CASE REPORT

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Disseminated tuberculosis presenting as febrile seizures with fatal evolution in an infant

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Abstract

Disseminated tuberculosis with the involvement of brain, liver and gut is a rare disease in immunocompetent infant. Early diagnostic and instauration of anti-tuberculosis therapy is capital because the outcome is poor. Here, we report the case of an 11-month-old boy with disseminated tuberculosis of brain, liver abdominal lymph nodes, small bowel and lung, which presented with fever, generalized tonic-clonic seizure, hemodynamic instability and a history of recurrent respiratory tract infections. His father was diagnosed with active pulmonary tuberculosis six month ago and family members completed an anti-tuberculosis chemoprophylaxis regimen.

Keywords: disseminated tuberculosis, tuberculosis, seizure, infant.

Introduction

Tuberculosis (TB) is an infectious disease caused by intracellular bacterium *Mycobacterium tuberculosis*. According to the latest estimation by *World Health Organization* (WHO), TB still remains a major problem of public health worldwide with 8.6 million new cases and 1.3 million death in 2012 [1]. The highest rates are in low-income countries and the 22 high burden countries account for 81% of all cases worldwide [1]. More than 500 000 new cases of TB occur in children less than 15 years [2]. However, TB is probably largely underestimated in children [3] mainly due to frequently non-specific presentation and difficulties in mycobacterium isolation. In TB-endemic areas, TB occurs mainly in poor and vulnerable populations, case identification being a major challenge [4]. Despite a progressive decline since 2003, the incidence of TB is still high in Romania (109.8 new cases/100 000 population and 28.7 new cases/100 000 population in children) compared with other countries of the European Union (1st place) [5]. Delayed diagnostic and therapy instauration are associated with a poor outcome.

It is estimated that one-third of world population is infected with *M. tuberculosis*. In most cases, immune system of adults manages to control bacterial growth but fails to completely eradicate the organism from the body. Asymptomatic infected adults show only 5–10% lifetime risk of developing active TB [6]. It is well known that infected children bear a much higher risk to develop active TB than adults [7]. Up to 50% of infected children develop active disease in the absence of prevention strategies [8]. Furthermore, the risk to develop active pulmonary TB is 30–40% in infants with familial exposure, in the absence of anti-tuberculous prophylaxis [8]. Postnatal achievement of gut microbiota requires downregulation of newborn immune system in order to assure germ tolerance. Unfortunately, this physiological mechanism also involves some immune pathways with major role in the protection against intracellular bacteria (poor phagocytic, microbicidal and chemotactic activity of macrophages; altered T-cell function; altered pro-inflammatory cytokine production) [9–11] including *M. tuberculosis*.

Here we report a case of disseminated TB with involvement of brain, liver, small bowel, abdominal lymph nodes and lung in an 11-month-old infant with family history of active TB. The patient presented with fever, generalized tonic-clonic seizures, coma, hepatomegaly, splenomegaly and malnutrition.

Patient, Methods and Results

An 11-month-old boy was referred to the Emergency Unit of the Emergency County Hospital of Craiova, Romania, with generalized tonic-clonic seizures and altered vigilance, on a background of 24-hour history of fever. He was immediately transferred to the Intensive Care Department. The infant was delivered by vaginal delivery at 36 weeks and had a birth weight of 2.26 kg. The first two months of life were uneventful. He had three episodes of pneumonia with a good outcome following antibiotic therapy at the age of three, four and six months, which resulted in failure to thrive. The mother had a history of syphilis infection in 2004 and 2013 receiving a correct therapy. The father was diagnosed with active pulmonary TB about five months ago and received an anti-tuberculosis treatment. The infant had no sign of active TB and received a preventive treatment with Isoniazid, 50 mg/day for four months. His mother was

disease-free and also received a prophylaxis treatment.

At hospital admittance, the general state of the infant was profoundly altered. The baby presented severe dystrophia (6000 g weight, with a weight deficit of 3000 g). During the physical examination, he was unresponsive (Glasgow score: 6), his temperature was 38.5^{0}C, respiratory rate was 40/min., heart rate was 50/min. and had no neck stiffness or focal neurological signs. The patient was pale, undernourished, dehydrated, had hepatosplenomegaly and bilateral cervical and axillary adenopathy. Intubation and mechanical ventilation were required. Laboratory studies were as follow: anemia (hemoglobin value of 9 g/dL), leukocytosis (25 000/mm^3) with neutrophilia (20 000/mm^3), thrombopenia (41 000/mm^3), erythrocyte sedimentation rate 60 mm/h, severe hypoalbuminemia (16 g/L), slightly increased transaminases (GOT 86 IU, GPT 54 IU), normal blood sugar, normal electrolyte levels and normal renal function. Arterial blood gas showed mild metabolic acidosis. HIV antibodies were negatives. Urine analysis results were normal. Because there was suspected a meningocerebral inflammation, there was performed a lumbar puncture, but the analysis of the cephalorachidian liquid excluded the presence of meningitis. These laboratory findings raised the suspicion of sepsis and appropriate antibiotic treatment was immediately started.

