Radiological findings of tuberculosis in HIV positive patients

Poster No.: C-0404
Congress: ECR 2013
Type: Educational Exhibit
Authors: N. Pujic Stanisavljev; Sremska Mitrovica/RS
Keywords: Lung, Abdomen, Conventional radiography, CT, Ultrasound, Diagnostic procedure, AIDS, Infection
DOI: 10.1594/ecr2013/C-0404

Any information contained in this pdf file is automatically generated from digital material submitted to EPOS by third parties in the form of scientific presentations. References to any names, marks, products, or services of third parties or hypertext links to third-party sites or information are provided solely as a convenience to you and do not in any way constitute or imply ECR's endorsement, sponsorship or recommendation of the third party, information, product or service. ECR is not responsible for the content of these pages and does not make any representations regarding the content or accuracy of material in this file.

As per copyright regulations, any unauthorised use of the material or parts thereof as well as commercial reproduction or multiple distribution by any traditional or electronically based reproduction/publication method is strictly prohibited.

You agree to defend, indemnify, and hold ECR harmless from and against any and all claims, damages, costs, and expenses, including attorneys' fees, arising from or related to your use of these pages.

Please note: Links to movies, ppt slideshows and any other multimedia files are not available in the pdf version of presentations.

www.myESR.org
Learning objectives

Illustration of typical and atypical imaging features of pulmonary (PTB) and extrapulmonary tuberculosis (EPTB) in HIV positive patients that could help us to make proper diagnosis and introduce adequate therapy.

Background

The greater virulence of Mycobacterium tuberculosis, compared to other opportunistic pathogens that complicate HIV infection, means that most patients who develop tuberculosis (TB) have not experienced other AIDS-defining infections (22). TB occurs at relatively high levels of CD4+ T-lymphocyte counts, although its frequency markedly increases in patients with more severe immunosuppression. HIV-TB cases have been observed to have severe immunosuppression at presentation, with several studies reporting CD4 counts of less than 200/µL (2). Clinical presentation of TB in HIV-infected individuals depends on the level of immunosuppression (22). Symptoms and signs are often nonspecific, and it is difficult to distinguish the disease from other opportunistic infections (20, 26, 78). The exclusive lung disease occurs in less than 50% of cases, and the rest have the extrapulmonary disease only or both (22).

HIV / TBC INTERACTION - IMMUNOLOGY

Mycobacterium tuberculosis and HIV are intracellular microorganisms. Th1 type immune response characterised by adequate cell-mediated immunity is the crucial host defence against intracellular infections like tuberculosis. (70) HIV infection primarily affects those components of host immune response responsible for cell-mediated immunity, and increases the susceptibility to M. tuberculosis. HIV causes depletion of CD4+ TLy cells and functional abnormalities in CD4+ TLy and CD8+ TLy, which play an important role in providing protection against active TB disease. (86, 90) Gp120 virus component is occupying CD4 receptors and interfere presentation of mycobacterial antigens by MHC II molecules that leads to hyposensitivity to soluble tuberculin antigens. Decreasing in cell mediated immune response is damaging reaction of late hypersensitivity and granuloma formation. Also, development of memorial clones of T cells that could replace clones lost during immunodeficiency caused by HIV, is damaged - there is no control of disseminated Mycobacterium tuberculosis infection in AIDS patients in future. (65) Thus in HIV infected individuals with latent TB infection (LTBI), the fine balance between M. tuberculosis and
the host immunity gets tilted in favour of the former, resulting in reactivation \(^{(18)}\). Moreover, the infection is poorly contained following reactivation, resulting in widespread dissemination causing extrapulmonary disease \(^{(81)}\). Compared with CD4\(^+\) count-matched HIV-infected controls without TB, the relative risk of death and development of other opportunistic infections is higher in HIV-TB co-infected patients \(^{(88)}\).

Unlike other opportunistic infections which occur at CD4\(^+\) counts below 200/µL, active TB occurs throughout the course of HIV disease \(^{(27)}\). The presence of bacilli is tolerated by the HIV-infected patients with low CD4\(^+\) cell counts as the host is unable to mount an inflammatory response needed to control their growth. However, the sudden increase in CD4\(^+\) T cells in AIDS patients receiving highly active antiretroviral treatment causes an aggressive granulamatous response and active TB disease. \(^{(46, 51)}\)

Pulmonary or extrapulmonary TB in HIV-infected patient is sufficient for the diagnosis of AIDS. M. tuberculosis infection accelerates the progression of asymptomatic HIV infection to acquired immunodeficiency syndrome (AIDS) and eventually to death. The course of HIV infection is accelerated subsequent to the development of TB. \(^{(81)}\) Mycobacterium tuberculosis is stimulating cellular immune response, and HIV is replicating only in activated lymphocytes \(^{(74)}\). That indicates that accelerated HIV progression is partly attributable to the increased systemic immune activation in patients with HIV-TB \(^{(83)}\). Macrophages in tuberculosis, with their proinflamatory cytokines, such as tumour necrosis factor-a (TNF-a) and chemokines such as monocyte chemotactic protein 1 (MCP1) result in transcriptional activation of HIV genes through activation of nuclear factor-kB (NF-kB) and mitogen-activated protein (MAP) kinase pathways that leads to replication of HIV, systemically and locally in affected lung and pleural fluid \(^{(59, 81, 82)}\). The local immune activation also favours the development of latent HIV infection of macrophages and dendritic cells, thereby potentially enhancing dissemination of HIV \(^{(9, 59, 81)}\). Thus in HIV-infected persons with active TB, the sites of active TB infection act as epifoci of HIV replication and evolution independent of systemic HIV disease activity \(^{(81)}\). Treatment of tuberculosis reduces a number of HIV and improves immune response \(^{(6)}\).

Changes in immune response in TB/HIV co-infection can explain the differences in clinical presentation of tuberculosis, radiology findings and possibility of diagnosis in HIV seropositive patients.

