Increasing Pathomorphism of Pulmonary Tuberculosis. An Observational Study of Slow Clinical, Microbiological and Imaging Response of Lung Tuberculosis to Specific Treatment. Which Role for Linezolid?

Roberto Manfredi[1], Anna Nanetti[2], Paola Dal Monte[2] and Leonardo Calza[1]

Departments of Infectious Diseases[1], and Microbiology[2],"Alma Mater Studiorum" University of Bologna, S.Orsola-Malpighi Hospital; Bologna, Italy

During recent years, a progressive emerging of tuberculosis occurred, related to the overall increased age of general population, primary and secondary (iatrogenic) immunodeficiencies, the availability of invasive procedures, surgical interventions and intensive care supports, bone marrow and solid organ transplantation, and especially the recent immigration flows of people often coming from areas endemic for tuberculosis, and living with evident social-economical disadvantages, and with a reduced access to health care facilities. Since January 2006, at our reference centre we followed 81 consecutive cases of pulmonary tuberculosis, with 65 of them which remained evaluable for the absence of extrapulmonary complications, and a continuative and effective clinical and therapeutic follow-up. The majority of episodes of evaluable pulmonary tuberculosis (49 cases out of 65: 75,4%) occurred in patients who immigrated from developing countries. In two patients multiresistant (MDR) *Mycobacterium tuberculosis* strains were found, while two more subjects (both immigrated from Eastern Europe) suffered from a disease due to extremely resistant (XDR) *M. tuberculosis* strains. Although enforcing all possible measures to increase patients' adherence to treatment (empowerment, delivery of oral drugs under direct control, use of i.v. formulation whenever possible), over 72% of evaluable patients had a very slow clinical, microbiological, and imaging ameliorement (1-6 months), with persistance of sputum and/or bronchoalveolar lavage (BAL) fluid positive for *M.tuberculosis* microscopy and/or culture for over 1-4 months (mean 9.2±3.2 weeks), during an apparently adequate treatment. When excluding patients suffering from XDR and MDR tuberculosis, in four subjects we observed that off-label linezolid adjunct together with at least three drugs with residual activity against tuberculosis, led to a significantly more rapid clinical-radiological improvement and negative microbiological search, with consequent possibility to led to a protected discharge, supported by a sequential, oral therapy. Linezolid was also successfully employed in all the four patients with XDR or MDR pulmonary tuberculosis: among these patients, a definitive or temporarily negativization of respiratory secretions, and consequent discharge, was achieved only after linezolid adjunct. Notwithstanding the maintained microbiological susceptibility of *M. tuberculosis* strains responsible of the great majority of cases of pulmonary tuberculosis to first-line drugs, an unexpected tendency of patients to have a persistingly positive sputum and/or BAL, and to experience prolonged hospitalization for cure and isolation, has been recognized in the last years. No particularly suggestive radiological imaging seems predictive of a so prolonged course, so that we presently lack of clinical and imaging elements which may be predictive of this slow treatment response. The same is for demographic and epidemiological issues, eventual underlying diseases, and clinical presentation, so that a major problem for health care providers is to distinguish upon admission patients who will be prone to have slow therapeutic response and a related prolonged hospitalization. The novel oxazolidinone linezolid is characterized by an affordable *in vitro* activity against *M. tuberculosis*, and an extremely elevated intracellular concentration in respiratory tissues. Worldwide, increasing microbiological, pharmacological, and clinical evidences may recommend the use as linezolid adjunct as an off-label salvage treatment of pulmonary tuberculosis refractory to treatment, although not necessarily determined by resistant (MDR-XDR) *M. tuberculosis* strains. Randomized clinical trials including initially patients with ascertained chemioresistant tuberculosis, are strongly warranted.

Key-Words: Pulmonary tuberculosis, microbiological assessment, radiological assessment, clinical-therapeutic issues, selected antimicrobial treatments, controlled observational study, linezolid.

A progressive emerging of both pulmonary and extrapulmonary tuberculosis occurred during recent years in Italy as well as in the majority of developed countries [1]. When assessing the possible pathogenesis of clinical manifestations descending from a previously latent (known or unknown) tubercular disease, these case are attributable to the increased age of general population, to the emerging role or primary and especially secondary immunodeficiencies (including the frequent and/or prolonged exposure to immunosuppressive and cytotoxic treatments), to the supporting role of invasive procedures, surgical interventions, bone marrow and solid organ transplantation, and intensive care assistance delivered to compromised patients [1-6]. On the other hand, the recent immigration flows of people coming from areas which are endemic for tuberculosis, and the social-economical distress of recently immigrated persons, greatly support the dissemination of tuberculosis in the community of immigrants and their contacts. To complicate this last

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Address for correspondence: Dr. Roberto Manfredi. Infectious Diseases, S. Orsola Hospital. Via Massarenti 11. I-40138 Bologna, Italy. Telephone: +39-051-6363355. Telefax: +39-051-343500. E-mail: Roberto.manfredi@unibo.it.