The pulmonary radiological examination highlighted numerous micronodular lesions, suggesting miliary tuberculosis, while the computer tomography scan of the brain revealed disseminated parenchymal nodules suggestive of calcified tuberculoma. Spinal fluid cultures came out negatives for *M. tuberculosis*. Unfortunately, the fourth day of hospitalization the patient died of sudden cardiac arrest before initiating anti tuberculosis treatment.

For the histopathological diagnosis of vital organs lesions, during necropsy there were harvested fragments of lung, liver, intestine and abdominal lymphatic ganglia. These fragments were fixed in 10% neutral formalin and included in histological paraffin. The sectioning of the biological material was performed with the rotary microtome Microm HM350. For the histological study, there were used the classical stainings of Hematoxylin–Eosin (HE) and the trichromic Goldner–Szekely (GS), and for highlighting tuberculous bacilli there was performed the Ziehl–Neelsen (ZN) staining.

For the immunohistochemical study, there were performed sections with a thickness of 4 μm, collected on poly-L-Lysine covered blades and introduced in a thermostate at 37^{0}C for 24 hours in order to increase the adherence of the biological material to the slide blade. Then, there followed the deparaffinzation and hydration of histological sections, followed by the incubation of the biological material for 30 minutes in 3% oxygenated water solution (hydrogen peroxide). The sections were then washed in tap water, and, for antigen unmasking, there were boiled in the microwave oven, in a sodium citrate solution, pH 6, for 21 minutes (seven cycles of 3 minutes). After boiling, they were cooled down for 15 minutes, then the biological material was washed in a bisaline phosphate buffer (BSP) for 5 minutes, followed by the stage of blocking non-specific sites in 2% fat-free milk for 30 minutes. The sections were incubated with primary antibodies for 18 hours over night, in a refrigerator, at 4^{0}C. The second day, there was applied the biotinylated secondary antibody (αMs/αRb) for 30 minutes, followed by passing in HRP Streptavidin for 30 minutes. The signal was detected with 3.3'-Diaminobenzidine (DAB) (Dako), followed by the contrasting processing with Hematoxylin, alcohol dehydration, xylene clarifying and fixation of blades in a DPX (Fluka) environment.

In our study, we used the following markers (Table 1):

Table 1 – ***Antibodies used in the immunohistochemical study***

Antibody	Code	Clone	Antigen retrieval	Specificity	Dilution	Source
CD20	M0755	L26	Sodium citrate buffer, pH 6	B-lymphocytes	1:100	Dako
CD3	A0452	F7.2.38	Sodium citrate buffer, pH 6	T-lymphocytes	1:100	Dako
CD68	M0814	KP1	Sodium citrate buffer, pH 6	Macrophages	1:200	Dako

The histopathological study highlighted the presence of severe lesions in all the organs. Thus, at lung level, there were highlighted numerous confluent tuberculous granulomas, characterized by the presence of some multinucleate giant cells (Langhans cells), mature or young ones, mainly located at the periphery of granulomas, with sizes varying from 50 to 120 μm, with multiple hyperchromic nuclei, located at the periphery, acidophilic cytoplasm (Figures 1 and 2). The center of tuberculous granulomas was mainly covered by the caseification necrosis that appeared as a fine granular, unstructured, intensely eosinophilic and heterogenous area. The light green staining (Figure 3) allowed a more clear demarcation of the area of caseification necrosis. In the tuberculous granuloma there were also identified epithelial cells, long-shaped, with unspecific limits, a fusiform nucleus and eosinophilic cytoplasm. At the periphery of granulomas there was highlighted the presence of numerous lymphocytes, placed "in a crown", and an intense fibrous reaction of the conjunctive tissue.