**TUBERCULOSIS IN HIV POSITIVE PATIENTS - DIAGNOSTIC CHALLENGE**

Diagnostic approach to TB in a HIV infected individual is similar to that in immunocompetent patients, except that invasive diagnostic procedures are more often
required to establish the diagnosis. However, the diagnosis of TB in HIV-infected patients is considered to be difficult due to several reasons: frequently negative sputum smears (as HIV/AIDS patients usually have a lower sputum concentration of M. tuberculosis, or may not be able to produce good quality sputum), atypical or absent radiographic findings, higher prevalence of EPTB especially at inaccessible sites, and resemblance to other opportunistic infections. \(^{(1, 28, 73, 93)}\) Fewer than half of TB cases in HIV infected patients are diagnosed before death \(^{(52)}\) and the new guidelines have to be reassessed in the first place to reduce the delay in diagnosing TB in HIV-positive patients.

Suspected pulmonary tuberculosis requires chest radiography. CT scan and magnetic resonance imaging (MRI) have facilitated the detection and characterisation of occult foci of both PTB and EPTB \(^{(74)}\). Furthermore, CT and/or MRI can also be useful for locating the affected areas accessible for a diagnostic puncture \(^{(22)}\). Extrapulmonal foci, like abdominal tuberculosis and peripheral lymphadenopathy can also be detected by ultrasonography.

**Pulmonary TB**

In patients with relatively intact immune function - CD4+ count > 200/µL, (in study of Long at al. \(^{(47)}\) above 300 in µL) tuberculosis presents in the same fashion as tuberculosis in patients who are not infected with HIV, and pulmonary TB is more frequently seen than extrapulmonary TB \(^{(31, 36, 95)}\). Moreover, within the pulmonary disease, clinical presentations more similar to the classic are more common and often resemble postprimary pulmonary tuberculosis \(^{(20, 26, 78)}\). Typically, the patient has symptoms for several weeks, consisting of coughing, expectoration, sometimes haemoptysis, chest pain and general symptoms (mild fever or fever, sweating, anorexia and weight loss). It is essential to suspect the disease, and for all patients with respiratory symptoms and/or general symptoms over 2-3 weeks, to initiate diagnostic procedures, as well as in all patients with haemoptysis, regardless of the duration. TB, in any of its locations, must always be considered in the differential diagnosis of fever with an unknown origin. Pulmonary TB can even be detected in asymptomatic individuals. \(^{(22)}\)

Radiological findings of pulmonal tuberculosis on chest x-ray differ according to fact if it is first contact with bacilli or it is reactivation or secondary infection and according to status of immune response of patient. The main primary TB radiological findings, either alone or in combination, are: parenchymal infiltrates or opacities, corresponding to the initial pneumonic outbreak, with small and occasionally lobar segments, lymphadenopathy (especially paratracheal and hilar, with the initial focus usually in the right chest area - this is the basis of diagnosis in children), segmental atelectasis (due to compression of the lymph node of the bronchial lumen or due to endobronchial TB, especially in the middle lobe, conditioning bronchiectasis), pleural effusion, usually unilateral (more common
in the young and may be the only radiological finding), and miliary TB, which is rare (corresponding to a self-limiting initial haematogenous spread). The main radiological findings of reactivation, secondary or postprimary TB are: bronchopneumonic type condensation, patched without air bronchograms, normally found in posterior segments of upper lobes, cavitations, single or multiple, of various sizes, with or without air-fluid level, usually located in posterior segments of upper lobes, secondary pleural effusion to bronchopleural fistula, leading to a pneumothorax or pneumothorax if the focus is cavitated, miliary, haematogenous and diffuse dissemination of 1-3mm diameter micronodules which may be imperceptible in the beginning or coalesce and coexist with cavitated lesions in later stages, tuberculomas, which are nodules or tumour-like masses of various sizes, with calcifications and fibrosis (attempt to heal infiltrates and cavities by calcification and fibrous retraction of the segment or lobe affected, with a distortion in its structure, it causes bronchiectasis and may lead to a destroyed lung pattern). In immunocompetent patients, the radiological findings common in children, adolescents and young adults are often those characteristic of primary TB, while in adults and the elderly they are post-primary TB. In immunosuppressed patients, as happens in HIV infection, the findings vary depending on the degree of suppression. Patients with normal CD4 count have findings similar to those described for immunocompetent individuals. Authors reported in patients with PTB and preserved immune response upper lobe infiltrates and/or bilateral infiltrates, cavitations, pulmonary fibrosis, shrinkage, and calcification similar to those in HIV negative individuals with PTB. Sputum smears are often positive for acid-fast bacilli (AFB). Upon comparing different radiological patterns with the CD4+ T-lymphocyte count, an association between the radiological pattern of post-primary tuberculosis and a CD4+ T-lymphocyte count of > 200 cells/µL was found. All patients with CD4+ T-lymphocyte counts < 200 cells/µL presented radiological patterns that were different from the post-primary ones. The chest x-ray may even be normal or it can be more like findings in primary TB. It shows interstitial infiltrates, unilateral or bilateral, localised more often in the lower lobes than in the upper lobes, air-space consolidation similar to bacterial pneumonia with no features of cavitations and fibrosis. There are reports about typical cavities that were seen in only 25% of patients. Diffuse pulmonary infiltrates/opacities are the dominant radiological presentation. Miliary pattern and pleural effusion are more common. Intrathoracic lymphadenopathy is often evident in these patients, resembling primary TB, regardless of the prior TB exposure status. TB in the hilar and mediastinal lymph nodes can be found together with other thoracic and/or cervical lymphadenitis. When the immune suppression is intense, the sputum smear can often be negative. Other studies also reported more frequent diffused or localized infiltration, as well as hilar or mediastinal lymphadenopathy, and pleural infiltration, cavitations and normal radiography that were the least common findings. All these results are in accordance with studies, where cavitations, post-primary or typical tuberculosis radiological patterns
were correlated with CD4+ T-lymphocyte counts > 200 cells/µL and lymphadenopathy and atypical findings with lower CD4 counts \(^{(24, 58, 64)}\). However, studies conducted in Africa \(^{(8, 58)}\) demonstrated greater frequency of cavitations in HIV-positive patients with tuberculosis. One explanation for this could be the high incidence of tuberculosis in that region \(^{(10)}\), where tuberculosis infection appears at earlier stages of HIV infection, when the immune system is relatively preserved \(^{(23, 53)}\).