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situation, we also have to take into consideration the significant increase in the incidence and prevalence of drug-resistant tuberculosis [2, 3, 6, 7]. This is the case of the demanding, moderately-resistant or extremely resistant (MDR-XDR) *Mycobacterium tuberculosis* strains, which usually are the result of a low therapeutic adherence or an incomplete drug delivery and treatment monitoring, which have been recognized especially among patients immigrated from disadvantages countries, where the health care system was not able to ensure an adequate disease control, and a reliable delivery of combined anti-tubercular therapy [1, 8].

Aim of our study is to assess all consecutive cases of isolated, confirmed pulmonary tuberculosis which came to our attention since January 2006, in order to assess the features of disease presentation during time and especially its tendency to hospital discharge and microbiological cure, in relation with a number of demographic, epidemiological, microbiological, clinical, imaging, and especially therapeutic issues. Our objectives are to focus on an apparently increased duration of admissions and continued isolations procedures for patients with a recently detected pulmonary tuberculosis, in order to possibly contribute to understand the pathogenesis of this recent disease pathomorphism, and especially to look for possible epidemiological, clinical, microbiological, and radiological clues which may help predict a long-term evolution of lung tuberculosis, together with its concerns (i.e. need for a very prolonged and extremely costly hospitalization, resort to prolonged isolation measures, determination of eventual bacterial resistance to common first-line antimycobacterial agents, and need for administration of some novel antimicrobial compounds which showed interesting activity against *M. tuberculosis* – i.e. novel fluoroquinolones and linezolid).

Material and Methods

Patient Population and Treatment Guidelines

Starting from January 2006, until March 2008, 81 consecutive patients with a pulmonary tuberculosis were diagnosed on clinical and imaging basis, and confirmed with a positive *M. tuberculosis* isolation and culture obtained on either bronchoalveolar lavage fluid and/or induced sputum.

Sixteen patients (19.7%) were excluded from further evaluation in this present study, due to one of the following conditions:

- patients who were lost to follow-up for more than one month during their clinical history (9 cases),
- subjects suffering from a disseminated tuberculosis involving organs and sites other than the respiratory tract (i.e. central nervous system, bone and joints, abdomen, kidney, and/or genital tract) (6 patients on the whole), or patients with concurrent respiratory pathogens other than *M. tuberculosis* (one case only).

The 65 evaluable patients with isolated pulmonary tuberculosis were hospitalized and/or followed on Day-Hospital basis at our tertiary care Division of Infectious Diseases, by the same staff physicians skilled in the treatment of tuberculosis and respiratory tract infections.

Anti-tubercular therapy was conducted according to current guidelines of treatment of pulmonary tuberculosis, and each drug was administered under a directly observed regimen on both inpatient or Day-Hospital basis, and intravenously when possible, in order to ensure the maximum adherence and bioavailability to both oral and i.v. prescribed regimens, during hospitalization at our centre, and later on Day-Hospital basis.

Microbiological Assays

After checking the suitability of pathologic specimens (sufficient amount, sputum consistency with sputum and lower respiratory tract secretions, and not saliva), the smears selected for direct microscopy examination were prepared according to the Ziehl-Nielsen-carbolfuchsin heated standardized staining technique. After microscopical search of alcohol-acid resistant bacteria, the specimens have been fluidified and decontaminated with a N-acetil-L-cysteine solution additioned with 4%NaOH (Snap'n'Digest-SDL/BBL, MYCO-PREP, Becton-Dickinson). A semiquantitative estimate of the amount of mycobacterial load was given for each examined specimen: all specimens were assessed by at least two skilled Microbiologist. As commonly established, a patient was considered cured only after three consecutive, negative microscopical searches for acid-fast bacilli performed on reliable sputum and/or bronchoalveolar lavage specimens, obtained in different days.

