Table 4. Up- or down-regulated Gene Ontology (GO) cellular processes.

(>2.0-fold)		
GO Accession	**GO Term**	**corrected p-value**
GO:0007566	embryo implantation	1.08E-05
GO:0005788|GO:0016022	endoplasmic reticulum lumen	1.33E-05
GO:0006694	steroid biosynthetic process	4.30E-04
GO:0044432	endoplasmic reticulum part	4.30E-04
GO:0005783	endoplasmic reticulum	4.30E-04
GO:0016126	sterol biosynthetic process	8.81E-04
GO:0008202	steroid metabolic process	0.004734457
GO:0008610	lipid biosynthetic process	0.008595788
GO:0016125	sterol metabolic process	0.011360187
GO:0015630	microtubule cytoskeleton	0.028312529
GO:0044444	cytoplasmic part	0.036239438
GO:0000278	mitotic cell cycle	0.039986596
GO:0016614	oxidoreductase activity, acting on CH-OH group of donors	0.053449787
GO:0016616	oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor	0.06185003
GO:0007049	cell cycle	0.063007735
GO:0005793	ER-Golgi intermediate compartment	0.073000684
(<0.5-fold)		
GO Accession	**GO Term**	**corrected p-value**
GO:0008092	cytoskeletal protein binding	2.59E-04
GO:0007156	homophilic cell adhesion	2.59E-04
GO:0005515|GO:0045308	protein binding	0.001197793
GO:0016337	cell-cell adhesion	0.004711885

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cytokeratin-18 expression. The reduction of genes of cell adhesion process might be a consequence of the loss of epithelial property and/or polarity. It should be noted that we selected only probes scored as "present call" in all samples, which allows relatively accurate comparison of expression levels between samples. However, this means that genes with very low expression in either PT or the SAGM-grown cells were probably excluded even though their difference in expression levels was far greater.

In summary, we have developed a novel system to propagate multilineage progenitor cells from adult normal human thyroid tissues. This seems to be achieved by dedifferentiation of thyroid follicular cells without any gene delivery. Since integration of transgene(s) may cause unpredictable problem, our system has an advantage in terms of safety. The presently described culture system may be useful for regenerative medicine, but the primary importance will be as a tool to elucidate the progression of thyroid disease. Moreover, this phenomenon could be induced *in vivo* because it can be achieved without introducing foreign genes. However, as we have not confirmed full functional differentiation of the cells, further study is necessary for regenerative application.

Supporting Information

Figure S1 Asymmetric division of the SAGM-grown cells. A, Scheme of cell divisions. Representative data obtained by time-lapse imaging of cell cycle are shown. ACD: asymmetric cell division, SCD: symmetric cell division. 6.1% of the cells showed asymmetric division after first division. Total 198 cells were analyzed. B, Representative images after asymmetric division. (TIF)

Table S1 Time-course expression of lineage-specific markers. (PDF)

Table S2 Colony formation in SAGM after FACS. (PDF)

Author Contributions

Conceived and designed the experiments: KS NM VS YN SY. Performed the experiments: KS NM MS MM. Analyzed the data: KS NM MS MM AO AK TU HY. Contributed reagents/materials/analysis tools: KS NM VS AO AK TU HY SY. Wrote the paper: KS NM VS YN.

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Short communication

Molecular characterization and phylogenetic analysis of full-genome HBV subgenotype D3 sequences from Serbia

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ABSTRACT

Hepatitis B virus (HBV) is classified into 8 genotypes with distinct geographical distribution. Genotype D (HBV/D) has the widest distribution area and is comprised of 7 subgenotypes. Subgenotypes D1, D2 and D3 appear worldwide, while D4–D7 have a more restricted distribution. Within the Mediterranean area, HBV/D and subgenotype D3 are the most prevalent. The purpose of this study was to characterize the full genome of Serbian HBV/D3 isolates by comparison and phylogenetic analysis with HBV/D3 sequences (66 samples) found in GeneBank/DDBJ databases from different parts of the world. Isolates were obtained from three patients diagnosed with chronic hepatitis B (HBsAg +). All three isolates have two very rare nucleotide substitutions, A929T and T150A, which indicate the same ancestor. Phylogenetic analysis of HBV/D3 genome sequences throughout the world follows an ethno-geographical origin of isolates with rare exceptions, which could be explained by human travelling and migration. The geographically close but ethnically different Serbian and Italian isolates clustered in the same subnode, and on a common branch with strains from Northern Canada. To test the apparently close HBV phylogenetic relationship between completely separated patients from Serbia and Northern Canada we analyzed in depth a 440 bp region of the HBsAg from Canadian (n = 73) and Serbian (n = 70) isolates. The constructed parsimony tree revealed that strains from Serbia and Northern Canada fell along the same branch which indicates independent evolution within regions of each country. Considering that HBsAg sequence has limited variability for phylogenetic analyses, our hypothesis needs further confirmation with more HBV complete genome sequences.

1. Introduction

One of the main features of hepatitis B virus (HBV) is a complex genetic heterogeneity. By phylogenetic analysis of the complete HBV genomic sequence, eight genotypes of HBV, with a putative ninth genotype, ranging from A to I, have been determined and divided into approximately twenty-five subgenotypes (Bartholomeusz and Schaefer, 2004; Norder et al., 2004; Schaefer et al., 2009; Pourkarim et al., 2010a). HBV genotypes differ by more than 8% at the nucleotide level and they show distinct geographical distributions (Norder et al., 2004; Schaefer, 2007a,b).

According to epidemiological data, the prevalence of worldwide HBV infection varies greatly with the highest estimated values in central Asia where genotypes B and C are the most frequent. Balkan countries, including Serbia, are highly endemic for HBV infection (Chironna et al., 2001; Kondili et al., 2005; Papaevangelou et al., 2006; Voiculescu et al., 2010), with a predominance of genotype D (HBV/D), which is customarily observed throughout the Mediterranean, Middle East, and Indian subcontinent (Norder et al., 2004; Bozdayi et al., 2005; Schaefer, 2007a; Baig et al., 2009; Chandra et al., 2009). Genotype E is restricted to Africa, while genotypes F and H, the most divergent of all HBV genotypes, are found in South and Central America and Mexico, respectively. Finally, genotypes G and

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putative I were recently identified in parts of Europe, North America, and Southeast Asia (Stuyver et al., 2000; Norder et al., 2004; Schaefer et al., 2009; Colson et al., 2009). Genotyping of HBV is of high interest because there is increasing evidence that HBV genotypes may be associated with HBeAg seroconversion rates, mutational patterns in the precore and core promoter region, severity of liver disease and treatment response (Schaefer, 2007a,b; Wiegand et al., 2008; Deterding et al., 2008). It has been shown that genotypes C and D are associated with more severe liver injuries and with a higher incidence of HCC than genotypes A and B (Kramvis and Kew, 2005; You et al., 2008; McMahon, 2009). In addition, HBV/C and/or D carriers have a much lower response rate to interferon therapy than those infected with A or B genotypes (Erhardt et al., 2005; Wiegand et al., 2008). Some studies have shown subtle differences in frequency and type of lamivudine resistant variants that occur in genotype A and D infections (Schaefer, 2007a).