At the level of abdominal lymphatic ganglia, there were highlighted the same type of lesions, namely confluent tuberculous granulomas occupying almost the entire surface of the ganglion (Figure 4). For identifying the remaining ganglionary parenchyma, we used the immunohistochemical reaction to CD20 antibody that highlights the lymphoid follicles of the lymphatic ganglion. Through this method, we could highlight isolated remainings of lymphoid parenchyma, namely positive CD20 areas, disseminated among the tuberculous granulomas (Figures 5 and 6).

At the level of small intestine, in the mucosa chorion and in the submucosa, there were identified diffusely disseminated tuberculous granulomas, and the Ziehl–Neelsen staining allowed us to identify the tuberculous bacilli isolated in the mucosa chorion (Figures 7 and 8).

The largest lesions were identified at liver level. Here, there were identified tuberculous granulomas mainly developed at the level of Kiernan portobilliary gaps, associated with severe macrovesicle liver steatosis (Figures 9 and 10). The CD68 antibody immunomarking showed that multinucleate giant cells, as well as the

epithelial cells, are cells belonging to the macrophage system (Figures 11 and 12), and the use of CD3 antibody allowed us to establish that the lymphocyte "crown" at the periphery of the tuberculous granuloma is formed of T-lymphocytes (Figure 13). Unlike the T-lymphocytes, B-lymphocytes were quite rarely identified around the tuberculous granuloma (Figure 14).

Alongside the diffusely disseminated tuberculous granulomas in the liver, the histopathological examination identified numerous areas of focal hepatitis characterized by intense hepatocytolysis, disorganization of Remack cordons, infiltration of the liver parenchyma with lymphocytes and granulocytes (Figure 15). Also, some Kiernan gaps presented an intense collagenous fibrosis (Figure 16) and moderate and intense inflammatory infiltrates, rich in T-lymphocytes (Figure 17), characteristic to a chronic form of hepatitis.

Discussion

We report the case of an 11-month-old infant diagnosed with disseminated TB and cerebral tuberculoma revealed by febrile seizures. Hematological dissemination is commune following primary infection, but progression to disseminated TB is rare except in immune compromised children and infant. The most severe forms of disseminated TB associate cerebral TB and/or tuberculous meningitis. Clinical presentation is often non-specific especially in children and unexplained recurrent fever and failure to thrive may be the only signs of the disease. Furthermore, the sputum smear is positive in less than 15% of cases [12]. Therefore, diagnostic is mostly based on a history of contact with an infectious index patient and presence of suggestive lesions on the chest X-ray. The recent onset of fever associated with neutrophilia, seizures and hemodynamic instability falsely oriented the diagnostic towards a sepsis and acute meningitis, which prompted instauration of an empiric antibiotic therapy. This hypothesis was not supported by the normal lumbar puncture results, absence of neck stiffness and negative bacterial cultures.

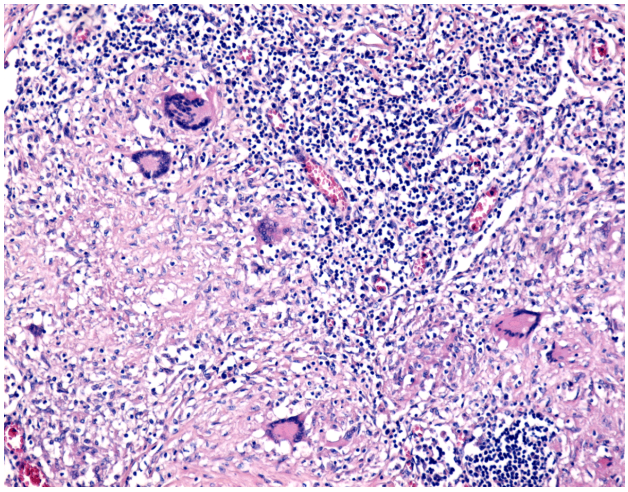

Figure 1 – ***Overall image at lung level, highlighting the presence of numerous confluent granulomas, characterized by the presence of multinucleate giant cells (Langhans cells), caseification necrosis and lymphocyte crown at periphery. HE staining, ×100.***