Patients with HIV and PTB have apparently normal-looking chest radiographs in 5-10% of HIV+ individuals \(^{(23, 35, 44, 80)}\), and in up to 20% of the patients with an advanced level of immunodepression, yet M. tuberculosis can be demonstrated or isolated from their sputum or bronchoalveolar lavage fluid \(^{(24, 45, 64)}\). It remains uncertain whether the absence of findings represents early stages of either primary disease or reactivation, or disease caused by intrathoracic adenopathy that has not been detected by simple radiographic examination \(^{(64)}\). HIV seropositive PTB had various CT demonstrations, including miliary foci, pulmonary nodules, tuberculoma, ground-glass, patching flakes, fiber cord, masses, pleural thickening, pleural fluid, intrathoracic lymph nodes enlargement, etc. \(^{(64, 94)}\)

Differential diagnoses for pulmonary TB include Pneumocystis carinii pneumonia (PCP), non-tuberculous mycobacteria, nocardiosis, fungal infections and non-infective complications like lymphocytic interstitial pneumonitis.

**Extrapulmonary TB**

As immunosuppression progresses, EPTB becomes increasingly common \(^{(92)}\). In developing countries, EPTB is the commonest cause of pyrexia of unknown origin among HIV-infected patients \(^{(68)}\). In contrast to HIV-negative patients with EPTB, the disease is often disseminated involving two or more noncontiguous organs concomitantly \(^{(72)}\). Radiology and other imaging techniques can help in diagnosing extrapulmonary TB \(^{(22)}\).

The most common forms of extrapulmonary involvement include lymph node TB, both peripheral, intrathoracic and intraabdominal, pleural and pericardial TB, abdominal TB (involved spleen, liver, kidneys, prostate, intestine), central nervous system (CNS) TB (involved meninges or brain parenchyma), TB of joints and bone marrow \(^{(16, 22, 44)}\). Once the diagnosis of extrapulmonary TB is made, association with pulmonary TB should always be investigated, as they can coexist. Although the treatment basis is the same, the contagious nature of pulmonary TB means close associates must be tested for the disease. \(^{(7, 25)}\)
The most common location of EPTB lymphadenitis is in the lymph nodes of the cervical and supraclavicular region, although it can occur at any location. In advanced HIV/AIDS, lymph node involvement is characterised by poor granuloma formation with abundant acid-fast bacilli in a background of neutrophils and florid necrosis. The peripheral enhancement with a low attenuated centre on the CT is as highly specific in the diagnosis of TB, although this is not pathognomonic.

Pleural TB can occur in isolation or concomitant with pulmonary TB. In contrast to HIV-negative patients in whom pleural effusion due to TB often resolves spontaneously, in HIV-positive patient it is progressive and remains culture-positive for M. tuberculosis for prolonged period of time. In addition, pleural fluid shows abundant mesothelial cells in these patients, a finding reflecting poor inflammatory response due to HIV/AIDS. Pleural infiltration as a manifestation of tuberculosis in HIV-positive patients is more commonly reported in patients with a T-lymphocyte count of CD4 > 200 cells/µL, which reflects a strong immune reaction in the pleura, but there are also studies that report the vast majority of cases with pleural infiltration in patients with a T-lymphocyte count of CD4 < 200 cells/µL.

Another form of TB manifestation in the chest is endobronchial affectation which, as with the previous ones, may occur alone or in combination with others. The usual bronchoscopy procedures provide diagnostic confirmation. It manifests as localised lesions and occasionally may have an endobronchial affectation derived from a bronchial perforation due to an adenopathy.

Another chest structure that can be affected by TB is the pericardium, which manifests as a pericardial effusion or constrictive pericarditis. This may appear alone or associated
with pleural or another form of TB. Tuberculous aetiology in the pericarditis of long
evolution, not responding to anti-inflammatory treatment, must be especially considered.
Echocardiography is useful for detecting the presence of pericardial fluid and
features such as collapse of right atrial or right ventricular free wall in diastole which is
diagnostic sign of cardiac tamponade. In fact, these features may sometimes precede
the other clinical evidence of pericardial tuberculosis. Echocardiogram is not an accurate
test to detect pericardial thickening. Sometimes, CECT of the thorax has been
used to assess pericardial thickening in patient with pericardial effusion. Indirect
echocardiographic signs such as flat posterior left ventricular wall motion in the diastole,
premature opening of the pulmonary valve, may suggest chronic constrictive pericarditis.

Abdominal tuberculosis in HIV-infected patients characterizes visceral lesions and
intraabdominal lymphadenopathy with necrosis and ascites. Hepatosplenic focal lesions
(granulomas or abscesses) and intraabdominal lymphadenopathy are more common in HIV-infected patients with abdominal TB; on the other hand, ascites and omental
thickening are less common when compared to HIV-negative patients with abdominal
TB. Granulomas or abscesses in pancreas, mesenteric infiltration, omental masses,
peritoneal enhancement/thickening and disorganised masses of soft tissue densities may
also be seen.

The lymph nodes in abdomen are either discrete or appear as matted conglomerate
masses. Retroperitoneal, peripancreatic, porta hepatis and mesenteric/omental lymph
node enlargement may be evident. Enlarged nodes usually contain central hypoechoic
areas on ultrasound. Pattern of contrast enhancement in CTs variable: the most frequent
is peripheral, and the rest could be heterogeneous, homogeneous, or absent, and larger
nodes may have a multilocular appearance. Abdominal CT scan also detects caseous
necrosis of lymph node which appears as low attenuated, necrotic centres and thick,
high attenuated inflammatory rim. Abdominal CT scan scores over ultrasonography for
detecting high attenuated ascites.

Involvement of the liver and spleen is common in miliary tuberculosis, and it is found in
80-100% of autopsied patients. Hepatosplenic tuberculosis develops under micro- or
macronodular forms. Macronodular form of lesions can be seen as multiple hypoechoic
and occasionally hyperechoic nodules on ultrasounds and as low attenuated small mass
with minimal peripheral contrast enhancement on CT examination. Macronodular splenic
involvement occurs in 15% of cases with HIV positivity. Other form, micronodular
form, is most common and manifests usually only as moderate hepatosplenomegaly
- tiny low attenuated foci scattered throughout the involved organs on CT and diffuse
hyperechogenicity of liver or spleen on ultrasound. The most common presentation
of hepatosplenic tuberculosis is non-specific hepatosplenomegaly, because lesions
are usually in a fine miliary pattern and most of the time below the resolving capacity of
Splenic lesions may be the only sign of tuberculous involvement. In these cases lymphoma should be kept in mind in the differential diagnosis. Splenic involvement is common among HIV-positive patients and in the miliary type appears as non-specific homogeneous splenic enlargement on ultrasound. Multiple tuberculosis abscesses in spleen have been described in patients with HIV infection.