Due to the genetic diversity of HBV, numerous subgenotypes have been described which differ by at least 4% over the entire genome with most also having a distinct geographic distribution. However, this is not true for HBV/D subgenotypes, where D1, D2 and D3 are found widespread throughout the world. HBV/D3 has been observed in South Africa (Kimbi et al., 2004), North America (Garfein et al., 2004; Panessa et al., 2009), Europe (Schaefer, 2007a) and Asia (34% of HBV/D in East India; Chandra et al., 2009), although rarely in Turkey or Pakistan (Bozdayi et al., 2005; Baig et al., 2009). Based on European published data, HBV/D3 is most prevalent in Italy with a frequency of 40.4% (72.7% of HBV/D, De Maddalena et al., 2007), with lower prevalence rates observed throughout the rest of Europe, from 16.3% in Belarus (Olinger et al., 2008), 11.3% in Belgium (Pourkarim et al., 2010b) to 6% or less in England, (Sloan et al., 2009), Turkey (Bozdayi et al., 2005), Russia and the Baltic region (Tallo et al., 2008; Olinger et al., 2008). As Serbia is part of the Mediterranean area, subgenotype D3 is highly prevalent at 39.3% (47.9% of HBV/D). Prevalence rates of subgenotypes D1, D2, and D4 in Serbia are very similar to other European regions, with frequencies of 4.5%, 32.6%, and 2.2%, respectively (Lazarević et al., 2007).

In order to investigate the molecular characteristics of prevalent HBV/D3 strains in Serbia, we sequenced the complete genome of HBV/D3 isolates from Serbia and compared them with isolates of the same subgenotype from geographically close and distant regions throughout the world.

2. Materials and methods

Clinical characteristics of analyzed patients are shown in Table 1. Serum samples were collected in the Institute for Infectious and Tropical Diseases, Clinical Center of Serbia, Belgrade. We randomly selected three therapy naive patients for HBV characterization to avoid selection pressure and therapy-derived nucleotide substitution within the HBV genome. Above that, we chose patients with HBV/D genotypes as determined in the clinic by the Inno-LIPA method (Innogenetics, Ghent, Belgium). The sera were tested for HBsAg/Ab, HBeAg/Ab and HBcAb IgM by commercially available enzyme-linked immunosorbent assay (ELISA) kits from Siemens Healthcare Diagnostics Products GmbH Marburg, Germany. HBV DNA copy number was measured by the Roche COBAS AMPLICOR HBV Monitor assay (Roche Diagnostics, Gmbh, Mannheim, Germany). The transmission route for examined patients is speculative: for RS1 and RS2 the transmission route could be vertical (their mothers had been HBsAg positive before delivery) or family contact; RS3 received a blood transfusion several times during 1999–2000, suggesting transfusion as a possible transmission route. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade, and all patients provided written consent.

Serum samples for HBV DNA analysis were kept at −80 °C prior to extraction. Viral DNA was extracted from 200 μL of serum using QIAamp MinElute Virus Spin Kit (QIAGEN GmbH, Germany), according to the manufacturer's instructions. Whole genomes were sequenced directly after amplification by PCR from both strands using primers and method described in Von Meltzer et al. (2008). The full length HBV sequences were submitted to GenBank with accession numbers HQ236014 through HQ236016.

2.1. Phylogenetic analyses

Subgenotyping of the three fully sequenced HBV/D Serbian isolates was performed by phylogenetic comparison with 95 complete genome sequences representing 9 HBV genotypes including 7 HBV/D subgenotypes using MEGA 4.0 software (Tamura et al., 2007). We selected a higher number of HBV subgenotype D1 (16 isolates), D2 (12 isolates) and D3 (29 isolates) sequences for better differentiation between closely related subgenotypes of HBV/D genotype. ID numbers of sequences used for phylogenetic analyses and references where these isolates were cited and genotyped are shown in Supplemetary Table A.1. HBV genome sequences were multiple-aligned using and ClustalW software (Aiyar, 2000). A Neighbor Joining tree was constructed using the Kimura 2 parameter algorithm and a bootstrap value of 1000 replicates (Tamura et al., 2007) revealed that all three selected isolates from Serbia were D3 subgenotype (phylogenetic tree is not shown).

With the aim to enlarge the number of HBV/D3 isolates for phylogeographic comparison we conducted a BLAST similarity search test with the above-mentioned phylogenetic analyses and

Table 1
Clinical characteristics of analyzed patients (RS1–RS3) and characteristics of detected HBV mutations.

	RS1	RS2	RS3
Gender/age (years)	f/27	m/7	f/10
ALT/AST[a]	222/427	121/187	24/30
HBsAg/Ab	+/−	+/−	+/−
HBcAb-IgM	−	ND	ND
HBeAg/Ab	−/+	ND	+/−
Viral load (log copy/mL)	7	5	4
HBV genotype	D	D	D
Other diseases	No	No	Acute lymphocyte leukemia
HBV mutations			
Basal core promotor			
A1762T	A1762	1762T	A1762
G1764A	G1764	1764A	G1764
Pre-C region			
G1896A (P gene, TGG28TAG nonsense)	G1896	1896A	G1896

f—female; m—male; ND—not determined.
[a] Reference ALT/AST value: 37/41 U/mL.

found further 37 HBV/D3 complete genome sequences in DDBJ/EMBL/GeneBank which were previously untyped (Supplementary Table A.1). Finally, we collected 66 HBV/D3 complete genome sequences comprising: 24 from South Europe, 5 from East Europe, 8 from West Europe, 6 from South Africa, 13 from Asia and 10 from North America (accession numbers, genotypes and geographical locations are listed in Supplementary Table A.1).

For phylogeographic analyses of the collected HBV/D3 sequences (Fig. 1), alignment was performed with ClustalX software (Thompson et al., 1997) and correction of alignment ends and gaps was done by BioEdit version 7.0.9.0 (Hall, 1999). ModelTest version 3.8 software (Posada, 2008) was used to determine the best-fit nucleotide substitution model for the data using the full set of 88 models. Maximum Likelihood analysis was done using PhyML version 3.0 software (Guindon and Gascuel, 2003) and recommended General Time Reversible substitution model (GTR), with default parameters except for the gamma shape parameter which was changed to fixed (0.628) and the proportion of invariant sites set to 0.342. Initial parsimony tree construction was supported by 100 bootstrap replicates. The tree was widened and annotated and the Canadian HBV/D3 subtree was rotated on the node to make the Serbian–Italian cluster clearer using TreeGraph version 2 (Stöver and Müller, 2010).

With the aim to evaluate results for phylogenetic similarity between Serbian and Canadian isolates we analyzed a 440 bp region of the HBsAg gene of 182 isolates provided in the GeneBank database: 73 isolates from the Canadian Arctic (Osiowy et al., 2011) and British Columbia (Panessa et al., 2009), 70 isolates from Serbia (Lazarević et al., 2007), as well as 39 HBV/D3 HBsAg sequences from different parts of the world (Supplementary Fig. 1.A). The most appropriate substitution model for the alignment data was determined by ModelTest (v3.8). Maximum Likelihood analysis was performed using PhyML (v.3.0) with a GTR substitution model having a gamma parameter of 0.52 and fixed proportion of invariant sites set to 0.723. The phylogenetic tree was constructed by the Parsimony method with 100 bootstrap replicates.

