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Resumos/Abstracts

(Continuação)

Follow-Up of SMEAR Positive Pulmonary Tuberculosis (TB) patients during treatment: can Cobas amplicor PCR be used in a Semi-quantitative manner to acess the efficacy of treatment?

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Patients and methods: Smear, culture and PCR results on respiratory specimens obtained on a monthly basis during treatment of 22 TB patients were compared. PCRs were performed using Cobas Amplicor for co-amplification of DNA from M. tuberculosis (MTB) and an internal inhibition control (MCC). After hybridisation to probes specific for MTB and MCC respectively, the optical density (OD) of each of the hybridisation products was measured. The ratio between the optical densities (MTB/MCC) was calculated.

Results obtained April 1st 1997 to May 15th 1998 are included: Statistical analysis (rank correlation coefficient by Spearman) showed that MTB/MCC ratio correlated significantly better with smear and with culture results than the MTB OD value alone. When correlating to smear grading (4+, 3+, 2+, +, negative), mean MTB/MCC ratios were 8.1, 2.8, 1.5, 1.1, and 0.3, respectively. For culture (3+, 2+, +, negative), mean MTB/MCC ratios were 5.2, 3.0, 0.9, and 0.3, respectively. When comparing analyses at different times during treatment, we found the following results:

Week after diagnosis	Smear pos./ all patients	Culture pos./ all patients	PCR pos./ all patients
1-7	22/22	22/22	22/22
9-17	8/22	3/22	17/22
18-26	1/20	0/17	8/22

Conclusions: The results show that PCR remains positive during a long period after initiation of treatment and indicate that Cobas Amplicor PCR might be used in a semi-quantitative manner to monitor the efficacy of treatment.

Evaluation of Mmb Redox Medium for Growth detection of *Mycobacterium*

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We undertook a study to evaluate a new liquid medium, MB Redox (Biotest), for recovery rate and time to detection of mycobacteria from respiratory specimens in comparison to conventional Löwenstein-Jensen (LJ) egg-based medium. We also look for evaluating the

workload and ease-to-use of the MB redox medium. A total of 742 consecutive respiratory specimens (320 sputa, 265 gastric aspirates, 77 bronchoscopic aspirates, 80 bronchoalveolar lavage fluids) were digested and decontaminated by the NALC-NaOH procedure. After centrifugation, the deposit was equally divided and inoculated under the volume of 0.5 ml onto LJ and MB media. After incubation at 37°C for 42 days (MB) and 56 days (LJ), positive cultures were identified by Ziehl-staining, Accuprobe testing and conventional identification. Contamination rate was 2% on LJ and 3% in MB. Twenty specimens (2.7%) were positive for M. tuberculosis complex (MTB), of which 8 were smear-positive and 12, smear-negative. Out of the 20, 16 were positive in both media including the 8 smear-positive, one was positive only on LJ, and 3 only in MB. In addition, 24 specimens (3.2%) were positive for nontuberculous mycobacteria or NTM (7 M. kansasii, 4 M.avium complex, 3 M. xenopi, 5 M. gordonae, 1 M. fortuitum, 3 M. chelonae, 1 unknown). Of them, 14 were positive in both media including the 2 smear-positive, 6 were positive only on LJ, and 4 only in MB. Mean time to detection for MTB was 23.6 days in MB and 28.9 on LJ, but respectively only 17 days and 20.7 days for the smear-positive specimens. Accuprobe identification was performed directly on MB positive cultures for 13 MTB and 6 NTM, resulting in 16 true-positive, 2 false-negative and 1 false-positive results, gold standard being conventional identification. Although the MB redox medium is neither easier-to-use nor more difficult-to-use than LJ, its performance might be better than that of LJ in terms of recovery rate and time to detection of mycobacteria. Its overall appraisal would require a more extensive evaluation.