Gastrointestinal TB may involve any part of the digestive tract from the mouth to the anus, with ileocecal localisation as the most common in HIV-negative population. An ulcerated image can be seen at endoscopy, which sometimes suggests a carcinoma. It can also manifest as warty or fistula images. Radiological signs of fibrosis, intestinal stenosis or retraction can be found. Isotopic techniques, such as scintigraphy and positron emission tomography (PET), which estimate the inflammatory activity, detect the disease in its early stages, although the resulting findings are non-specific for the diagnosis of extrathoracic TB. The most common symptoms related to gastrointestinal TB are diarrhoea and weight loss. Severe weight loss is a common presenting feature of HIV-infected patients presenting with TB. Many patients with AIDS, particularly in Africa, develop severe wasting and this has been called "slim disease". In African study weight loss greater than 10% was strongly correlated with CD4+ T-lymphocyte counts < 200 cells/μL for patients with both tuberculosis and HIV. Since these patients usually have chronic diarrhoea, the condition was thus thought to be a consequence of HIV-enteropathy. However, at autopsy, nearly half of HIV/AIDS patients who died with "slim disease" were found to have disseminated TB as compared with just over a quarter of those dying without such wasting, suggesting that cryptic disseminated/miliary TB may be an important cause of wasting in these patients. It is more common in disseminated TB in a gastrointestinal location. Neither leukocytosis nor biochemical alterations are usually found.

Urinary TB has non-specific or even absence symptoms, possible dysuria and haematuria or if fibrosis occurs, symptoms of narrowing or obstructing the urinary path with cramps that can lead to hydronephrosis. In renal TB, affection of the calyces is often the first radiologically objective manifestation. The lesion may progress causing calyceal deformity, ulceration, fibrosis and stenosis, which in the end lead to caliectasis, hydronephrosis and ureteral hydronephrosis, secondary to renal parenchymal destruction. Intravenous pyelography and percutaneous nephrogram are useful in imaging genitourinary TB. Radiography may demonstrate calcification within the renal parenchyma. Intravenous urography can demonstrate a "moth-eaten" calix, phantom calyx, Kerr kink of renal pelvis. CT is helpful in identifying the manifestations of renal tuberculosis. Calcifications, various patterns of hydronephrosis and include focal caliectasis, caliectasis without pelvic dilatation, and generalized hydronephrosis, cortical thinning, parenchymal scarring, fibrotic strictures of the
Infundibula, renal pelvis and ureters at contrast-enhanced CT is highly suggestive of tuberculosis and depict the extension of disease into the extrarenal space (67).

Genital TB in males can affect the prostate, epididymis and less frequently the testicles and seminal vesicles. It is often associated with urinary tract TB. Ultrasound techniques may be of diagnostic help in tuberculous epididymitis and other forms of genital location. Genital TB shows hypoechoic foci and a low degree of blood flow in the Doppler study. (91) In women, the fallopian tube is the most likely location, accounting for more than 80% of female genital TB. It is usually bilateral and also affects the endometrium. (22)

Osteoarticular TB is usually the result of haematogenous spread of bacilli. The most frequent sites are the thoracic spine in young patients and the lumbar in the elderly. Usually, the disease affects the anterior vertebral section. If the disease progresses, it leads to the extension of the lesion to adjacent vertebrae and progressive kyphosis by destruction of the anterior vertebral body. In advanced cases the infection spreads to the adjacent soft tissue, causes cold abscesses or towards the back of the vertebra and can affect the spinal cord, causing compression. Less frequently, bone TB affects other regions, with an emphasis on the metaphyseal portion of the long bones. From this point, it may also extend to the adjacent joint space, resulting in arthritis of a tuberculous origin. A special form of joint disease is called Poncet’s syndrome. Periarticular demineralisation is an early, but non-specific, radiological sign. MRI is more sensitive than CT in detecting lesions of tuberculous origin. (22)

Central nervous system involvement is also more common in HIV-positive patients (3). Tuberculous meningitis is the most common presentation of TB in the CNS (22). TB meningitis is accompanied by TB elsewhere in the body in most of the patients with HIV-TB. The cerebrospinal fluid (CSF) is often acellular; at times, CSF may be completely normal both in cellular and biochemical characteristics (3, 37, 41). In patients with acellular CSF, meningeal signs may not be evident clinically (41). Symptoms from the cranial, optic or oculomotor nerve (pairs III, IV and VI) can be present, because they are most often involved. The diverse manifestations of CNS TB are reflected in both CT and MRI images (22), although there are no specific signs for differential diagnosis from other mass lesions of the CNS space (30).

Focal neurotuberculosis may present as areas of cerebritis, granulomas - tuberculomas (enhancing nodules on CECT), caseating granulomas (lesions with irregular ring enhancement on CECT), or as a tuberculous abscess (a lesion with thin ring enhancement on CECT and mass effect and edema) (85). Granulomas may have little or no mass effect and little associated edema. They are typically multiple. Abscesses are generally solitary. (89) Tuberculomas are usually supratentorial in location, show peripheral ring enhancement and appear with a hypo- or isointense signal on the T1 sequence and hyperintense on the T2; or, hypointense in caseating tuberculomas.
They may be surrounded with varying degrees of oedema. MRI spectroscopy shows prominent lipid peaks, which are in fact quite characteristic of intracranial tuberculomas. (56)

Abscesses are low attenuated on CT scans, low signal intensity on T1W-MR images, and high signal intensity on T2W-MR images. They have thin enhancing rims, usually induce considerable surrounding edema, and can be identical in appearance to pyogenic abscesses. Hydrocephalus with intracranial hypertension and ischaemic stroke secondary to vasculitis are other serious forms of presentation of CNS-TB. (22)