3. Results and discussion

Fig. 1 shows phylogeographic analyses of 69 HBV/D3 isolates from different parts of the world. From the phylogenetic tree it is observed that most strains cluster within a defined ethnogeographic group. Some exceptions are expected, due to differences in transmission risk factors or immigration and travel, which may shape the genotype distribution within non-endemic regions of the world (Panessa et al., 2009; Sloan et al., 2009; Komatsu et al., 2010). Generally, the phylogenetic relation of sequences under study reveals several groupings of HBV/D3 subgenotype strains: Asian and Eastern European strains appear separate from a clade with several sub-nodes supported by a bootstrap value of 94%. The sub-nodes include isolates from Indonesia and South and West Europe, North America and South Africa. Italian and Serbian isolates appear clustered together suggesting a common ancestor (Fig. 1). Italian and Serb people are geographically close but with different ethnicities. Namely, Serbs settled on the Balkan Peninsula in the 6th century from a region where the Ukraine is now, and ethnically they belong to a Slovenian heritage (like Ukrainian, Belarus, Russian, etc.). In these areas with Slovenian ethnicities the most frequently observed sub-genotype is HBV/D2 (Tallo et al., 2008; Olinger et al., 2008). We observed that HBV/D3 isolates from these regions clustered separately in relation to the Serbian isolates (Fig. 1). As branching of the Italian and Serbian isolates was not supported by a high bootstrap value (node bootstrap of 15%), we can conclude that the association between these isolates may indicate a clustering trend of the Serbian HBV/D3 pool from Eastern European to Mediterranean areas.

In our phylogenetic dendrogram the Italian/Serbian cluster grouped with a Canadian sub-node (Fig. 1). This was surprising, as the Canadian sequences (GQ922000 through GQ922002) originated from archived patient samples from the Canadian Arctic. To help resolve this observation, we further analyzed a 440 bp region of the HBsAg gene: 73 isolates from the Canadian Arctic (Osiowy et al., 2011) and British Columbia (Panessa et al., 2009), 70 isolates from Serbia (Lazarević et al., 2007), as well as 39 HBV/D3 HBsAg sequences from different parts of the world provided in the GeneBank database (Supplementary Fig. 1.A). The constructed parsimony tree revealed that strains from Serbia and Northern Canada fell along the same branch and were separate from the smaller sub-clades formed by Italian sequences (Supplementary Fig. 1.A). For example, RS1 and RS2 isolates were interspersed among Canadian Arctic (for example: 1B3925, 1B4116, GQ92000, GQ92001, etc.) and Serbian sequences (for example: EF177856, EF177877, EF177893, etc.) while RS3 clustered separately. However, as bootstrap values were less than 60%, specific inferences of association among Canadian and Serbian HBV/D3 strains cannot be made. Interestingly, most HBV/D3 strains from Eastern Europe and Asia did not cluster with RS1–3 or other Serbian sequences, which might be expected based on mentioned geography and migration. Furthermore, several Serbian and Canadian (British Columbia [BC]) sequences clustered separately and apart from sequences of the same respective country. This observation may be due to independent evolution within regions of the country or different transmission risk factors (Panessa et al., 2009; Van Steenbergen et al., 2002). In accordance with the limited variability of the preS/S HBV region for phylogenetic analyses, more complete HBV genome isolates should be investigated to obtain a definite conclusion (Meldal et al., 2009; Pourkarim et al., 2010a).

Alignment of complete genome sequences demonstrated 31–46 nucleotide differences between isolate RS1, 2 and 3, with intergroup nucleotide divergence values of 1.19 ± 0.16. Isolate RS2 had the dual core promoter mutations, T1762/A1764, and the Pre-C stop codon mutation G1896A (Table 1), both of which are frequent for the D genotype especially in the Mediterranean area (Schaefer, 2007a; Elkady et al., 2008; Chandra et al., 2009). A1896 appears in 26 isolates in our examined group of HBV/D3 (18 from Italy, 2 from Spain and all USA isolates). However, within the 66 HBV/D3 isolates analyzed, the T1762/A1764 double mutation appears rarely (AJ132335-Italy, EU594435-Estonia, EU414142-Belarus and isolates from USA: AY902768, AY902769, AY902772, AY902774, AY902776, AY902777).

By detailed visual survey of aligned sequences, we found two rare nucleotide substitutions common for all three RS isolates. Firstly, we detected the A929T nucleotide change which induces Q266L replacement in the reverse transcriptase part of P protein. The same nucleotide change was found in five non-subgenotyped HBV/D isolates from Italy and USA (ID: EF514284, EU908839, EF514292, GQ486332, GQ486138), one isolate from Brazil (ID: EU221473, unknown genotype), and one isolate from USA with genotype C (ID: GQ486855). Tallo et al. (2008) established the typical amino acid profile for HBV genotypes and found Leu at rt266 in strains of HBV/B and HBV/C, but Q/H appeared in HBV/D isolates. Nucleotide substitution T150A found in RS isolates is also very rare, appearing in 16 non-subgenotyped HBV/D isolates from different parts of the world (Netherland, Germany, Mongolia and Brazil), as well as Iranian subgenotype D1 strains and two isolates from Papua Indonesia with putative D6 subgenotype. This transversion induces L173Q alteration in the preS protein and for the P gene is a silent mutation. PreS 173Leu has been found as a genotype-specific amino acid residue for HBV/D in the survey of Tallo et al. (2008), where the authors compared 80 wild-type