The microbial culture and isolation of *M. tuberculosis* has been obtained on a commercial Lowenstein-Jenssen solid or liquid medium (additioned with polymixin B, amphotericin B, carbenicillin, and trimethoprim), performed according to the manufacturer's instructions, or with automated liquid mediums (MB/BactT, BioMerieux). All positive cultural examinations were submitted to a further microscopy control (performed with a Ziehl-Nielsen-carbolfuchsin stain), and subsequently identified in a shorter time compared with standard growth, with the aid of a commercial assay for the search of *M. tuberculosis* DNA (Amplified-MTD, Gen-Probe, Biomerieux).

The presence of genetic determinants of drug resistance against rifampicin and isoniazid was systematically determined by a commercial assay (Inno-Lipa Mycobacteria RIF-TB, Innogenetics), which includes a polymerase chain reaction (PCR) amplification of the *rpoB* gene (which confers rifampicin-isoniazid resistance), and a subsequent hybridization with a series of gene probes, containing or not containing specific mutations related to potential drug resistances of the examined *M. tuberculosis* strains to the available first-line antimycobacterial drugs.

During clinical follow-up of our patients, sputum specimens and/or bronchoalveolar lavage fluid specimens were periodically submitted to our reference Microbiology laboratory, where microscopical and culture examination were performed systematically, as described above.

Non-tubercular mycobacteria and other concurrent microbial pathogens (i.e. bacteria, fungi), were carefully excluded, at every passage.

Imaging Evaluation

After obtaining a standard chest X-ray film, all patients with a microbiologically ascertained pulmonary tuberculosis (confirmed by bacteriological assays and culture, as described above), underwent a chest computerized tomography (CT) scan (high-resolution CT scan, and also contrast-enhanced CT scan in selected cases), before treatment initiation.

All CT examinations were performed using a multidetector scanner (Light Speed 16 QX Scanner, General Electric Medical System, Milwaukee, WI, USA). The images were obtained at end-inspiraton by using a 1,25 mm collimation at 5 mm interval and reconstucted using a high-spatial-frequency algorithm at 1.25 mm thickness and 1 mm interval for transaxial images (120-140 kVp, 200-250 mA). All images were directly displayed on separate workstation (Advantage Windows 3.1., General Electric Medical System, Milwaukee, WI, USA) and viewed at window settings optimized for assessment of lung parenchyma (window width, 1500-1800 H; window level, -500 -700 HU) and mediastinal structures (window width, 400 HU; window level, 40 HU). Intravenous injection of contrast medium were administered only when considered useful. Two independent chest radiologists, unaware of any clinical information analysed the HRCT scans and reached final decisions regarding the findings by consensus.

During the subsequent follow-up, high-resolution and contrast-enhanced thorax CT scans were obtained in parallel with antimycobacterial treatment, in order to follow the clinical and microbiological course of disease. In particular, the following aspects have been carefully examined: tree-in-bud opacities, nodules, consolidations (lobular, segmental, ad peribronchial), cavities (single or multiple), bronchiectasis, lobar volume decrease, lymphadenopathy, pleural effusion and thickening. A comparison with all prior CT examinations was determined, in order to discuss evolutive images during time.

All radiologic imaging was assessed separately by at least two Radiologists skilled in the interpretation of chest CT imaging, who were aware of the diagnosis of pulmonary tuberculosis, but did not have access to current microbiological and therapeutic data. In the event of some conflicting interpretation, a consultancy was arranged with a third Radiologist expert in chest CT consultancy, and an univocal interpretation was finally expressed on a joined basis.

Group Comparison

Our 65 evaluable patients with isolated pulmonary tuberculosis were compared essentially according to a shorter (within 30 days), *versus* a longer time of microbiological cure (more than one month), since discharge was deemed possible only after three confirmed sputum smears testing negative for acid-fast bacilli. Upon discharge from the Hospital/Day Hospital units, all patients were followed-up periodically with their continued anti-tubercular therapy performed on outpatient basis, during a period ranging from 6-12 months.

Statistical Assessment

Continuous variables were assessed through Student *t* test, while Mantel-Haenzel chi-square test or Fischer exact test were adopted to evaluate non-continuous variables. Statistical significance was posed at p values $<.05$.

Results

Among the 65 evaluable patients followed at our Metropolitan centre from January 2006 to March 2008, 49 (75.4%) occurred in subjects who recently immigrated from outside of the European Union. In majority, patients came from Eastern Europe (21 cases), followed by Subsaharian-Central Africa (11 patients), Northern Africa (8 cases), Southern-Eastern Asia (7 cases), and Southern America (2 patients).