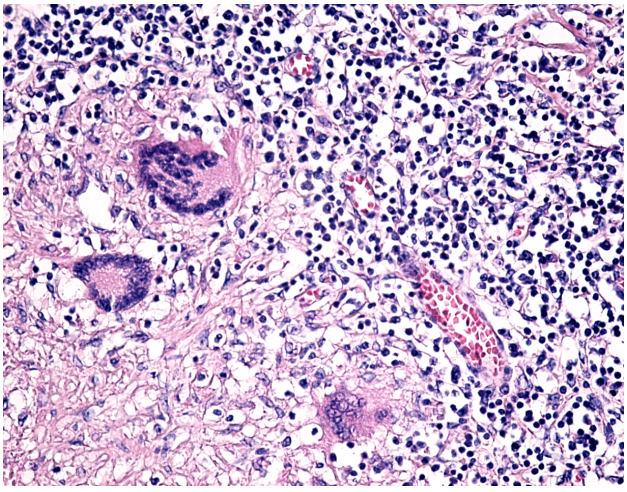

Figure 2 – ***Detail from previous image, from the periphery of tuberculous granuloma, with mature and young Langhans cells, perilesional "lymphocyte crown" and vascular congestion. HE staining, ×200.***

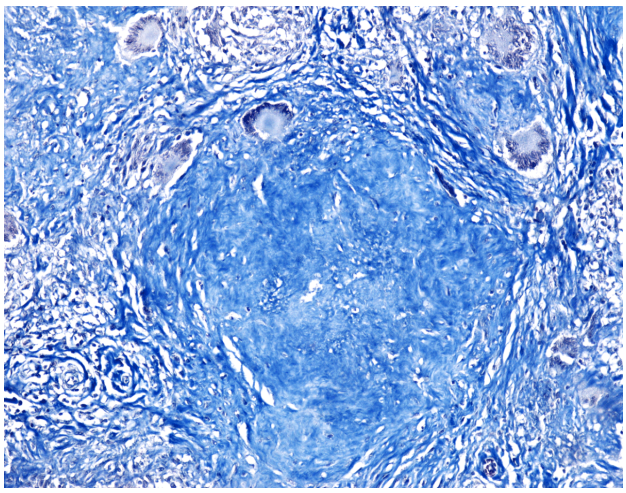

Figure 3 – ***Lung tuberculous granuloma, with multinucleate giant cells, located at lesion periphery and extended areas of caseification necrosis. Trichromic GS staining, ×100.***

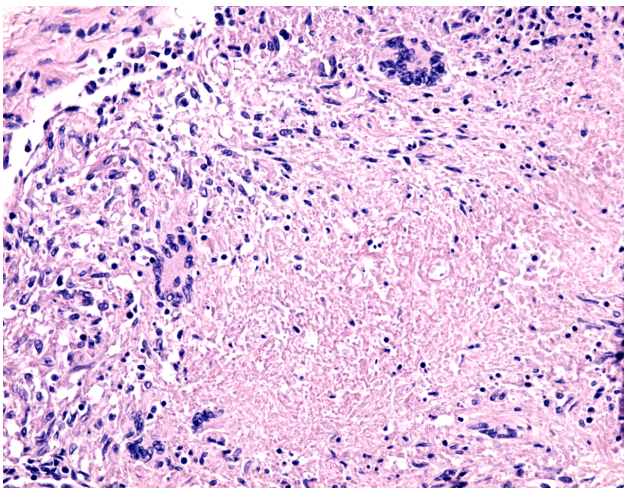

Figure 4 – ***Microscopic image of the tuberculous granuloma located at the level of abdominal lymphatic ganglia. HE staining, ×100.***

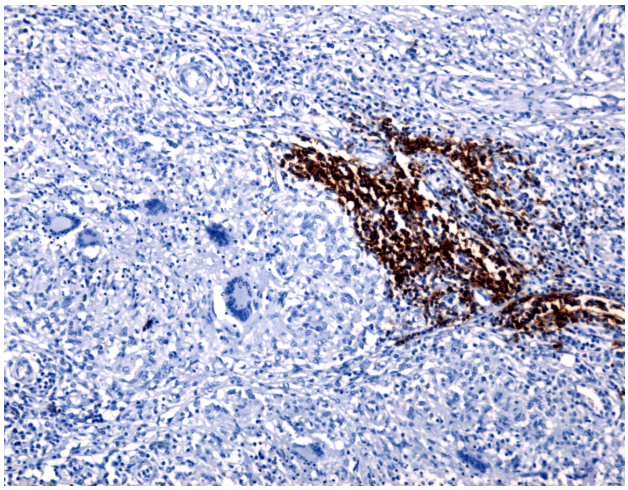

Figure 5 – ***Microscopic image at the level of abdominal lymphatic ganglion, where there may be observed the destruction of the ganglionary structure by the development of tuberculous granulomas and the presence of some isolated islands of lymphatic parenchyma, formed of B-lymphocytes. Immunomarking with anti-CD20 antibodies, ×100.***