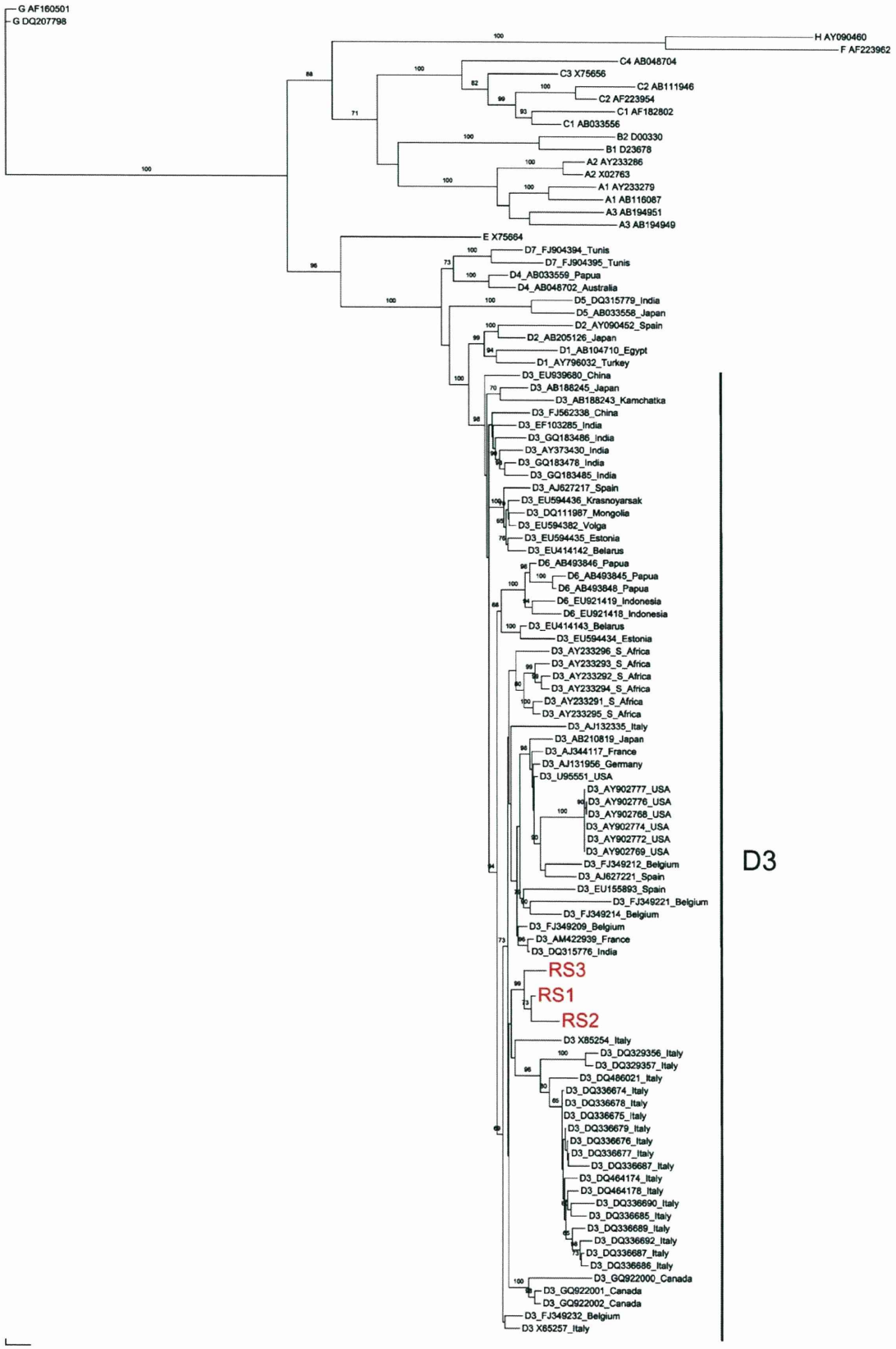

Fig. 1. Phylogenetic analysis of 3 full length Serbian HBV/D3 isolate genomes (RS1–RS3). One hundred comparative full length HBV sequences are designated by the HBV genotype/subgenotype, and GenBank accession number. Bootstrap confidence values of ≥60% are given. Bar branch length for a pairwise distance equal to 0.02.

genotype D strains. Presence of very rare nucleotide substitutions in all three RS isolates indicates that they likely have the same common ancestor.

Based on the full-genome phylogenetic tree (Fig. 1), Indonesian subgenotype D6 isolates (Utsumi et al., 2009) were found to be positioned between Eastern European/Asian sub-clades and Western/South African clades, and within a subgenotype D3 cluster with sequences from Belarus and Estonia on the same branch. Nucleotide distance between the D6 and D3 sequences included in the phylogenetic analysis was 3.0 ± 0.2%. On the basis of our analysis which is the first phylo-geographic comparison of a large number of full length subgenotype D3 genomes, we propose the reevaluation of subgenotype D6 as a distinct subgenotype of genotype D following rules offered recently by Pourkarim et al. (2010a).

Our phylogenetic analyses of 69 HBV/D3 isolates throughout the world show that almost all nucleotide sequences follow ethno-geographical relations. Rare exceptions could be explained by human travelling or migration and different risk factors. Although this is not likely the case for the observed Serbian and Canadian Arctic HBV/D3 phylogenetic association, it is possible that independent evolution of HBV in these two very distinct populations occurred. However, this assumption needs further confirmation with analysis of additional full HBV genomes obtained from these geographical regions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.meegid.2011.05.004.

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15

Pathology of HIV/AIDS: Lessons from Autopsy Series

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1. Introduction

HIV infection is a global disease and despite considerable efforts of the international community it is a main cause of human mortality (UNAIDS, 2009). Morphological insights into HIV/AIDS are based on the study of clinical cases by means of biopsy and autopsy. Morphological changes during development of HIV infection and, especially, through AIDS progression are variable and specified mainly by characteristics of widespread secondary infections and tumors. Opportunistic infections account for approximately 80% of deaths in patients with AIDS and their spectrum is constantly changing, as a result of improvements in treatment options and prophylaxis along with the increasing life span of HIV-infected individuals. Postmortem examinations provide important diagnostic and epidemiological data and represent a most reliable source for estimation of the full spectrum of diseases in individual patients and the general population.

2. Pathomorphology of HIV/AIDS

Morphology of HIV/AIDS is manifested by wide range of indicative (secondary) diseases while specific changes caused by HIV are mainly detected in immune system at the early stages of infection and in central nervous system.

Lymphadenopathy is the marked feature of acute HIV infection defined as generalized enlargement of lymph nodes. Histologically, this process passes through a series of changes: hyperplasia, involution, depletion, and sclerosis (Baroni & Uccini, 1993). In the stage of hyperplasia, the lymph nodes are characterized by disorderly grouped multiple follicles with 'starry sky' pattern due to arrangement of macrophages. Formation of multinuclear cells resembling symplasts is result of merging of lymphocytes infected by virus. With the progression of disease, lymphoid depletion becomes extensive and a fibrovascular carcass appears more evident along with increasing vascularity (angiomatosis). Finally, lymph nodes harbor 'burnt-out' appearance. Although profound depletion of lymphoid tissues is driven by cytotoxic effect of HIV but there is no histologic picture diagnostic of this condition (O'Murchadha et al., 1987).

Large proportion of patients at different stages of disease has morphological proofs of HIV-induced brain damage (Kibayashi et al., 1996). HIV neuropathology is comprised of

following patterns (in order of appearance): lymphocyte infiltration of the leptomeninges, microglial nodules formation and HIV encephalitis. The latter lesion consists of numerous foci with mononuclear cells typical of small macrophages, microglia, and multinucleated giant cells. The giant cells are the hallmark of HIV infection since viral antigens can be detected in their cytoplasm (Gyorkey et al., 1987).
Development of indicative diseases which include opportunistic infections and secondary neoplasms which reflects severe deficiency of immune system and in most of cases determines progression of the disease to full-blown AIDS. Morphological descriptions given below represent our common findings of infectious and neoplastic diseases in AIDS.

2.1 Morphology of mycobacterial Infections in HIV patients

Tuberculosis in HIV-patients is characterized by a prevalence of its generalized form with extensive dissemination and acute progression of specific processes. Notable histological features are loss of granuloma formation and abundance of necrotic changes. Generally, all the forms of tuberculosis seen in the terminal stages are actively progressive. The main forms of tuberculosis are generalized, pulmonary (often disseminated) and extrapulmonary. Thus, various organs are affected, most often the lungs, lymph nodes, liver, kidneys, spleen, intestine, and central nervous system (Smith et al., 2000). Tissue reaction in the terminal stage shows typical tuberculous granulomas with giant and epithelioid cells in only 20% of lesions, whereas the remaining 80% demonstrates numerous foci of nonreactive caseous necrosis abundant of acid-fast bacilli (Parkhomenko et al., 2003).
Pulmonary tuberculosis is manifested as a bilateral disseminated type or polycavernous variant. In disseminated tuberculosis, foci of specific lesions (granulomas) comprise large central zones of caseous necrosis surrounded by a few inflammatory cells. Giant cells are uncommon. Ziehl-Neelsen staining shows numerous acid-fast bacteria in the foci of caseous necrosis. All these histological signs characterize tuberculosis as progressive and highly active. Macroscopic study of the lungs often reveals miliary disseminated tuberculosis, while macrofocal dissemination and caseous pneumonia are rare. The pattern of dissemination is bilateral, with a predominance of micronodular, miliary and submiliary types. Tubercles evenly spread to the whole organ or localized to one of the lobes. In a large proportion of cases, macroscopic detection of tuberculous changes in lungs is difficult, but histological examination reveals miliary and submiliary necrotic foci (Berdnikov et al., 2011). The characteristic microscopic picture is a predominance of alterative and exudative changes with the lack of a productive component of inflammation or its minimal manifestation (Fig. 1a). The latter is marked by the absence of signs of encapsulation and organization of inflammatory foci. Classic granulomas are infrequent and only few of them contain giant cells of Langhans (Fig. 1b). Initially, there is formation of colonies of *Mycobacteria* in the pulmonary parenchyma, which is accompanied by cellular infiltration with a significant predominance of polymorphonuclear leucocytes. The cells phagocytose the bacteria and this step is marked by karyorrhexis. Later, this process is associated with massive breakdown of leucocytes resulting in necrosis and microabscesses. Tissue sections stained by Ziehl-Neelsen showed numerous acid-fast bacteria in the foci of caseous necrosis. An exudative reaction in the form of serous-fibrinous pneumonia or fibrinous-purulent pneumonia with predominance of neutrophilic leucocytes is detected at the periphery of caseous foci. Such