Several demographic and epidemiological data are summarized in Table 1: the two patient groups did not differ significantly according to gender (although the overall number of males doubled that of females), age at disease presentation, and their condition of immigrants *versus* native patients, although the mean age was significantly lower for immigrants (29.2±9.8 years) *versus* native Italian residents (53.2±14.1 years; $p<.0001$). Several known predisposing conditions related to the respiratory tract, such as a history of tuberculosis, a known exposure to tuberculosis, one-more chronic, underlying pulmonary diseases, cigarette smoking (>5 cigarettes per day), and use of inhalatory steroids (>3 months per year). With regard to systemic disease, also some predisposing conditions like diabetes mellitus, collagen vascular disease (either treated or not with systemic steroids), HIV infection, and solid or hematologic malignancies, were assessed (Table 1).

The initial treatment of pulmonary tuberculosis was basically conducted with four drugs of common use (isoniazid at 300 mg/day i.v. or orally, rifampicin at 600 mg/day i.v. or orally, ethambutol at 1000-1200 mg/day i.v. or orally, plus oral pirazynamide at 1,500 mg/day, or i.m. streptomycin at 1 MU/day), but again no significant differences occurred between the two study groups, as to initial anti-tubercular therapy (including pirazynamide *versus* streptomycin). On the other hand, oral moxifloxacin (at 400 mg/day) and especially off-label linezolid (at 600 mg twice daily, i.v. followed by a sequential oral route), were likely to be introduced in patients in patient with an already ascertained bacterial resistance, as well as in some, selected cases of patients with a very late microbiological response (Table 1).

Some data may descend also from initial radiological (chest CT) control: although the figure is not statistically significant, the only 7 patients who experienced a worsening 3-4 weeks after the first chest CT scan were all included in the group of "late" microbiological responders (Table 1).

Table 1. Disease features of pulmonary tuberculosis, compared according to am established threshold of time of bacteriological cure (within one month, *versus* over than one month).

Patients' and disease characteristics	Overall evaluable patients with pulmonary tuberculosis (n=65)	Episodes of pulmonary tuberculosis cured in one month or less (n=18)	Episodes of pulmonary tuberculosis cured in over than one month (n=47)	p value
Gender (males / females)	44 / 21	12 / 6	32 / 15	n.s.
Age (years ± SD)	46,2±11,8	45,7±13,2	48,8±9,6	n.s.
Immigrated patients / resident (Italian) subjects	49 / 16	14 / 4	35 / 12	n.s.
Prior pulmonary disease and predisposing conditions				
history of prior tuberculosis	14	5	9	n.s.
known exposure to tuberculosis	21	4	17	n.s.
chronic, underlying pulmonary disease (i.e. COPD)	16	5	11	n.s.
cigarette smoking (≥5 cigarettes per day)	22	6	16	n.s.
frequent use of inhalatory steroids (≥3 mo per year)	5	1	4	n.s.
Underlying, systemic illnesses	19	6	13	n.s.
frank diabetes mellitus	9	3	7	n.s.
HIV infection/AIDS	2	1	0	n.s.
collagen vascular diseases or sarcoidosis	5	1	4	n.s.
(requiring immunosuppressive therapy)	5	1	4	n.s.
solid or hematologic malignancies	3	1	2	n.s.
(requiring chemotherapy/radiotherapy/transplantation)	3	1	2	n.s.
Initial radiological assessment (thorax CT scan)				
n. patients with CT worsening within 3-4 weeks of therapy	9	0	7	n.s.
Mycobacterium tuberculosis strains encoding for:				
rifampicin and isoniazid resistance	2	0	2	n.s.
multiple drug resistance (MDR strains)	2	0	2	n.s.
extremely extensive resistance (XDR strains)	2	0	2	n.s.
Administered anti-tubercular treatment combinations				
n. patients starting with baseline three drugs* plus pirazynamide	42	11	31	n.s.
n. patients starting with baseline three drugs* plus streptomycin	23	7	16	n.s.
n. patients adding moxifloxacin to their regimen	17	2	15	n.s.
n. patients adding linexolid to their regimen	4	0	4	n.s.
n. patients adding moxifloxacin plus linezolid	4	0	4	n.s.
Time to microbiological cure§ (weeks±SD) with a standard 4-drug therapy (patients with MDR-XDR tuberculosis are excluded)	5.3±2.9$	3.0±0.9$	9.2±3.2$$	p=.001$ to p<.001$$
time to microbiological cure§ (weeks±SD) in patients with moxifloxacin adjunct only	4.8±1.6&	3.1±1.1&	7.8±2.3	p<.001&
time to microbiological cure§ (weeks±SD) in patients with linezolid adjunct only	4.1±1.1^	n.a.	6.2±1.8^	p<.001^
time to microbiological cure§ (weeks±SD) in patients with moxifloxacin plus linezolid adjunct	4.2±1.3°	n.a.	5.1±1.1°	p<.001°
Time to microbiological cure§ (weeks±SD) in patients with XDR tuberculosis	78.3±41.5	n.a.	78.3±41.5	n.s.
time to microbioogical cure§ (weeks±SD) in patients taking 1-5 second-third line drugs (including fluoroquinolones), according to the *in vitro* susceptibility testing	>68	n.a.	>68	n.s.
time to microbiological cure§ (weeks±SD) in patients with linezolid adjunct	78.3±41.5	n.a.	78.3±41.5	n.s.