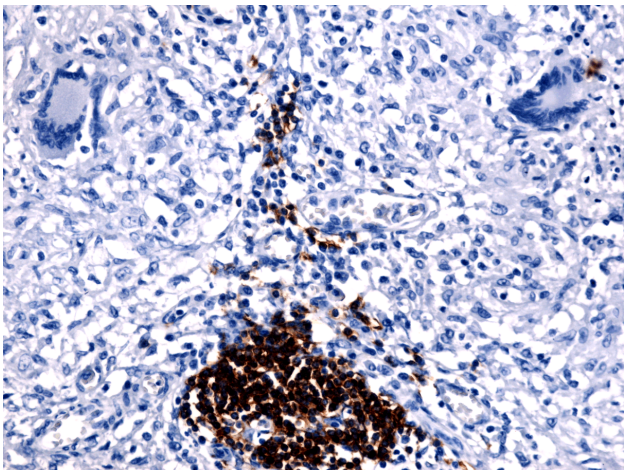

Figure 6 – ***Island of remaining lymphoid parenchyma at the level of abdominal lymphatic ganglion. Immunomarking with anti-CD20 antibodies, ×200.***

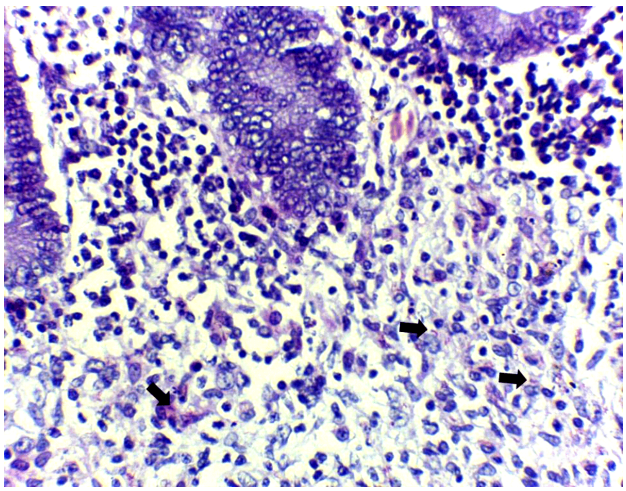

Figure 7 – ***Small intestine mucosa with multiple tuberculous bacilli disseminated in the chorion (arrow). ZN staining, ×200.***

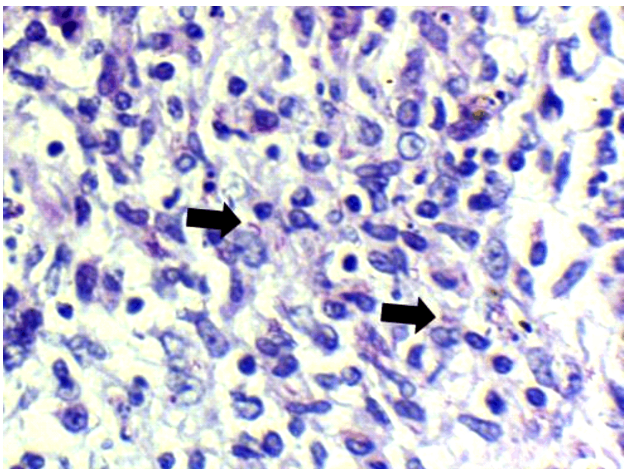

Figure 8 – ***Detail of previous image. ZN staining, ×400.***

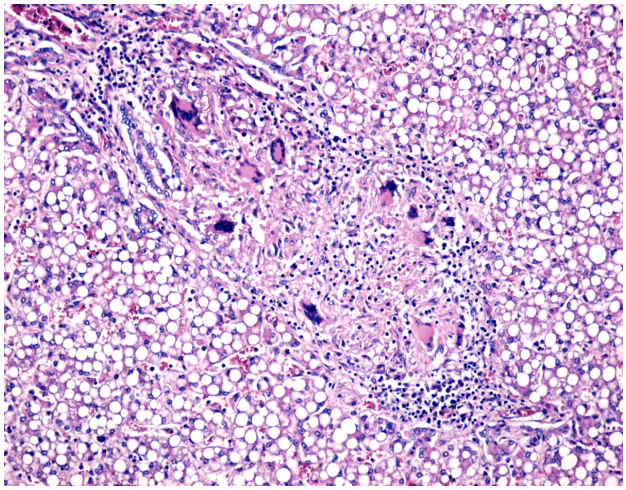

Figure 9 – ***Tuberculous granuloma developed in the Kiernan portobiliary gap, with multiple multinucleate giant cells, associated with an inflammatory infiltrate and severe liver steatosis. HE staining, ×100.***