Significantly increased killing efficacy of Acylated INH against INH-Resistant Mycobacteria

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Apart from a genetic basis, multidrug resistance of Mycobacterium tuberculosis or Mycobacterium avium is assumed to be cell wall mediated by alteration of envelope layers. In this study, we tested whether acylation of the hydrophilic drug Isoniazid (INH) with different chain lengths could improve the killing efficacy of the parent compound against INH-resistant mycobacteria. The lack of mutations in the genes coding for enoyl-ACP-reductase (inhA) and katalase (katG) in M.tuberculosis ATCC 35825 was determined by amplification, sequencing of the inhA fragment, or AciI digestion of the katG fragment. N-acyl-INH derivatives were synthesized and tested against M.tuberculosis ATCC 35825 and the a priori multidrug-resistant M.avium ATCC 25291 grown in either Middlebrook 7H9 medium or phagocytosed into the mouse macrophage cell line RAW 264.7. The bacterial viability after drug treatment was determined mass spectrometrically (LAMMA) by measuring the intrabacterial Na+/K+-ratios of typically 300 individual organisms of the treated and untreated bacterial populations. INH acylated with C8-C10 and C14-C16-residues had a significantly higher killing efficacy against M.avium and M.tuberculosis in extracellular susceptibility tests than the nonacylated INH. In intracellular tests the INH killing efficacy against M.avium was highest for the C12-C16 derivatives. Against intracellular M.tuberculosis, the N-acyl-INH derivatives C8, C9, and >C14 were the most effective. Although it could be demonstrated that N-acyl-INH derivatives of particular chain lengths can improve the killing efficacy of the hydrophilic parent molecule in vitro, a therapeutic benefit is at present not predictable. In any case, they might be useful tools for probing a possible role of cell wall alterations in drug resistance.

Isolation of Atypical Mycobacteria in HIV patients in an automatic growth System

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Objective: To evaluate the prevalence of atypical mycobacteria in an HIV population in Southern Spain by an automatic growth system based in liquid medium.

Material and methods: Over two years (may 1996-may 1998) we cultured 1825 samples from HIV patients in the automatic system MB/BACT, which uses culture bottles containing 10 ml of modified 7H Middlebrook medium. This system allows higher detection of mycobacteria and an important time saving compared to Lowenstein medium.

Results: We isolated a total of 61 atypical mycobacteria, with 46 M. avium-intracellullare, 4 M. flavescens, 3 M.chelonae, 2 M. gordonae, 2 M. scrofulaceum, 1 M. fortuitum, 1 M. simiae, 1 M. smegmatis and 1 M. xenopi.

The growth of atypical mycobacteria meant a 3.34% of the samples from the HIV population.

Conclusions: The prevalence of atypical mycobacteria in our HIV population is higher than expected, as measured by the MB BACT method that proves its utility in the detection of these mycobacteria.

Evaluation of the BACTEC MGIT 960 Mycobacteria Detection System

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The BACTEC(r) MGIT(tm) 960 is an automated system for the growth and detection of mycobacteria with a capacity to incubate and continuously monitor 960 MGIT(tm) culture tubes. We compared 562 specimens collected from 321 patients, inoculated to the BACTEC(r) MGIT(tm) 960, MGIT(tm), BACTEC(r) 460TB systems, as well as Lowenstein Jensen, Middlebrook 7H11 and Middlebrook 7H11 Selective solid media and compared the results. Specimens tested included 458 respiratory and 104 non-respiratory samples. A total of 156 specimens were positive for mycobacteria in all systems: BACTEC(r) MGIT(tm) 960 - 127 (81.4%); MGIT(tm) - 112 (71.8%); BACTEC(r) 460TB - 110 (70.5%); solid media - 93 (59.6%). A total of 34 specimens were positive for M. tuberculosis (Mtb): BACTEC(r) MGIT(tm) 960 - 23 (67.6%); MGIT(tm) - 27 (79.4%), BACTEC(r) 460TB - 29 (83.2%). The time to detection in days (TTD) for Mtb in each system were: BACTEC(r) MGIT(tm) 960 - 11.2d; MGIT(tm) - 16.1d, BACTEC(r) 460TB - 14.1d. A total of 116 specimens were positive for MAC: BACTEC(r) MGIT(tm) 960 - 99 (85.3%); MGIT(tm) - 84 (72.4%), BACTEC(r) 460TB - 79 (68.1%); solid media - 68 (58.6). The TTD for MAC in each system were: BACTEC(r) MGIT(tm) 960 - 9.2d; MGIT(tm) - 10.8d; BACTEC(r) 460TB - 9.6d. A total of 6 specimens were positive for MOTT: BACTEC(r) MGIT(tm) 960 - 5; MGIT(tm) - 1, BACTEC(r) 460TB - 2. Contamination rates for each system were: BACTEC(r) MGIT(tm) 960 - 9.3%; MGIT(tm) - 8.9%), BACTEC(r) 460TB - 5.1%. In sum, the greatest number of positive specimens was detected in the shortest period of time with the BACTEC(r) MGIT(tm) 960 system.