exudation may extent from lobular up to sub-lobar area. Some alveoli contain accumulations of foamy macrophages that are characteristic for typical tuberculous inflammation. There is an increase in the thickness of the pleura caused by extensive hyperemia and edema. The intrathoracic lymph nodes are also affected, enlarged (3–4 cm in diameter), and aggregated. Partial or total caseous lymphadenitis is detected with the spread of inflammatory processes to the surrounding soft tissues. Evident reduction of follicular structures and lymphoid depletion is a characteristic feature of these lymph nodes.

Extrapulmonary tuberculosis is detected as a component of a generalized type of tuberculosis. Monomorphic miliary foci of caseous necrosis are found in various internal organs, more often in the spleen, kidneys, liver, and rarely in the meninges, peritoneum, exo- and endocrine glands (pancreas, adrenals, prostate, thyroid, ovaries). As a whole, in cases of generalized tuberculosis, *Mycobacteria* cause alterative and exudative reactions simultaneously in several organs with the mean number of organs involved is 5.4 (own data). Most of the foci are suspected to be spread via hematogenous dissemination from lungs. Histopathology of the parenchymatous organs reveals monomorphic miliary nodules of caseous necrosis with rare giant cells, as in the lungs. In many cases, tubercles are not visible by visual inspection. In the spleen, the foci of caseous necrosis have a tendency to fuse and may cover up to 50% of the cut surface.

Tuberculous meningitis is characterized grossly by typical basilar localization with poorly detected gray-white exudates and tubercles in the subarachnoid space. Microscopic examination of the meninges reveals evident hyperemia and edema accompanied by alterative reactions. The latter is manifested as areas of caseous necrosis extensively infiltrated by polymorphs, lymphocytes, and macrophages. Various types of vasculitis such as endovasculitis, panvasculitis, thrombovasculitis, and perivasculitis are evident. Perivasculitis is more often present with edema and excessive mononuclear, neutrophilic, eosinophilic, and plasma cell infiltration in all layers of the vessel wall (Fig. 1c). Destructive process may extent into brain tissue with formation of localized abscesses.

Mycobacterium avium-intracellulare (MAI) infection leads to massive necrotic destruction of lymph nodes with minor involvement of the lungs. Mostly granulomas are difficult to detect throughout the inner organs by naked eye. The only exception is spleen which is filled with miliary granulomas in roughly half of cases. Different groups of visceral lymph nodes are enlarged and show characteristic yellow tone of cut surface. Microscopically, proliferation of large round to elliptical striated pale blue macrophages is noted. Cytoplasm of these cells is packed with huge number of acid-fast bacilli. Well-formed granulomas with fibrosis, necrosis, and epithelioid histiocytes are present in less than one third of cases (Klatt et al., 1987).

2.2 Morphology of bacterial infections

Bacterial Pneumonia

There is a broad spectrum of causative agents of pneumonia revealed by microbiology. Besides typical microflora, bacterial pneumonia can be caused by opportunistic agents, which are activated under immunodeficiency. The most common causative agents of pneumonia are *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pneumoniae, Haemophilus influenzae* and *Escherichia coli* (Afessa et al., 1998).

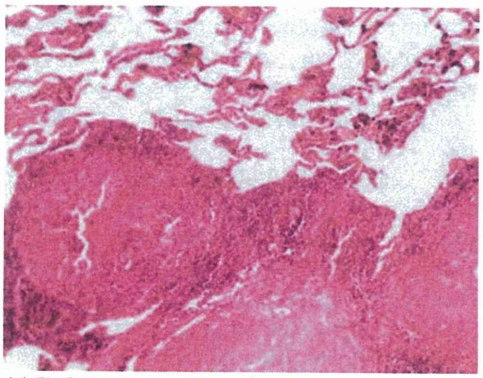
Hematoxylin and eosin together with Gram staining of sections of lungs helps in revealing nonspecific microflora. At autopsy, staining of smears of lung sections using Romanovskii-Giemsa and Gram stains is also useful in establishing the nonspecific character of microflora in cases of bacterial pneumonia. Bacteriological culture of lung tissue helps in revealing the nature of the causative agents of pneumonia most accurately. Grossly, patchy areas of red or grey consolidation involve more often the lower zones of the lungs. On cut surface, these patchy consolidated lesions are dry, granular, firm, red or grey in color, slightly elevated over the surface and are often located around a bronchiole. Histologically, suppurative exudate, consisting of neutrophils with admixture of fibrin, fills alveoli and alveolar septa are dilated by congested capillaries and leucocytic infiltration. Often, the course of pneumonia in HIV-infected patients has a tendency to form microabscesses, and in such cases, the microscopic changes resembles to microfocal dissemination in pulmonary tuberculosis. In microabscesses, purulent necrotic foci are found with expressed perifocal exudative reaction, which strengthened their resemblance to pulmonary tuberculosis in HIV-infected patients.

Sepsis

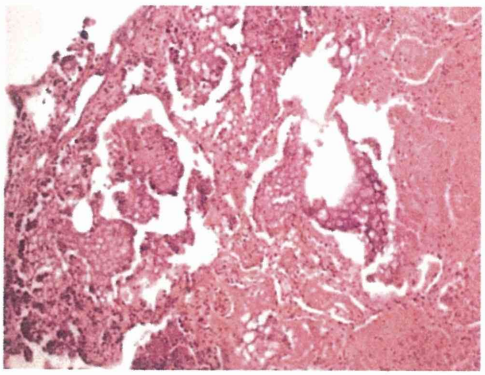
Pneumonia and primary bloodstream infections are the main sites of infections for almost all patients with sepsis, followed by angiogenic-related bacteremia originated from thrombophlebitis in intravenous drug users or from venous catheter in bedridden patients and urinary tract infections. Nosocomial infections compose the major part of etiology of severe sepsis. Microbiology of infections is comprised of different species, mostly *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus spp.*, while *Streptococcus spp., Escherichia coli* and *Salmonella spp.* are detected with lower frequency (Japiassú et al., 2010). Microorganisms impact small vessels in the primary site and cause local injury both by obstructing the vessels and by releasing toxins. Subsequently a combination of necrosis, hemorrhage and suppuration occurs, with further formation of pyemic abscesses in the various organs and their distribution depends on the site of the original septic thrombosis. Microscopically, pyemic abscesses are typically surrounded by a zone of hemorrhage and an early lesion may show a central zone of necrosis often containing huge numbers of bacteria. This is surrounded by a zone of suppuration and an outermost zone of acutely inflamed and often hemorrhagic tissue. In septic thrombosis of major veins, larger fragments may be released into the circulation, and by impacting in arteries give rise to correspondingly larger foci of necrosis and suppuration (septic infarcts). In case if it involves heart, pyogenic bacteria may produce endocarditis and severely damage cardiac valves. The vegetations on valve are tend to break down and the valve cusps are largely covered by crumbling masses, which consist of layers of fibrin containing clumps of bacteria enclosed by a zone of leukocytes, macrophages and granulation tissue. The substance of the cusps may be extensively destroyed by suppuration.