Intracerebral mass lesions are more commonly present in HIV-infected patients with TB meningitis (15). The simultaneous occurrence of basilar meningitis in association with focal parenchymal lesions or with an abscess greatly increases the likelihood of a diagnosis of neurotuberculosis (89). In meningitis TB, the presence of a triad consisting of contrast uptake in the basal meninges on CT scans and MR images, with thickening of the meninges, hydrocephalus, and images of vascular origin infarcts secondary to vascular stenosis or occlusion are typically described. However, none of them is pathognomonic of TB and only a small percentage of meningitis TB cases present all three signs. (22)

Possible findings are also periventricular oedema and exudates, with common sites such as basal cisterna ambiens, suprasellar cistern and sylvian fissures. Serial CT scans are very helpful in assessing the course of tuberculomas and hydrocephalus. MRI is the imaging technique for earlier detection of lesions and is therefore preferred if available. Gadolinium enhanced MRI is superior to the CT scan in detection of basal meningeal enhancement and small tuberculomas. Contrast enhanced MRI has been found to be superior to the contrast enhanced CT scan in detection of diffuse and focal meningeal granulomatous lesions, in delineating focal infarcts of the basal ganglia and diencephalon. Further, MRI is superior to CT in defining the presence, location and extent of associated brainstem lesions. MRI of the spine is also useful in the diagnosis of lesions of spinal tuberculosis. (73)

Miliary TB occurs after haematogenous spread of bacilli to multiple organs. The hallmark of acute disseminated miliary TB is the miliary pattern on the chest radiography. The term miliary refers to the "millet seed" size of the nodules (2-3 mm) seen on classical chest films, representing the typical pattern of granulomas. (22, 69, 75) The diagnosis of miliary TB is easier when the patient presents with classical miliary shadowing on chest radiograph in an appropriate setting. However, the diagnosis may be difficult in those situations where chest radiograph does not show classical miliary shadows. (73) Some patients with miliary TB, however, may have normal chest radiographs and some may have patterns that are indistinguishable from interstitial pneumonia (22, 75). Some of the patients may manifest coalescent opacities. When patients with miliary TB develop ARDS, the chest radiograph may be identical to that seen in ARDS due to other causes (75). If there is a high index of suspicion of the diagnosis of miliary TB and the chest radiograph is atypical, it is suggested that high resolution computed tomographic scan (HRCT scan) be done to support the diagnosis. Computed tomography (CT) is also useful for showing lesions
not visible on conventional radiology. HRCT scan is superior to the conventional CT scan in defining the parenchymal detail. Further, HRCT of the chest with contrast can also be useful in detecting lymph nodal enlargement, calcification and pleural lesions. In patients with disseminated/miliary TB, CT scan and MRI scan may reveal evidence of neurotuberculosis, intraabdominal lymphadenopathy, infiltrative lesions in liver, spleen and kidney.

**IRIS**

The rapid expansion of antiretroviral therapy programmes (HAART) in high TB burden areas, mainly in southern Africa, has been associated with an unexpectedly high occurrence of TB during the first 3 months of HAART, suggesting a potential role of HAART-driven immune restoration in "unmasking" subclinical TB. A growing body of indirect evidence suggests that the phenomenon is the result of a complex interaction between HAART-driven immune restoration, especially cellular immunity and Mycobacterium antigen load. The resulting disease spectrum is very wide: we could found isolated instances of fever, exacerbation and extension of pulmonary and extrapulmonary disease, particularly nodal affection in multiple areas, increased or initial appearance of lymphadenopathy, new or worsening pulmonary infiltrates on chest radiography, typical clinical TB presentations or TB presenting as an exaggerated inflammatory response due to dysregulated immune restoration, forms of TB with explosive development after starting antiretroviral treatment in patients with diagnosis and treatment of TB prior to initiating antiretroviral therapy, serositis, cutaneous lesions, and new or expanding central nervous system mass lesions. Consequently, some patients may develop acute renal failure or acute respiratory distress syndrome (ARDS).

All this different symptoms and findings are commonly referred to as immune reconstitution inflammatory syndrome (IRIS), also called paradoxical reactions or immune restoration syndromes. IRIS can be brief or prolonged with multiple recurrences. It has been reported in 32 to 36 per cent of patients with HIV-TB, within days to weeks after the initiation of antiretroviral treatment. The main risk factor appears to be the simultaneous administration of antiretroviral and tuberculosis therapy, with symptoms appearing within the first two months of treatment. These TB exacerbation do not imply a worse development or prognosis. Some authors claimed that IRIS require no specific treatment, although administration of steroids may be useful, while others think that the clinical management of this manifestations can be particularly challenging, due to the difficulties in its diagnosis and the uncertainties regarding its...
optimal management and it appears to be a major threat to global health and poses additional barriers to successful HIV/AIDS care and treatment programmes. (43)

**Imaging findings OR Procedure details**

A retrospective study was performed to document the radiological appearances of newly diagnosed pulmonary and extrapulmonary tuberculosis and its relationship with CD4+ T-lymphocyte count in patients with human immunodeficiency virus (HIV) co-infection. Patients were hospitalized between 2007 and 2012 in Clinic for Infective and Tropical Diseases of Clinical Centre of Serbia in Belgrade. Study included 17 patient, 12 males and 5 females with their age ranging from 21 years to 52 years, who had 31 independent episode of tuberculosis. Information on demography, history, signs and symptoms of tuberculosis and imaging results was collected by review of medical records. Patients treated in ambulant conditions did not enter the study. HIV serological status was assessed by enzyme-linked immunoassay (ELISA) methods and Western blot. The diagnosis of tuberculosis was based either on a positive Ziehl-Neelsen staining of a first morning sputum or liquor specimen, or positive culture of sputum or other specimen on Lowenstein-Jensen medium (together with sensitivity to antibiotics), or the combination of both a clinical presentation and a chest radiograph or other needed imaging method for both pulmonal and extrapulmonal tuberculosis or response to antitubercular chemotherapy. In some cases Mycobacterium tuberculosis was proved by hybridisation reaction, MGIT and PCR. A posteroanterior chest radiograph was taken of every subject, as well as abdomen ultrasonography, blood analysis, biochemical analysis of serum and liquor, microbiological analysis of sputum, liquor, blood, urine and feces, and number of CD4 and CD8 lymphocytes in µL and CD4/CD8 ratio. If it was needed, CT, MRI, biopsy of lymph node or bone marrow, bronchoscopy, oesophagogastroduodenoscopy or colonoscopy and histology examination and culture of specimen were added to protocol. Most patients were also tested for hepatitis B and C co-infection, Toxoplasma gondii, Treponema palidum (sexually transmitted diseases), as well as other suspected opportunistic infections if needed (such as Criptococcus neoformans, Candida sp., Microsporidiosis, Hystoplasmosis). In this report only diversity in radiology findings are presented.