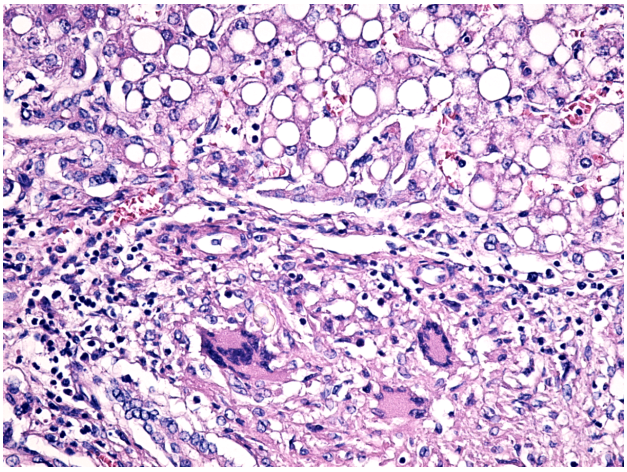

Figure 10 – ***Detail of previous image. There may be noticed the presence of Langhans cells at one pole of the tuberculous granuloma developed in the Kiernan gap and the macrovesicle steatosis lesions present at hepatocyte level. HE staining, ×200.***

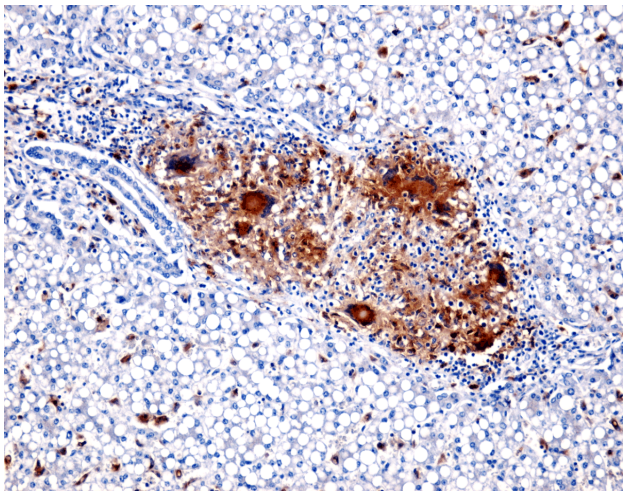

Figure 11 – *Microscopic image of a liver tuberculous granuloma, with an intense reaction to CD68 antibody. Immunomarking with anti-CD68 antibodies, ×100.*

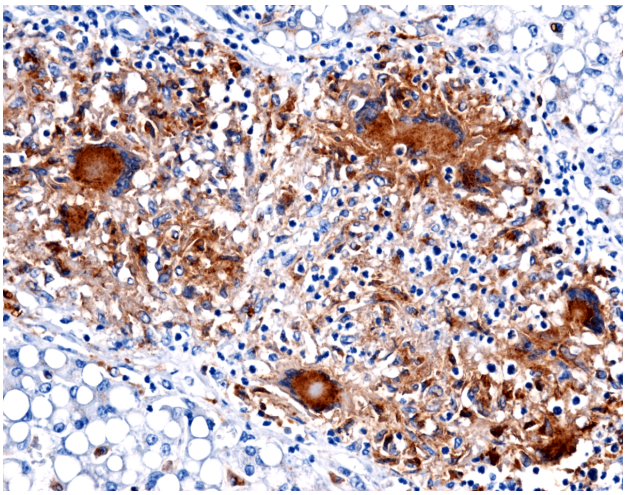

Figure 12 – *Detail of previous image, where there may be noticed that multinucleate giant cells, as well as epithelial cells are intensely positive to CD68, demonstrating their origin in the monocyte-macrophage system. Immunomarking with anti-CD68 antibodies, ×200.*