2.3 Invasive fungal infections

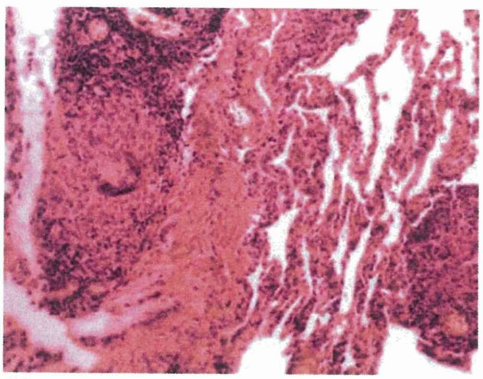
Pneumocystis jirovecii (former *Pneumocystis carinii*) typically produces pneumonia that is widespread throughout the lungs with a chronic course of disease and rapid progression. Pulmonary pneumocystosis is a disease caused by intense multiplication of relatively pathogenic single-celled saprophyte *Pneumocystis jirovecii* in the human respiratory tract.



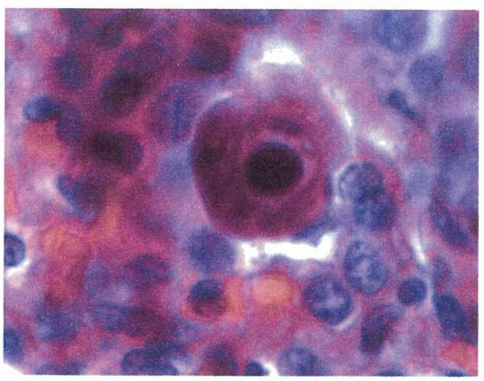
(a) Pulmonary tuberculosis,
foci of caseous necrosis. H&E, × 100



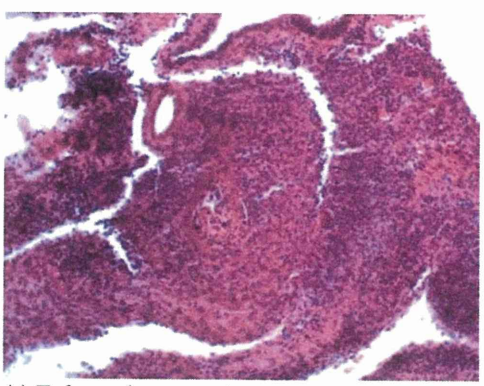
(d) Pneumocystic pneumonia,
foamy exudate. H&E, × 100



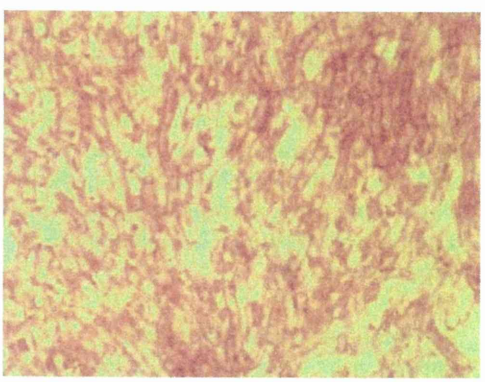
(b) Pulmonary tuberculosis,
granuloma with giant cell. H&E, × 140



(e) Cytomegalovirus infection of lung,
cell with 'owl-eye' appearance. H&E, × 1200



(c) Tuberculous meningitis,
panvasculitis with alteration. H&E, × 140



(f) Pulmonary aspergillosis,
branching hyphae of fungus. PAS, × 600

Fig. 1. Microscopic patterns of opportunistic infections in AIDS

The terminal period of pneumocystosis is pneumonia, manifested in the later stages of HIV infection, which often leads to death. The gross appearance resembles to pneumonic consolidation. The cut surface of the lung is pale pink with scattered areas of congestion and rarely hemorrhages. Microscopically, in the edematous stage, characteristic homogenous, foamy protein-containing eosinophilic exudate is found in the alveolar lumen (Fig. 1d). This is a pathognomonic sign of pneumocystic pneumonia. Neutrophils, macrophages and plasma cells are detected around the collections of *Pneumocystis jirovecii.*

Cryptococcus neoformans in immunocompromised hosts may spread from lungs, which is the site of primary infection, to distant organs anh most frequently affecting the central nervous system and causes meningitis. Pulmonary manifestations exhibit pneumonitis, pulmonary nodules or less commonly pleural effusions. Sometimes variably sized pale soft granulomas are grossly visible in the lungs. If fungi with capsules are numerous, a grossly apparent mucoid exudate may be seen in the cerebral ventricles or on the meninges. Microscopically, the yeast cells appear pale blue and ovoid while the capsule is round and clear. Inflammatory reaction is weak and represented by a few scattered lymphocytes or macrophages with phagocytized organisms. PAS stain is effective for detection of the capsule and nucleus of the organisms.

Candida albicans infection is one of the most prevalent in patients with AIDS, ranging from localized skin and mucosa lesions to widely disseminated disease. Characteristic gross findings of candidiasis are prominent in the pharynx, larynx, and trachea with invasion into principle bronchi, which includes a pseudomembranous form with white, elevated mucosal plaques. Bronchopulmonary aspergillosis and candidiasis are characterized by the collection of fungal mycelia in the lumen of small bronchi and invasion of fungus into the acini. *Candida* microabscesses are common and they had a typical polymorphonuclear leucocytes infiltration. Histologically, *Candida* organisms could be identified by their size, budding property, and pseudohyphae. The pseudohyphae could be distinguished from *Aspergillus* hyphae by the lack of branching, the smaller size, and frequent absence of true septations in the former. Histological diagnosis may be confirmed using the Romanovskii staining technique, which is helpful in differentiation between *Candida* and *Aspergillus.* Bronchopulmonary aspergillosis is characterized by the collection of branching mycelia of *Aspergillus* in the bronchial lumen with involvement of the bronchial wall and further invasion of the fungus into the acini (Fig. 1f).

2.4 Viral and parasitic infections

Cytomegalovirus (CMV) *infection* is one the most prevalent secondary diseases in AIDS. It is featured by multiple organs involvement, including lungs, digestive system, brain and eyes. CMV infection proceeds diversely from latent infection to severe acute generalization in the later stages of HIV infection. Microscopically, CMV lesions appear as characteristic metamorphosis of alveolar and bronchial epithelium (Fig. 1e). The persistence of viruses in the epithelial cells leads to cytomegalic giant cell formation. Alveolar cells increase in size up to 25–40 μm. About 1–2 nuclear inclusions are detected containing viral particles in the chromatin in each cell and there is a thin perinuclear clear halo. The nucleus of each affected cell is usually eccentrically positioned and the cell border is not prominent. Additionally, the cytoplasm of affected cells may contain coarse dark basophilic bodies. Characteristic infiltrative changes and CMV transformations are numerous. Moderate cytomegalic transformation of alveolar and bronchial epithelial cells (2–3 typical cells in the form of an

'owl-eye' in the field of view) is accompanied with focal accumulations of serous fluid and protein masses in the alveolar cavities along with admixtures of macrophages and weak infiltration of interstitial tissue. If the lung changes consist of diffuse persisting alveolitis with CMV transformation (up to 20 cells per field of view), then this process is accompanied by extensive fibrosis, but uncommonly leads to the formation of a 'honeycomb-like' appearance of the lungs. The outcome of CMV infection of the lungs is peribronchial and widespread interstitial fibrosis with thickening and vast deformation of the interalveolar septa. Thus, heterogeneous patterns of CMV infection of the lung represent continuous progression of disease and include the following events as virus-induced transformation of the cells, pneumonias with cavity formation, productive granulomatous alveolitis and eventually pulmonary fibrosis (Parkhomenko et al., 2004).