Only one patient had typical radiography of lung postprimary tuberculosis, while all other patients had atypical chest radiography findings, no matter what was the level of their immunodeficiency. Three cases of tuberculosis had normal lung radiography and all three had number of CD4 cells under 200/µL. The commonest form of extrapulmonary manifestation in our study was lymphadenopathy, thoracic, cervical or abdominal. Lymphadenopathies, enlargement of liver or spleen, focal lesions in spleen and pathologic findings in brain were seen more frequently in patient with severe immunodeficiency. After HAART introduction four cases had symptoms of IRIS, and among them only one patient had number of CD4 cells above 200/µL (exactly 268/µL).
Cultivation of sputum smear in two patients found Mycobacterium avium intracellulare infection instead of Mycobacterium tuberculosis, and one patient had multidrug resistant species of Mycobacterium tuberculosis.

Typical radiography of lung postprimary tuberculosis with parenchymal infiltration of right upper and apical lung field was seen in patient that had CD4 count above 200/µL. Patient was 28 years old female, use to be male, so besides infiltrates in parenchyma, we can see silicon implants in patient's breast on chest radiography. There were no abnormalities on abdominal ultrasound. Two months after beginning of tuberculosis treatment patient (who also received HAART) was hospitalized again because of worsening of symptoms and radiology findings, defined as IRIS. On chest x-ray (figure no. 1), we could see spreading of infiltrates that were localised now in apical, upper and medial lung field. CT was also done and it showed infiltrative tumour-like lesion, irregular in shape, with spiculated margins situated in posterior segment of right upper lobe, with extension to superior segment of right lower lobe, 4,6x3,5x5,6mm in size, without enlargement of mediastinal lymph nodes, with several axillary lymph nodes size up to 1cm. Abdominal ultrasound was still without pathologic changes. Treatment of tuberculosis was continued.

Figure no. 1
Other patients had atypical presentation of pulmonal tuberculosis, extrapulmonal tuberculosis or combination of both.

Atypical pulmonal tuberculosis was presented on chest x-ray as infiltration of right middle lung field (figure no. 2) in male patient, age 31, besides his high CD4 count level (630/µL), or parenchymal infiltration of left parahilar region (figure no. 3) in also male patient, age 27, that had CD4 count 116/µL.
Fig. 26

**References:** Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

It was also presented as left basal parenchymal infiltration on chest x-ray (figure no. 4) in male patient, age 37, with CD4 count 262/µL, and as massive bilateral infiltrations (figure no. 5) in male patient, age 30, with no data about CD4 count at the moment of hospitalization.
Fig. 27

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS
Fig. 28

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

Female patient, age 52, with unknown CD4 count in patient chart had bilateral infiltration in parahilar localisation in combination with bilateral pleural effusion on chest radiography findings (figure no. 6) and another female patient, age 39, with CD4 count 21/µL, had bilateral miliary pattern of infiltration, (confirmed by CT of thorax) with pneumothorax as later complication (figure no. 7).
Fig. 29

**References:** Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS
Fig. 30

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

Male patient, with heroin and alcohol abuses, and chronic hepatitis type B and C, age 35, CD4 count 214/µL had radiology findings that included parenchimal consolidation as in pneumonia in right parahilar region but with abscess. It was confirmed on CT of the thorax that found parenchimal consolidation in right lung near hilus with lateral extension, located in anterior segment of upper lobe, with abscess cavum 4.5cm in size and 18mm wall thickness, two smaller cavitations and enlargement of hilar lymph nodes. (Figure no. 8). These cases of tuberculosis had no signs of extrapulmonal tuberculosis on clinical examination and performed abdominal ultrasonography.
Combination of atypical pulmonal and extrapulmonal tuberculosis was found in male patient, age 49 that had CD4 count 32/µL and chest x-ray with massive bilateral infiltrations mostly on lower lung fields. CT was indicated and it had zone of infiltration of right lung in perihilar and basal region, with nodal lesion in anterior segment of right upper lobus 15mm in size that had central excavation zone, and another nodal lesion beside the first one, 13mm in size and with blured margins. Nodal lesions were also in laterobasal region of right lung 7mm in size, in apical region of the left lung, paramediastinal in localisation and 15mm in size, and also in left lung, in posterobasal region 10mm in size. There were also large mass of increased lymph nodes in right hilus, and enlargement of lymph nodes localised in paratracheal (19 mm), left prevascular (13-20 mm) and subcarineal (25 mm) area. Abdominal ultrasound had hepatomegaly with no other findings (figure no. 9).
Next patient with pulmonal and extrapulmonal tuberculosis was male, age 23, and CD4 count 18/µL. Chest x-ray was done and abdomen ultrasonography. They found enlargement of left hilus and infiltration of lung parenhym near that hilus, with enlargement of lymph nodes in liver hilus (10 mm in size) and retroperitoneum as well as hepatosplenomegalia (figure no. 10).
Abdominal lymphadenopathy was found on ultrasound in female patient, 35 years old, five years living with HIV, three years without HAART (at her own decision), with CD4 count only 3/µL. She came to hospital with chest x-ray that had left side pneumonia with cavitation. Infiltration was in both upper and lower lobe with ring shadows that had thick wall, located in region of second and forth intervertebral space (Figure no. 11).
Another case of atypical pulmonal tuberculosis that could be misdiagnosed was found in male patient, 21 years old, with CD4 count that was 50/µL. He had interstitial pattern of diffuse infiltration on chest x-ray. CT of the thorax was done and it showed discreet shadows in parenchymal window like "milky glass", located in upper lobes bilateral, more on left side, which can be seen in vascular lesions and viral pneumonia. There was no enlargement of thoracic lymph nodes or changes on pleura. Hepatosplenomegalia was also found on abdominal ultrasound as manifestation of extrapulmonal tuberculosis (figure no. 12).
Female patient, 33 years old, with CD4 count that was 56/µL had infiltration of basal part of left lung with small pleural effusion because of pleuroneumonia on chest x-ray, confirmed by CT (figure no. 13). Splenomegaly with hypoechogen lesions 0.4 - 1.1 cm in size and retroperitoneal lymphadenopathy (parapancreatic 2 cm in size, at least two with central necrosis, and in hepatic hilus 11 mm in size) were seen on abdominal ultrasound and CT of the abdomen (figure no. 14). The same patient was received to hospital again two weeks after initiation of HAART because of symptoms of IRIS, with CD4 count 22/µL and progression of findings showed on chest x-ray.
Fig. 36