SD = standard deviation; n.s.= not significant; n.a. = not applicable; *Baseline three drugs: i.v. or oral isoniazid, ethambutol, and rifampicin; §At least three consecutive respiratory secretions (sputum and/or bronchoalveolar lavage fluid), testing negative at microscopy search of alcohol-acid fast bacilli, as described above.

The time to microbiological cure (represented by at least three consecutive, reliable sputum/bronchoalveolar lavage fluid specimens testing negative at microscopical examination, as described above), did not show any significance when comparing the two standard initial regimens (isoniazid, ethambutol, rifampicin, plus pirazynamide, *versus* isoniazid, ethambutol, rifampicin, plus streptomycin). Some patients with a very slow microbiological response (over 8-10 weeks), were more likely to add moxifloxacin, and finally linezolid to their initial regimen (Table 1).

The time to microbiological clearance of acid-fast bacilli from the sputum tested significantly faster in all patients with moxifloxacin and/or linezolid adjunct ($p<.001$), taking into consideration that both drugs were administered only to selected patients with a very slow bacteriological response (Table 1). As a consequence, the two fluoroquinolones and oxazolidinone compounds respectively, are expected to add significantly to the treatment of MDR-XDR tuberculosis, but also to obtain a more rapid microbiological cure of non-drug-resistant, but long-lasting tuberculosis, too.

Furthermore, from a drug resistance point of view, six overall patients carried *M. tuberculosis* strains encoding for genetic rifampicin plus isoniazide resistance, as described above. Of these six cases, two strains tested resistant to multiple anti-tubercular drugs (the so-called "MDR" strains) at the *in vitro* susceptibility assays, and two more strains proved extremely drug resistant (the so-called "XDR" strains). Second-choice drugs were tested by culture and *in vitro* assays of *M. tuberculosis* strains shipped to the deputed laboratories of the "Istituto Superiore di Sanità" in Rome, Italy. All these patients were cured in an extremely prolonged time, and deserve some separate consideration (see below), although the proportionally low number of episodes does not make significant their comparison with fully susceptible *M. tuberculosis* strains (as represented in Table 1).

In particular, a 30-year-old male from Moldova and a 24-year-old female patient from Ukraine underwent very prolonged hospitalizations due to XDR tuberculosis. The first patient, with tubercular disease known since six years, developed XDR *M. tuberculosis* strains due to frequent treatment discontinuations, and the radiological chest CT picture was extremely severe (Figure 1). On the ground of *in vitro* supplementary sensitivity assays, cycloserine, para-aminosalycilic acid, capreomycin, ethionamide, and linezolid were added, obtaining clinical-microbiological cure after 15 consecutive month of hospitalization. Three months after the first discharge, our patient maintained an effective six-drug regimen on Day-Hospital basis, but three months later another five-month hospitalization was needed after retrieval of a positive sputum. An outpatient treatment was conducted on Day-Hospital basis for three months, but positive sputum prompted a third admission ending with repeated microscopical negative assays six months later. The very prolonged linezolid treatment prompted emerging myelotoxicity, which required a red blood cell transfusion in two cases, while an overwhelming peripheral neuropathy was prompted by the large amount of potentially neurotoxic anti-tubercular agents. Our second "XDR" patient who came to Italy with an extremely resistant tuberculosis strain, had an initial unfavorable course, and was tested *in vitro* for second-choice drugs, which suggested a cycloserine, para-aminosalycilic acid, capreomycin, ethionamide, moxifloxacin, and linezolid adjunct. On the ground of this last combination, our patient achieved clinical-bacteriological cure of XDR tuberculosis and hospital discharge after five months, despite a concurrent chronic hepatitis C which hampered liver tolerability of the multiple, administered anti-mycobacterial compounds. During the subsequent seven-month follow-up carried out at our Day-Hospital, a five-drug association including both moxifloxacin and oral linezolid ensured a stabilized cure, still under follow-up at our Institution. Table 1 shows the extremely prolonged time to microscopical cure of XDR tuberculosis in both our patients: a mean time exceeding 78 weeks, reached only after off-label linezolid adjunct to a pre-existing 5-7-drug combination regimen. In these cases too, also the late linezolid adjunct apparently prompted microbiological negativization, which preceded clinical and imaging cure of XDR tuberculosis in these patients with an extremely severe and life-threatening condition.