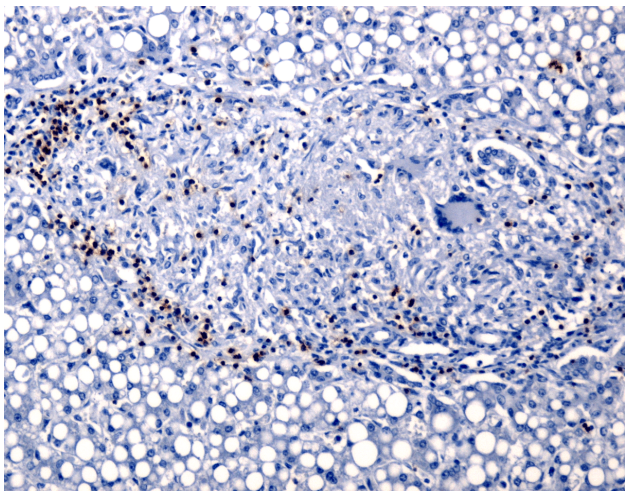

Figure 13 – *T-lymphocytes located in "a crown" at the periphery of tuberculous granuloma, but also inside it. Immunomarking with anti-CD3 antibodies, ×100.*

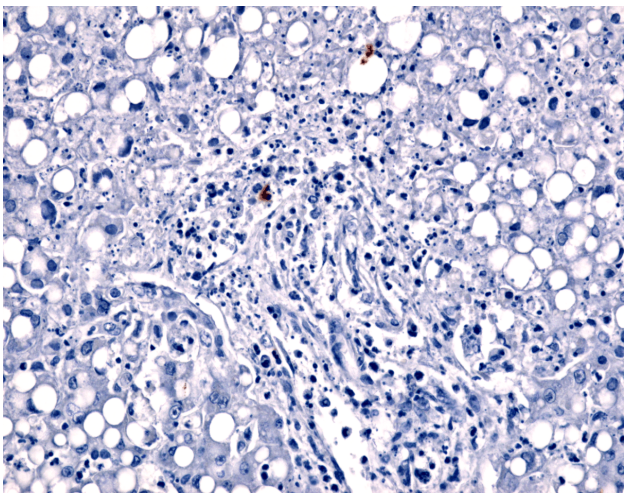

Figure 14 – *Microscopic image of the liver, with rare B-lymphocytes located at the periphery of the tuberculous focal. Immunomarking with anti-CD20 antibodies, ×200.*

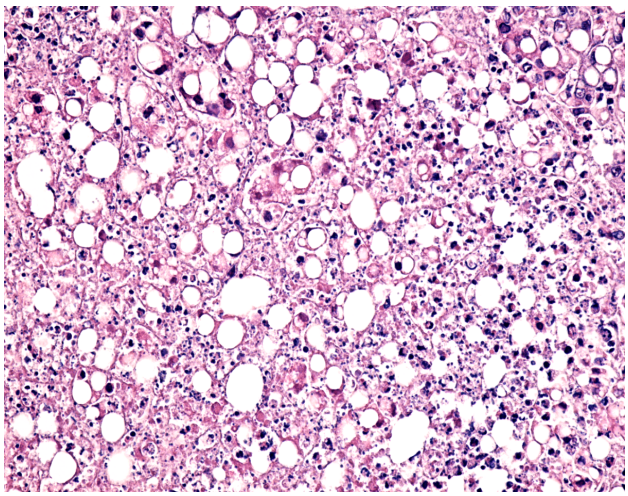

Figure 15 – *Image of focal hepatitis, with intense hepatocytary cytolysis, disorganization of hepatocyte cordons, associated with lesions of severe steatosis and liver parenchyma infiltration with lymphocytes and granulocytes. HE staining, ×200.*

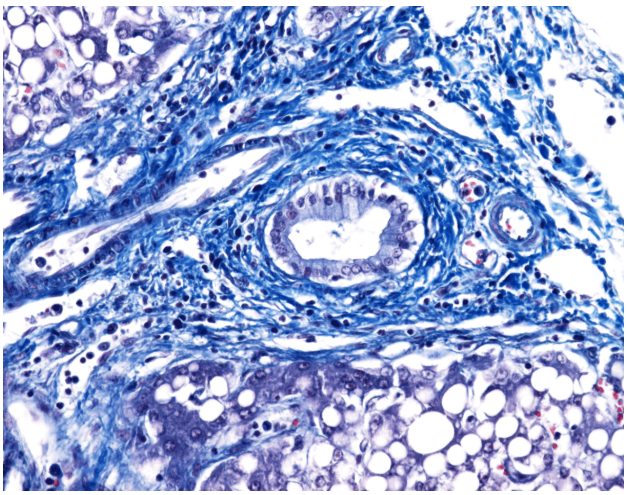

Figure 16 – *Intense collagenous fibrosis in the porto-biliary gap. Trichromic GS staining, ×200.*