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS
Fig. 37

**References:** Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

One case of multiresistant tuberculosis was found in male patient with age 47, and CD4 count 34/µL that had chest x-ray showing bilateral pulmonal infiltrates and abdomen ultrasound with enlargement of lymph nodes around portal vein and abdominal aorta, 1cm in size. Next year he left HAART and came with CD4 count, which was 97/µL. Chest x-ray had sequels from tuberculosis and abdominal ultrasound was normal. He was treated in the next few years for tuberculosis and came with resistance to antituberculotic drugs. In next few years his lung was destroyed and CT that was done showed big cavity in whole right upper lobe, with thick walls, as well as cavity in apicoposterior segment of left upper lobe that has soft tissue mass, 40x25mm in size and air. Lungs were fibrocystic. Upper parts of pleura were thick, and mediastinum was dislocated to the right. CD4 count was 371/µL (figure no. 15).
Fig. 38

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS
Fig. 39

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

Only extrapulmonary tuberculosis was confirmed in male patient, 40 years old, who came to hospital with CD4 count 14/µL and with normal chest x-ray. He received HAART and after one month he came with symptoms of IRIS. CD4 count was 64/µL and radiography of patient's lung was normal, but CT of abdomen had hepatosplenomegalia, with lacunar low attenuated multifocal lesion on spleen, and enlargement of retroperitoneal lymph nodes, paraaortal, infrarenal, nodes near both a. iliaca communis, and the biggest one in interaortocaval region 2 cm in size (figure no. 16). Inhomogen lesion in right m. psoas was haemorrhage because of haemophilia that patient had. His next hospitalization was three months later because of fistulization of neck lymph node and fever when abdomen ultrasound was done and showed hepatosplenomegalia and enlargement of retroperitoneal lymph nodes that had signs of central necrosis. TB lymphadenitis was treated in male patient, age 23, with CD4 count 39/µL, and enlargement of neck lymph nodes. The same patient came five years later with CD4 count below 200/µL and abdominal ultrasound that found infraumbilical soft tissue mass, hypo to unechogen, which consists of aggregate of increased lymph nodes, three at least, 30 mm, 20 mm and 21 mm in size.
Fig. 40

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

There were two cases of neurotuberculosis. First case was male patient with age 24 and CD4 count 76/µL, who had also splenomegalia on abdominal ultrasound and normal chest x-ray. His findings of endocranium CT were: interstitial oedema next to the frontal and occipital horn of lateral brain ventricle that was, as well as all other brain ventricles, normal in size. Brain sulcuses on the left frontotemporoparietal region were smaller, and there was oval low attenuated lesion on the left parietal and periventricular region without compressive effect. After application of intravenous contrast, meningeal opacification of left frontotemporoparietal region was found. All described findings correspond to localised meningoencephalitis (figure no. 17).
Fig. 41

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

Another case of neurotuberculosis, also with splenomegalia on abdominal ultrasound and normal chest x-ray was in male patient, age 25, and with CD4 count 169/µL. CT findings were left temporoparietal focal encephalitis and on NMR zone of encephalomalacia in left parietooccipital region as sequel of tuberculosis and brain infarct in zone of arteria cerebri media, with regression of focal lesion (tuberculoma) and glyosis as TB sequel on the right, without acute lesions (figure no. 18).
Two patients had sputum culture positive for Mycobacterium avium intracellulare. First one had only pulmonal pathology. It was male, age 36, without CD4 count in the chart. First lung radiography was normal but after beginning of HAART, progression of bilateral parenchymal infiltrate and appearance of cavitations, more at the left side of lung, was seen. ThoraxCT was done and showed bilateral bizarre consolidation of parenchyma, more expressed in subpleural and basal region, with gas inclusion in mediastinum and subcutan region of neck and right axilla (figure no. 19). ControlCT was done after two months of therapy and showed reduction of lesions. Bronchoscopia was done and findings were bronchitis chronica. Antituberculotic drugs were introduced ex iuvantibus.
Another Mycobacterium avium intracellularae positive patient was male, age 42, who started with HAART and came back again because of IRIS symptoms with CD4 count 44/µL. CT of the thorax was done and findings were massive lymphadenopathy in all lymph groups, especially in paratracheal (33 mm in size) and hilar (30 mm in size) group. Bilateral zones of consolidation in pulmonal parenchyma, mostly in right upper lobe and parahilar region like pneumonitis, were seen. Besides pulmonal changes his abdominal ultrasound showed hepatosplenomegalia and rectosigmoidoscopy was also done with no changes of intestinal mucosa. Antituberculotic treatment was introduced ex iuvantibus (figure no. 20).
Fig. 44

References: Clinic for Infective and Tropical Diseases, Clinical Centre of Serbia, Belgrade, RS

Images for this section:
Figure no. 1

- 28 years old, female, use to be male
- CD4 > 200/μL
- chest x-ray: typical postprimary tuberculosis (parenchymal infiltration of right upper lung field)
- two months after beginning of the treatment: worsening of symptoms and radiology findings (IRIS)
- chest x-ray: spreading of infiltrates that were localised now in apical, upper and medial lung field - silicon implants in patient’s breast
- CT: infiltrative tumour-like lesion, irregular in shape, with spiculated margins situated in posterior segment of right upper lobe, with extension to superior segment of right lower lobe, 4.6x3.5x5.6mm in size, without enlargement of mediastinal lymph nodes

Fig. 1
Figure no. 2

- male, age 31
- CD4 = 630/μL
- chest x-ray:
  - infiltration of right middle lung field

Fig. 2
Figure no. 3

- male, age 27
- CD4 = 116/μL
- chest x-ray: parenchymal infiltration of left parahilar region