Discussion

Given the recent re-emerging of tuberculosis (considered as a somewhat neglected disease in the developing world until the last decade) [1, 3, 6, 9], substantial interest should be concentrated on the early diagnosis, and mode and time of treatment, at least during the initial phase, when the bacillary disease is still contagious and hospital isolation is required in the majority of cases [9]. In fact, a sort of domiciliary isolation is actually impossible to be performed, since a large part of patients with pulmonary tuberculosis is represented by immigrants with housing difficulties (75.4% in our series), and a large part of residents are aged 50-60 years or more, and usually do not live alone.

The slow microbiological, radiological, and clinical response to an apparently appropriate anti-tubercular regimen, whose adherence was carefully enforced by patient empowerment, and drug delivery under direct health personnel control, or through the resort to i.v. formulations (where possible), in all treated patient, is a quite surprising feature, when compared with "historical" series, which pointed out that the large majority of patients are expected to become non-bacillary (and to have isolation measures removed), after 2-4 weeks of anti-tubercular therapy [10, 11].

As a consequence, one endpoint of our observational study was directed to look for demographic, epidemiological, clinical, radiological, and therapeutic variables supporting this unpredictable change of response of pulmonary tuberculosis to an appropriate and checked anti-tubercular therapy, since the international literature still lack of relevant evidences in this field.

Although we arbitrarily selected the already "prolonged" threshold of one month to separate "fast" responders from "slow" responders, however we surprisingly found that 72.3% of patients belonging to our single-centre cohort of patients coming from the same metropolitan area (Bologna, North-Eastern Italy), reached a microbiological cure beyond one month of hospitalization (mean time 9.2±3.2 weeks, excluding subjects with MDR-XDR tuberculosis) (Table 1).

Our very simple univariate analysis (summarized in Table 1), failed in identifying any demographic, epidemiological, clinical, radiological, and also therapeutic variable related to the risk of suffering from a prolonged lung tuberculosis requiring hospitalization, isolation, and long-term treatment sometimes including drugs other than first-line anti-tubercular ones. For instance, males were more frequent than females (but no significant differences were found in relation with time of microbiological cure), the number of immigrants overcame that of Italian residents, but again no difference was found as to cure rate and time. Moreover, a number of prior lower respiratory tract disorders were considered, together with systemic disorders, eventually requiring steroideal or immunosuppressive therapy, and diabetes mellitus [5], but once again no difference was found between the two study groups. Radiological assessment needs a deep implementation with appropriate algorithms [7, 12-14], although a gross worsening of the chest CT scan was already evident among a small (not significant) number of patients, all belonging to the "slow" responders to antitubercular treatment. When looking to the selected anti-tubercular regimens, the two "classic" four-drug associations were initially used in all study patients, without any difference when comparing subjects starting with a pirazynamide-including regimen, *versus* those starting with a streptomycin-containing association (Table 1). The adjunct of the fluoroquinolone moxifloxacin or the oxazolidinone linezolid occurred in the majority of cases among "slow" responders, which included also the six drug-resistant patients (and in particular, two cases each of "MDR" and "XDR" tuberculosis; only "MDR" and "XDR" episodes were treated with combined moxifloxacin and linezolid (Table 1).

The only statistically significant variables of our univariate analysis are concentrated in the area of "time to microbiological cure" (Table 1). Both patients with isolated moxifloxacin and linezolid adjunct, and those with associated moxifloxacin-linezolid adjunct experienced a mean longer time to bacteriological cure compared with overall patients ($p<.001$), but all of them they came from the "slow responder" group. Although the limited figures do not allow a statistical comparison, however the "slow responders" who received linezolid plus moxifloxacin (and at a minor extent those who received linezolid adjunct only), experienced a time to cure comparable to that of "rapid" responders, while it was not the case of patients who added moxifloxacin only (Table 1).