Toxoplasma gondii is a protozoan parasite which is highly prevalent among humans and animals throughout the world. Immunocompromised patients are especially prone to develop disseminated toxoplasmosis, either from acute exposure to the organisms or from reactivation of latent infection. Multiple organ systems are often involved, including the CNS, heart, lungs and skeletal muscle. Damage to the CNS by *Toxoplasma gondii* is characterized by numerous foci of enlarging necrosis and microglia nodules. The former are often resolved with cyst formation or calcification. Presence of many brain abscesses with almost universal involvement of the cerebral hemispheres is the most characteristic feature of toxoplasmic encephalitis in AIDS patients. The diagnosis of toxoplasmosis is readily made from histologic analysis of tissue specimens by observing any of the three infectious stages of *T. gondii*: tachyzoites in groups, bradyzoites (parasitic tissue cysts) or sporozoites within oocysts. Tachyzoites and cysts are seen in and adjacent to necrotic foci near or in glial nodules, perivascular regions, and even in uninvolved cerebral tissue. Reactive inflammatory reaction is comprised of mixed infiltrate distributed in patchy pattern.

2.5 HIV-associated neoplasia

Kaposi's sarcoma is a low-grade mesenchymal tumor which arises initially as an angioproliferative disorder caused by *Kaposi's sarcoma-associated herpes virus* (Du et al., 2007). Skin involvement is common and manifested by the presence of red to red-purple lesions ranging from flat patches to slightly raised plaques and nodules. Visceral involvement frequently includes the lung, lymph nodes, and gastrointestinal tract. Microscopically, Kaposi's sarcoma features clusters of tiny apparent capillaries budding off normal blood vessels. It grows as massed bundles of spindle cells, with red blood cells in slits between them. Hemosiderin pigmentation and hyaline globules usually accompany the spindle cell proliferation. Tumor has an ability to infiltrate around large vascular structures, near epithelial or mesothelial surfaces, or near the capsules of organs.

Non-Hodgkin lymphomas (NHL) risk in AIDS patients is increased more than 70 times comparing with general population (Frisch et al., 2001). Malignant non-Hodgkin's lymphomas (mostly intermediate- to high-grade tumors) exhibit two major patterns which include systemic and CNS lymphomas. These lymphomas often show evidence of *Epstein-Barr virus* as etiologic agent (Fassone et al., 2002). AIDS-related lymphomas consistently determine a B-cell phenotype and are histogenetically related to germinal center or postgerminal center B-cells in the vast majority of cases. About 80% of NHL's in AIDS arise systemically, either nodally or extranodally, while 20% arise in the central nervous system. Almost any extranodal site can be involved with predominance of bone

marrow, gastrointestinal tract and liver. NHLs appear as small infiltrates, focal nodular lesions, or larger tumor masses with accompanying necrosis and hemorrhage. Microscopically, tumors generally referred to diffuse large B-cell lymphomas or high-grade B-cell (small non-cleaved) Burkitt-like lymphomas, according to REAL classification. Immunohistochemistry is a routine diagnostic tool in typing of these lesions.

3. Autopsy series

AIDS was recognized in 1981 for the first time, resulting in the deaths of more than 25 million people. Since the late 1990s approximately 2 million HIV-infected persons are reported to have died annually (UNAIDS, 2009). Distribution of the regions with the highest death tolls is determined by the prevalence of HIV infection. Countries of Sub-Saharan Africa are the worst-affected, followed by South and South-East Asia. Most countries with high rates of AIDS prevalence have published reports on autopsy series to date (Fig. 2). Currently, there is a global decline of autopsy rates as a consequence of improved patient management, introduction of etiologic therapy and preventive measures. However such advances are available only in developed countries, where postmortem examination has lost the status of routine diagnostic procedure. Concurrently only few recent reports from Sub-Saharan Africa are available while the pace of HIV epidemics is still very high. Moreover, some countries with high AIDS burden (more than 500,000 infected) including Thailand, China and Ukraine are yet to present large autopsy series.

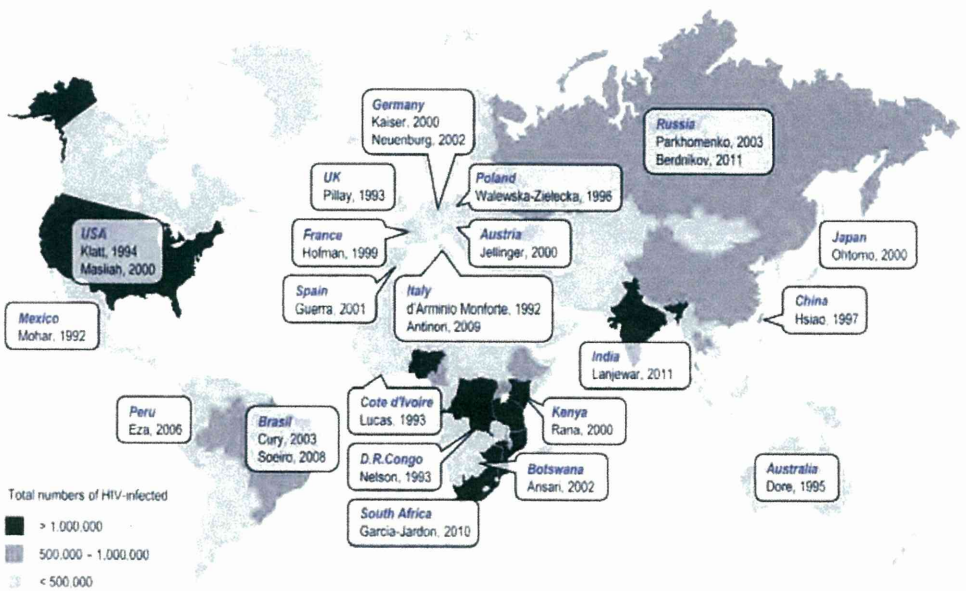


Fig. 2. Worldwide representative autopsy series

3.1 Value of autopsy

Since the first years of HIV/AIDS pathology has contributed significantly to study of new disease. Before mid-1990s various changes of organs and systems studied with different pathological techniques were described in numerous publications. The data taken from the study of autopsy series have shown that postmortem examination is extremely valuable for determining wide range of AIDS-related diseases. Diagnostic role of the autopsy is enhanced considerably by employment of histological examination of the organs. Such specimens can be further proceeded to staining with special techniques useful in detection of microorganisms or immunohistochemistry and even to molecular biological techniques. Thus, complete postmortem study allows determining the cause of death and contributed pathologies, it may identify diseases and etiological agents that were clinically unsuspected or undiagnosed. By providing these types of data, the autopsy serves as an important measure in monitoring the quality of care, basically comparing antemortem with postmortem findings. Autopsy remains established tool for obtaining epidemiological information about diseases and producing vital statistics, since systematic postmortem examination provides representative data on the main pathologies in distinct communities and permits evaluation of changes that may occur over time (Lanjewar, 2011; Lyon et al., 1996). Postmortem surveillance is vital for monitoring the course of HIV-infection and promotes clinicians awareness (Sehonanda et al., 1996).