Fig. 3
Figure no. 4

- male, age 37
- CD4 = 262/µL
- chest x-ray: left basal parenchymal infiltration

Fig. 4
Figure no. 5

- male, age 30,
- no data about CD4 count
- chest x-ray: massive bilateral infiltrations
Figure no. 6

- female, age 52
- CD4 count unknown
- chest x-ray, AP: bilateral infiltration in parahilar localisation and bilateral pleural effusion on chest radiography findings
Figure no. 7

- female, age 39
- CD4 = 21/μL
- chest x-ray:
  - bilateral miliary pattern of infiltration
  - pneumothorax as later complication

Fig. 7
Figure no. 8

- male, age 35
- CD4 = 214/μL
- chest x-ray: parenchymal consolidation as in pneumonia in right parahilar region with abscess
- CT thorax: parenchymal consolidation in right lung near hilus with lateral extension, located in anterior segment of upper lobe, with abscess cavum 4.5cm in size and 1.5cm wall thickness, two smaller cavitations and enlargement of hilar lymph nodes
- Male, age 49
- CD4 = 32/μL

CT:

RIGHT LUNG:
- Zone of infiltration in perihilar and basal region
- Nodal lesion in anterior segment of upper lobe (15mm) with central excavation
- Nodal lesion next to the first one (13mm), with blurred margins
- Nodal lesions in laterobasal region (7mm)

LEFT LUNG:
- Nodal lesion in apical region, paramediastinal (15mm) and posteroobasal region (10mm)

- Large mass of increased lymph nodes in right hilus, and enlargement of lymph nodes localised in paratracheal (19mm), left prevascular (13-20mm) and subcarinaeal (25mm) area
- age 23, male,
- CD4 = 18/μL
- chest x-ray: enlargement of left hilus and parenchymal infiltration of left parahilar region
Figure no. 11

- female, 35 years old
- CD4 = 3/μL

- chest x-ray: left side pneumonia with cavitations - infiltration in both upper and lower lobe with ring shadow that had thick wall, located in region of second intervertebral space and lower lung field.
Figure no. 12

- male, 21 years old
- CD4 = 50/μL
- chest x-ray: interstitial pattern of diffuse infiltration, more on left side
- CT: discreet shadows in parenchymal window like "milky glass" in upper lobes bilateral, more on left side, (which can be seen in vascular lesions and viral pneumonia) and enlargement of thoracic lymph nodes or changes on pleura
- Female, 33 years old
- CD4 = 56/μL
- CT: Infiltration of basal part of left lung with small pleural effusion (pleuropneumonia)
CT: splenomegaly with hypodense small lesion in spleen, retroperitoneal lymphadenopathy (parapancreatic 2cm, at least two with central necrosis, and in hepatic hilar 11mm)
Figure no. 15

- male, age 47
- CD4 = 34/μL
- chest x-ray: bilateral pulmonal infiltrates

- next year CD4 = 97/μL
- chest x-ray: sequels from tuberculosis
- in the next few years: resistance to antituberculotic drugs
• CD4 = 371/μL
• CT: big cavity in whole right upper lobe with thick walls, cavity in apicoposterior segment of left upper lobe with soft tissue mass (40x25mm) and air, lungs were fibrocystic, upper parts of pleura were thick, and mediastinum was dislocated to the right.

Fig. 16
Fig. 16

- Male, 40 years old
- CD4 = 14/μL
- chest x-ray: normal
- one month after HAART: IRIS
- CD4 = 64/μL
- chest radiography: normal
- CT of abdomen: hepatosplenomegalias, lacunar multiple low attenuation foci on spleen

enlargement of retroperitoneal lymph nodes, paraaortal, infra renal, nodes, and the biggest one in interaortocaval region (2cm)
inchoxygen lesion in right m. psoas
(haemorrhage because of haemophilia)

Fig. 17
- age 24, male
- CD4 = 76/μL
- chest x-ray: normal

- endocranium CT: localised meningoencephalitis
- native CT: interstitial oedema next to the frontal and occipital horn of lateral brain ventricle, brain ventricle normal in size,
  smaller brain sulcus on the left frontotemporoparietal region,
  oval hypodense lesion without compressive effect on the left parietal and periventricular region,
- postkontrast CT: meningeal opacification of left frontotemporoparietal region

Fig. 18
male, age 25
CD4 = 169/μL
chest x-ray: normal

CT endocranii: left temporoparietal focal encephalitis

NMR: zone of encephalomalacia in left parietooccipital region as sequel of tuberculosis and brain infarct in zone of arteria cerebi media, with focal lesion (tuberculoma) and gliosis as TB sequel on the right
Figure no. 19

- male, age 36
- no CD4 count in the chart
- sputum culture: *Mycobacterium avium intracellulare*
- first chest radiography: normal
- after beginning of HAART: progression of bilateral parenchymal infiltrate and appearance of cavitations, more at the left side of lung

Fig. 20
Thorax CT: bilateral bizarre consolidation of parenchyma, more expressed in subpleural and basal region, with gas inclusion in mediastinum
male, age 42  
- after HAART came with IRIS 
- CD4 = 44/μL  
- sputum culture: Mycobacterium avium intracellulare

- CT thorax: bilateral zones of consolidation in parenchyma, mostly right in upper lobe and parahilar region like pneumonitis  
  - massive lymphadenopathy in all lymph groups, especially paratracheal (33mm in size) and hilar (30mm in size)
Fig. 23
Conclusion

Study's significance is reflected in realizing the variability of appearance of tuberculosis in HIV seropositive patients, here presented in its radiology findings. It is because the diagnosis of TB in HIV/AIDS patients is difficult and current diagnostic tools perform badly in HIV-positive patients. However, WHO guidelines recommend screening for TB in all HIV/AIDS patients, so there is an urgent need to develop rapid, simple, specific and sensitive tuberculosis diagnostic tools for detection of TB in HIV-positive patients to reduce the delay in diagnosis, number of complications and mortality.

References


25. Grupo de Estudio de contactos de la Unidad de Investigación en Tuberculosis de Barcelona (UITB). Documento de Consenso sobre el


Personal Information

Dr Natasa Pujic Stanisavljev, Department of Radiology, General Hospital Sremska Mitrovica, Serbia, e-mail: pujicnatasha@yahoo.com