Effect of agitation on recovery and detection of *Mycobacterium SPP*. In seeded blood specimens in the MB/BACT blood cultutre bottle

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The MB/BacT Blood Culture Bottle in combination with the MB/BacT System is a fully automated, non-invasive, non-radioactive system designed to recover and detect Mycobacterium spp. commonly isolated from blood specimens. The purpose of this study was to compare the effect of agitation on recovery and detection of a variety of Mycobacterium spp. from MB/BacT Blood Culture Bottles versus culture bottles incubated under static conditions. Blood from healthy individuals was collected into Isolator Tubes (Wampole Laboratories, Cranbury, NJ) and seeded with the test organisms such that the initial inoculum was less than 100 CFU/bottle. Sets of six MB/BacT Blood Culture Bottles, each containing 1.0 ml of MB/BacT Enrichment Fluid, were inoculated with 5.0 ml of the seeded blood specimens. Three bottles of each set were incubated at 36° C in an MB BacT/Alert cabinet (static cultures) and three bottles were incubated in a BacT/Alert cabinet with an MB/BacT detection algorithm (agitated cultures). Average times to detection for M. avium were 11.7 days (agitated) and 14.5 days (static). Averages for M. intracellulare were 12.8 days (agitated) and 15.1 days (static). Agitation resulted in slight delays in times to detection for M. tuberculosis strains (21.4 days versus 20.6 days). Times to detection were improved with agitation for all other mycobacteria tested by an average of 2.5 days with the exception of M. gordonae, where there was a 19.5 day delay in the agitated culture. The results indicate that agitation of MB/BacT Blood Culture Bottles enhances the growth of MAC and may lead to shorter times to detection for several other mycobacteria species.

The Influence of environmental Mycobacteria in the cell mediated immunity against tuberculosis

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Environmental mycobacteria are widely distributed in nature. There is a hypotesis that these atypical mycobacteria can contribute to immunity against tuberculosis and negatively influence the protective effect of BCG vaccine. The mycobacteria species, the dose of the inoculum and the route of exposure (inhalation, swallowing, skin trauma) can be diverse and are associated with the climate and the individual life style. Interferon-gamma (IFN-g) is a crucial cytokine for macrophage activation and is associated with protection against tuberculosis, type 1 immune response. IL-10 is a cytokine with a potent inhibitory effect on macrophages, the main effector cells on the mycobacteria killing. The authors studied the cell mediated immune response (CMI) in vitro to 7 environmental mycobacteria.

Objectives: 1-Determine the lymphoproliferatve response to PPD from 7 environmental Mycobacteria.
2-Measure the production of IFN-g and IL-10

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from the blood of 10 healthy donors. Cells were incubated for 7 days with PPD of M.avium M.intracellulare, M.scrofulaceum, M.fortuitum, M.malmoense, M.vaccae, M.gordonae and M.tuberculosis. Lymphoproliferation was measured by 3H thymidine incorporation. IFN-g and IL-10 were determined by ELISA in the PBMC supernatant.

Results: M.scrofulaceum, M.avium and M.intracellulare gave highest lymphoproliferation and IFN-g production, but these differences between the species were not statistically significant.