Finally, the bottom lines of Table 1 summarizes the time to microbiological cure of the two patients with "XDR" tuberculosis, all treated with 1-5 second or third line drugs selected on the ground of the *in vitro* susceptibility testing performed at a centralized reference centre (Rome, Italy), with both patients receiving linezolid adjunct, after an *in vitro* assessment which showed very low minimal inhibitory concentrations (<0,05 mg/μL) of this last drug against the examined XDR *M. tuberculosis* strain.

Notwithstanding the maintained, extensive microbiological susceptibility of *M. tuberculosis* strains responsible of the majority of cases of pulmonary tuberculosis in our country and in our area too, an unexpected tendency of our patients to have a persistingly positive sputum and/or bronchoalveolar lavage fluid examination and experience prolonged hospitalization for cure and isolation, was recognized in the last three years (2006-2008). Neither bacteriological findings, nor particularly suggestive initial chest X-ray and chest CT imaging seems predictive of a so prolonged course, so that we presently lack of microbiological-clinical-radiological elements initially predictive of a particularly slow treatment response, taking eventual benefit from the adjunct of novel drugs characterized by an elevated anti-mycobacterial potency and a favourable respiratory tissue concentration, like moxofloxacin and especially linezolid. In fact, the oxazolidinone linezolid is characterized by an affordable activity against *M. tuberculosis*, and an extremely elevated respiratory tract concentration, intracellular activity, and post-antibiotic effect [15-22]. Well-based, and increasing microbiological, pharmacological, and clinical evidences may recommend the use as an off-label salvage treatment of pulmonary tuberculosis which tests refractory to treatment, although not necessarily determined by resistant (MDR-XDR) tubercular strains. In order to produce controlled, significant data, randomized clinical trials are strongly warranted in these particular patient groups. Also in our series, when excluding patients suffering from MDR-XDR tuberculosis (who systematically added off-label linezolid to an optimized background regimen), in four more cases we observed that off-label linezolid adjunct to at least three drugs with residual activity against tuberculosis, led to a rapid clinical and radiological improvement and negative microbiological search in a time comparable to that of "rapid" responders, with consequent possibility to led to a protected discharge, supported by a sequential, oral linezolid therapy [19]. Should one or more variables anticipating a condition of "slow responder" behaviour to standard anti-tubercular therapy (despite the absence of drug-resistant strains), may be identified by studies like ours, these patients could benefit from the introduction of potent and sufficiently safe drugs, whose crude cost is expected to greatly outweight the expenditures related to prolonged hospitalization and isolation measures, further instrumental and laboratory assays, and so on.

As anticipated, in our experience linezolid was also successfully employed in four patients with MDR or XDR tuberculosis (two cases each): in these subjects, a

negativization of respiratory secretions was achieved always and only after linezolid adjunct. As known, even MDR-XDR tuberculosis is a worldwide emergency, due to the increased number of patients immigrating from countries where the health care assistance could not ensure adequate anti-tubercular drug delivery and monitoring. The management of the emerging MDR-XDR tuberculosis encompasses elevated clinical suspicion, diagnostic accuracy, availability of reliable *in vitro* susceptibility assays of second and third line drugs, as well as "novel", potential anti-tubercular drugs [17-20, 22, 23], although some authors include the novel fluoroquinolones as substitute agents of first-line agents, more than adjunctive pharmacological weapons, and susbstantial differences emerge among the different available compounds [23, 24]. In particular, adequate isolation and public health issues become prominent, when prolonged hospitalizations or protected discharges are needed [6-8, 11]. The frequent involvement of foreign immigrants is burdened by further social-economical, cultural, and administrative problems. The easy development of life-threatening and contagious MDR-XDR tuberculosis in health care contexts where low-cost anti-tubercular drugs are not always available, is in contrast with the huge danger and the incredibly elevated costs of these episodes which need prolonged hospitalization and isolation, and enormous technologic and health care efforts. A systematic planning of the most adequate management and prophylactic measures aimed at containing-preventing MDR-XDR tuberculosis in the next future, is strongly needed, together with appropriate pharmacoeconomic evaluations which take care of all, comprehensive variables connected with tuberculosis-related hospitalization.