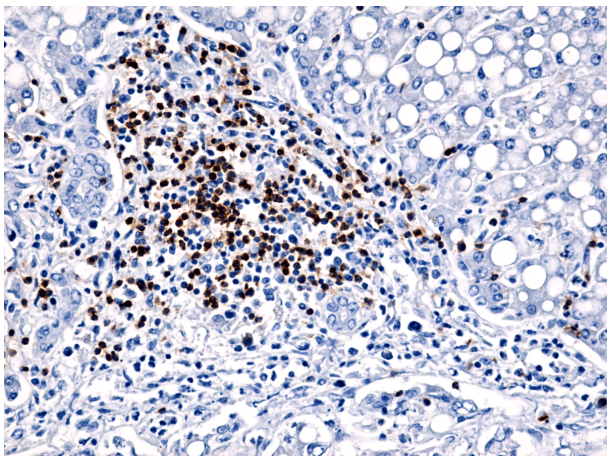

Figure 17 – ***Abundant inflammatory infiltrate in the Kiernan gap, rich in T-lymphocytes. Immunomarking with anti-CD3 antibodies, ×200.***

Familial contact with an index case (the father) of active pulmonary TB and the presence of disseminated nodules at lung level and on the computer tomography scan of the brain were the main clues raising the diagnostic of TB. Nevertheless, the infant received prophylactic therapy with Isoniazid at the time of his father's diagnostic. A chest X-ray was performed during previous hospital admissions for recurrent pneumonia, four months ago, no suggestive lesions of pulmonary TB were identified. Administration of Isoniazid chemoprophylaxis reduces the risk of active TB by up to 90% [13]. Nevertheless, our patient developed disseminated TB despite prevention therapy. The unusual clinical presentation with 24-hour history of febrile seizures and normal results of cerebrospinal fluid, further delayed anti-TB therapy initiation. Hemodynamic instability and altered vigilance were already present at hospital admission, the patient dying four days later.

The histopathological aspects surprised us through their *M. tuberculosis* infection in the baby occurred in the first months of life, and antituberculous chemoprophylaxis was inefficient. The dissemination of the tuberculous bacillus from the lungs to other organs was made in a lymphatic and hematogenic way.

The case presented by us shows that in Romania tuberculosis has become a serious public health problem. Numerous studies have shown that, worldwidely, tuberculosis continues to be a major medical and social problem, with high morbidity and mortality [14]. It is well-known the fact that in the second half of the 20th century, due to prevention methods and chemotherapy efficiency, the incidence of tuberculosis has significantly been reduced all over the world [15, 16]. Unfortunately, in the last years, the incidence of tuberculosis has increased in Western countries, due to an increase of HIV-infected patients and in developing countries by the reduction of tuberculosis surveillance and treatment systems [17, 18].

According to some studies, in 2011, approximately half a million children contracted tuberculosis, while 64 000 children died because of this disease [19]. Approximately 8–20% of deaths caused by tuberculosis occur in children [20, 21].

In our case, due to a young age (less than one-year-old) and to an inappropriately developed immune system, the tuberculous infection developed rapidly as a severe miliary form, with lymphohematogenic dissemination, simultaneously affecting various organs. To the development of severe form of tuberculosis there also contributed the baby's state of malnutrition, with a weight deficit of 3 kg. Various studies have shown that malnutrition is associated with severe forms of common infectious diseases and severe forms of some specific infections [22].

It is well known that the *M. tuberculosis* infection causes a significant depreciation of the liver function, both in adults and in children [23]. When the tuberculous lesions are also located at liver level, liver failure occurs rapidly. In our case, liver lesions were complex: diffusely disseminated tuberculous granulomas associated with severe steatosis, focal hepatocytolysis, fibrosis and inflammatory infiltrate in portobiliary gaps, characteristic to chronic hepatitis. We consider that the preventive administered Izoniazid was not sufficient enough, high bacillary populations allowing the development of a lung miliary tuberculosis, distance dissemination of the infection, with multiple liver, ganglionary, intestinal and encephalic localizations.

Conclusions

Disseminated tuberculosis affecting liver and the nervous system is a very severe form. Clinical symptoms were totally irrelevant, which led to a delay in the diagnosis. Early treatment initiation is extremely important once TB is suspected, to improve survival and prevent morbidity. It is imposed the revision of the recommendations of Izoniazid chemoprophylaxis in young children that come from intensely bacilli focals, with the risk of rapid dissemination and development of military, multiorganic tuberculosis. In these cases, chemotherapy would be more safe than chemoprophylaxis.

Author contribution

All authors have contributed equally to the present work.

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