Discussion: M.scrofulaceum, M.avium and M.intracellulare, also known as MAIS group, induced the highest response in this study. Although there was no statistical significance, these results can be explained by the high cross reactivity of antigens between these 3 species, and by the probable predominant environmental exposure to them. The high induction of IFN-g production by these mycobacteria, working as a natural vaccine, can bias the CMI to type 1 response, that is associated with protection against tuberculosis.

Differential Cytokine and Proliferative responses to challenge with Live and Killed *Mycobacterium bovis* BCG

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Specific resistance to Mycobacterium tuberculosis infection is only efficiently generated with live, but not with killed bacteria. This suggests that the antigens necessary to generate resistance are elaborate by live and metabolically active bacteria, but not by killed organisms.

Objectives: To establish whether live and killed M.bovis BCG induce different proliferative and cytokine responses.

Materials & Methods: Peripheral blood mononuclear cells from untreated tuberculosis patients with pulmonary and extrapulmonary disease, and from healthy BCG vaccinated controls were stimulated either with live or killed (irradiated) M. bovis BCG. Thymidine incorporation and cytokine production (IFN-g, TNF-a, and IL-10) were assayed.

Results: Our study demonstrates significant differences in the ability of live or killed M. bovis BCG preparations to elicit T-cell proliferation and cytokine production. Live bacteria were more efficient in induction of proliferation, IFN-g, TNF-a, and IL-10 than the equivalent killed preparation. This difference was observed in both, tuberculosis patients and healthy controls.

Conclusions: These results suggest that live M. bovis BCG secretes antigens which induce T cell proliferation and cytokine production by both T cells and monocytes.

Mycobacteriosis in Hospital de S. João (HSJ): Patients, strains and susceptibilities

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INTRODUCTION: The reemergence of tuberculosis in the last decade and the increase of Mycobacteria isolates other than *Mycobacterium tuberculosis* have been frequently connected with HIV infection. In this report, we review Mycobacteria isolates from our laboratory and their susceptibilities for one year.
MATERIAL AND METHODS: Samples from several biological products were processed and inoculated in Middlebrook 7H13 or Middlebrook 7H12 broth, or in Lowestein-Jensen (LJ) and were surveyed weekly for a total of six to eight weeks. The Accuprobe® DNA probes or the classic bioquimical methods in LJ were used for the identification of the strains in the positive cultures (positive smears for the presence of acid-fast bacilli). To access *Mycobacterium tuberculosis complex* (*Mtc*) susceptibilities SIRE (Streptomycin, Isoniazid, Rifampicin, Ethambutol) in Bactec 460 TB System was used; the other strains were surveyed with traditional methods for SIRE, cycloserine, rifabutin, para-aminosalicylic acid, ethionamide, kanamycin, ofloxacin and pyrazinamide.
RESULTS: A total of 359 samples from 162 patients were positive. Of these, 71 (43.8 %) belong to Infectious Diseases Department, patients who were mostly HIV positive. The remaining patients had been admitted to the Pneumology (55, 34.0%), Internal Medicine (25, 15.4%) and other departments (11, 6.8%). *Mtc* was isolated in 94.4% of the patients, while *Mycobacterium avium complex* (*Mac*) was isolated in the remaining 5.6%. Susceptibility testing performed in 100 strains of *Mtc* reveal the following drug resistance: isoniazid and streptomycin, 4.0%; isoniazid only, 2.0%; streptomycin only, 1.0%; and isoniazid, rifampicin and streptomycin, 1.0%. The other strains (92.0%) did not show drug resistance. As to the *Mac* isolates the susceptibility testing was performed in 6 strains: 1 revealed generalised resistance to all drugs and 5 were sensitive to cycloserine only.
CONCLUSIONS: The results emphasises the higher prevalence of *Mtc* strains in patients who are frequently HIV positive. They also show that first line drug resistance is present in 8% and only a 1% multidrug resistance was observed. In agreement with previous reports *Mac* is the second most frequent isolate.