References

1. Sabbatani S, Baldi E, Manfredi R. Time trends in health care needs of non-EU citizens from developing countries, admitted to a general hospital in northern Italy. Infez Med **2007**; 15:242-249.
2. Abdel Aziz M, Wright A. The World Health Organization/ International Union against Tuberculosis and lung disease global project on surveillance for anti-tuberculosis drug resistance: a model for other infectious diseases. Clin Infect Dis **2005**; 41:S258-S262.
3. Sabbatani S, Manfredi R, Legnani G, Chiodo F. Tuberculosis in a metropolitan area of northern Italy: epidemiological trends and public health concerns. Eur J Epidemiol **2004**; 19:501-503.
4. Sabbatani S, Manfredi R, Marinacci G, Pavoni M, Cristoni L, Chiodo F. Reactivation of severe, acute pulmonary tuberculosis during treatment with pegylated interferon-alpha and ribavirin for chronic HCV hepatitis. Scand J Infect Dis **2006**; 38:205-208.
5. Broxmeyer L. Diabetes mellitus, tuberculosis and the mycobacteria: two millennia of enigma. Med Hypotheses **2005**; 65:433-439.
6. Falzon D, Le Strat Y, Belghiti F, Infuso A. Exploring the determinants of treatment success for tuberculosis cases in Europe. Int J Tuberc Lung Dis **2005**; 9:1224-1229.
7. De Backer AI, Mortelé KJ, De Keulenaer BL, Parizel PM. Tuberculosis: epidemiology, manifestations, and the value of medical imaging in diagnosis. JBR-BTR **2006**; 89:243-250.
8. Ferrara G, Richeldi L, Bugiani M, et al. Management of multidrug-resistant tuberculosis in Italy. Int J Tuberc Lung Dis **2005**; 9:507-513.
9. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. BMC Public Health **2008**; 8:15.
10. Andresen D. Microbiological diagnostic procedures in respiratory infections: mycobacterial infections. Pediatr Respir Rev **2007**; 8:221-230.
11. Fortun J, Martin-Davila P, Molina A, et al. Sputum conversion among patients with pulmonary tuberculosis: are there implications for removal of respiratory isolation? J Antimicrob Chemother **2007**; 59:794-798.
12. Chung MJ, Lee KS, Koh WJ, et al. Drug-sensitive tuberculosis, multidrug-resistant tuberculosis, and nontuberculous mycobacterial pulmonary disease in nonAIDS adults: comparisons of thin-section TC findings. Eur Radiol **2006**; 16:1934-1941.
13. Van Mieghem IM, De Wever WF, Verschakelen JA. Lung infection in radiology: a summary of frequently depicted signs. JBR-BTR **2005**; 88:66-71.
14. Eisenhuber E, Mostbeck G, Bankier A, Stadler A, Rumetshofer R. Radiologic diagnosis of lung tuberculosis. Radiologe **2007**; 47:393-400.
15. Vera-Cabrera L, Brown-Elliott BA, Wallace EJ Jr, et al. *In vitro* activities of the novel oxazolidinones DA-7867 and DA-7157 against rapidly and slowly growing Mycobacteria. Antimicrob Agents Chemother **2006**; 50:4027-4029.
16. Hui M, Au-Yeang C, Wong KT, Chan CY, Yew WW, Leung CC. Post-antibiotic effects of linezolid and other agents against *Mycobacterium tuberculosis*. Int J Antimicrob Agents **2008**; 31:395-396.
17. Erturan Z, Uzun M. *In vitro* activity of linezolid against multidrug-resistant *Mycobacterium tuberculosis* isolates. Int J Antimicrob Agents **2005**; 26:78-80.
18. Tomioka H. Current status of some antituberculosis drugs and the development of new antituberculous agents with special reference to their *in vitro* and *in vivo* antimicrobial activities. Curr Pharm Des **2006**; 12: 4047-4070.
19. Ntziora F, Falagas ME. Linezolid for the treatment of patients with mycobacterial infections. A systematic review. Int J Tuberc Lung Dis **2007**; 11:606-611.
20. Barry PJ, O'Connor TM. Novel agents in the management of *Mycobacterium tuberculosis* disease. Curr Med Chem **2007**; 14:2000-2008.
21. Yew WW, Chau CH, Wen KH. Linezolid in the treatment of "difficult" multidrug-resistant tuberculosis. Int J Tuberc Lung Dis **2008**; 12:245-246.
22. Manfredi R. Update on the appropriate use of linezolid in clinical practice. Ther Clin Risk Manag **2006**; 2:455-464.
23. Moadebi S, Harder CK, Fitzgerald MJ, Elwood KR, Marra F. Fluoroquinolones for the treatment of pulmonary tuberculosis. Drugs **2007**; 67:2077-2099.
24. Zigashina LE, Squire SB. Fluoroquinolones for treating tuberculosis. Cochrane Database Syst Rev **2008**; 1:CD004795.