensis in vitro* and suggest that this allylamine can be considered a new option as drug therapy for paracoccidioidomycosis.

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DISSEMINATED PARACOCCIDIOIDOMYCOSIS: CORRELATION BETWEEN CLINICAL AND IN VITRO RESISTANCE TO KETOCONAZOLE AND TRIMETHOPRIM/SULPHAMETHOXAZOLE

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This study relates to a case of sub-acute multifocal paracoccidioidomycosis with involvment with the upper intestinal tract. The involvment in the digestive system of paracoccidioidomycosis has been described in clinical studies, supported by radiological and endoscopic examination, as well as post-mortem material, but involvment in the upper digestive tract is however uncommon. Recommended therapeutic treatment plans and the difficulty in the treatment of paracoccidioidomycosis are discussed in association with tests of susceptibility to antifungal drugs *in vitro*. This is the first report available in the literature showing, in parallel, clinical and *in vitro* resistance to ketoconazole and trimethoprim/sulphamethoxazole, studied during the course of the disease.

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Closing conference

RECENT ADVANCES IN THE STUDY OF *Paracoccidioides brasiliensis*

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Recent advances in the study of *Paracoccidioides brasiliensis* are reviewed focusing on contributions in the area of gene characterization and expression as related to morphogenesis, fungal biology and virulence. Also reviewed are recent results on immunological mechanisms induced in experimental paracoccidioidomycosis, particularly those involving chemokines. Perspectives of vaccination are discussed based on the cellular immune response in human patients induced by a series of peptides derived from the major antigen gp43 with prediction of presentation by human DR alleles using TEPITOPE computational analysis. Partial transcriptome characterization of *P. brasiliensis* has been developed by Felipe and coworkers (University of Brasília) with annotated proteins including HSPs (HSP70, HSP60, GroEL), prohibitin, polyubiquitin, multidrug resistance proteins (MDR), proteins of meiotic sister-chromatid recombination (Msc1p). Other genes identified by the group encoded a stage-specific PbY20 protein, kexin-like proteinase, calmodulin. The collaborating group of Soares (Federal University of Goias) cloned the cDNA encoding the L35 ribosomal protein as well as HSP60 and enzymes of carbohydrate metabolism, a genome clone encoding a ClpB chaperone homolog, and analyzed the expression of alpha-tubulin isoforms in yeast and mycelial forms. Goldman and coworkers (Faculty of Medicine, Ribeirão Preto, USP) out of 4,692 expressed genes of *P. brasiliensis* separated gene clusters differentially expressed in the phase transition mycelium-yeast, comprising RBT (homolog to *Candida albicans* gene), hydrophobin, isocitrate lyase, malate dehydrogenase, GPI, alternative oxidase, ubiquitin, delta-9-desaturase, HSPs 70, 82, 104, and two Pb Contigs. Results stress the importance of genomic analysis in human pathogenic fungi. Several *C. albicans* virulence/pathogenicity homologues were found in *P. brasiliensis.*

In another important development, the chemokines induced by *P. brasiliensis* yeast cells and cell wall and by the gp43 were determined in wild type and knock-out mice for IFN-gamma and TNF-alpha p55 receptor (Silva and coworkers, Faculty of Medicine, Ribeirão Preto, USP). Results indicated that CC as well as CXC chemokines are involved in the recruitment of leukocytes to the infection site and that their production is regulated by cytokines IFN-gamma and TNF-alpha. Finally, advances have also been made to foster the vaccine project (Iwai and coworkers, INCOR and UNIFESP). To identify immunodominant and potentially protective human T cell epitopes of the gp43, peptide sequences predicted to bind promiscuously to multiple HLA-DR molecules using TEPITOPE algorithm were selected. Eight gp43 peptides, each predicted to bind to 12-21 HLA-DR molecules at 3% threshold, were tested on proliferation assays with peripheral blood mononuclear cells (PBMC) from 19 HLA-DR typed, treated and healed nonanergic PCM patients and healthy controls. The results showed that PBMC from all patients responded to purified gp43 and 14 patients recognized at least one of the tested peptides while none of the gp43-negative healthy controls recognized any peptide. Peptide gp43 (180-194) was recognized by 53% of patients, while the other selected gp43 peptides were recognized by 32-47% of patients. The frequency of peptide recognition was proportional to the promiscuity of HLA binding, as determined by TEPITOPE analysis. In conclusion, epitopes neighboring P10 (181-196), which is protective in mice, are also immunodominant in humans. In silico prediction of promiscuous epitopes and combinations thereof may increase the coverage and allow the immunization of genetically distinct populations.