However, over the past decades, autopsy rates have markedly declined all over the world due to various reasons (Table 1). Advances in laboratory and radiological diagnostics contribute in recognizing different AIDS-related diseases and diminish diagnostic value of autopsy.

Benefits	Limitations
Diagnostic value: - gross findings, - histological analysis, - correlations with antemortem diagnosis *Epidemiological needs:* - death records, - trends over time periods *Educational goals:* - for students, - for professionals (clinical conferences) *Scientific potential:* - case reports, - series reports, - sample collection/further reevaluation	*Difficulties in obtaining consent* *High efficiency of clinical diagnostic tools:* - laboratory diagnostics, - diagnostic radiology, - endoscopy *Risk of infection for staff* *Choice of 'alternative autopsy' techniques:* - needle autopsy, - virtual autopsy, - verbal autopsy *High costs*

Table 1. The AIDS autopsies today: *Pros & Cons*

Health care systems of developed countries offer high-quality diagnostic opportunities for HIV patients, while in developing countries such options have limited availability. Another one concern is that most relatives of died patients are not willing to provide consent for an autopsy because of cultural, traditional and other beliefs (Garcia-Jardon et al., 2010). It is important for clinicians to approach families for autopsy consent. From the other hand, some pathologists and technicians avoid to carry out autopsies on HIV infected cases, because of risk to be infected (Lanjewar, 2011). A major challenge in applying autopsy for AIDS cases is the rising trend of so-called 'alternative autopsy' techniques. Whereas incomplete autopsies such as examination of selected organs or needle autopsy may be accepted partially as being equivalent to a full autopsy, non-invasive procedures like virtual autopsy and echopsy cannot substitute for conventional necropsy techniques (Burton & Underwood, 2007). Verbal autopsy which appears to be gaining acceptance in developing countries (Bhattacharya & Neogi, 2008) is in no way an objective diagnostic technique. Postmortem examination should include collection of organs specimens for histological study; any exception to this rule will markedly decrease the value of autopsy.

3.2 Results

We have analyzed the largest autopsy series from different continents that covered the time period from 1982 to 2011 (Table 2). The main focus was on the prevalence of AIDS-indicative diseases and their distribution according to time periods and geography. Opportunistic infections were the most common autopsy findings, followed by less frequent secondary neoplasms.

Mycobacterial infections were detected in all the series with the lowest frequency around 20% (Afessa et al., 1998; Guerra et al., 2001; Soeiro et al., 2008). In developed countries such as Italy, Germany and Japan, the prevalence of tuberculosis in autopsies of patients with HIV/AIDS is 5-7%, whereas these rates were found to be 38-59% mostly in developing countries (Ansari et al., 2002; d'Arminio Monforte et al., 1992; Hsiao et al., 1997; Kaiser et al., 2000; Lucas et al., 1993; Ohtomo et al., 2000). Pulmonary lesions tend to hematogenous spread, thus the disseminated variant was described in 60-90% cases (Ansari et al., 2002; Cury et al., 2003; Parkhomenko et al., 2003; Rana et al., 2000; Soeiro et al., 2008). Extremely high rates of tuberculosis were reported in recent studies from Russia and India, 82% and 68%, respectively (Berdnikov et al., 2011; Lanjewar, 2011). Emergence of tuberculosis became obvious only in the last decade, when dramatic increase of this infection was implicated as a prime cause of death in AIDS patients. The main reason for such burden in Russia is the overlapping of prior independent epidemics of HIV and tuberculosis with subsequent merging and fast spread through neglected population groups like intravenous drug users, prisoners, alcoholics, homeless persons. Modern HIV-associated tuberculosis is a highly aggressive destructive process in the lungs caused by multidrug resistant strains of *Mycobacteria* and characterized by widespread dissemination and extrapulmonary involvement (Berdnikov et al., 2011).

Currently MAI infections are not of major significance, but they featured notably in the early series from USA and Europe (Guerra et al., 2001; Jellinger et al., 2000; Klatt et al., 1994; Masliah et al., 2000).

Table 2. Autopsy findings from the largest autopsy series.

Country	USA	USA	USA	USA	USA	Austria	Spain	Botswana	Brasil	Russia	Brasil	SouthAfrica	Russie	India	Russia
Source	Klatt 1994	Lyon, 1993	Sehonanda, 1996	Afessa, 1998	Masliah, 2000	Jellinger, 2000	Guerra, 2001	Ansari, 2002	Cury 2003	Pavlovnenko, 2003	Soeiro, 2009	Garcia-Jardon 2010	Bondnikov, 2011	Lanjewar, 2011	own data (unpublished)
Period	1982-1992	1984-1992	1987-1993	1985-1996	1982-1998	1993-1999	1991-1997	1997-2000	1993-2003	1991-2001	1999-2006	2000-2008	2001-2010	1999-2009	2003-2011
Cases	565	279	168	233	390	260	150	104	92	321	250	86	264	223	155
Opportunistic Infections															
MycoBact		64 23%							25 27%						
TB	76 13%		45 27%	13 6%		15 6%	12 8%	42 40%		99 17%	36 14%	25 32%	216 82%	152 68%	72 46%
MAI	104 18%		26 15%	31 13%	85 22%	36 14%	17 11%	2 2%			15 6%		7 3%		1 1%
Bacterial		81 29%			149 38%	138 53%									
Pneum	52 9%			98 42%			14 9%	24 23%		48 15%	91 36%	17 20%	26 10%	48 21%	40 26%
Sepsis	32 6%						1 1%	4 4%	13 14%	23 7%	36 14%	4 5%	10 4%		6 4%
Meningitis								2 2%	5 5%	5 2%	4 2%	8 10%			1 1%
Viral															
CMV	286 51%	128 46%	36 21%	40 17%	196 50%	127 49%	19 13%	16 15%	16 17%	59 19%	33 13%		3 1%	35 16%	10 7%
HSV	92 16%	20 7%				11 4%	1 1%								1 1%
Fungal															
PCP	308 55%	80 29%	55 33%	56 24%	100 26%	30 12%	21 14%	11 11%	15 16%	6 2%	68 27%	7 9%	6 2%	11 5%	17 11%
Asperg	6 1%	3 1%	9 5%	2 1%		12 5%	3 2%				1 <1%		1 <1%		1 1%
Cryptoc	78 14%	25 9%	9 5%	23 10%	119 30%	25 10%	3 2%	7 7%	4 4%	3 1%	9 3%	6 7%	9 3%	18 8%	5 3%
Cand	240 42%	30 11%	23 14%	6 3%		46 18%	1 1%		6 6%	3 1%			44 17%	6 3%	9 6%
Histoplasm	13 2%	29 10%	3 2%	2 1%		2 1%			5 5%		3 1%	1 1%	3 1%		
Parasitic															
Toxo	51 9%	9 3%	5 3%		11 3%	18 7%	1 1%	1 1%	9 10%	22 7%	18 7%	1 1%		15 7%	
Cryptosp	5 1%	7 3%												6 3%	
Helminthes											5 2%	1 1%		3 1%	
Secondary Neoplasms															
KS		138 49%		19 8%	41 10%	41 16%	1 1%	11 11%		33 10%	11 4%	1 1%	2 1%	2 1%	
NHL		81 29%		3 1%	20 5%	17 7%	13 9%	3 3%	2 2%	3 1%	5 2%		6 2%	8 4%	2 2%
Frequency hierarchy	CMV PCP-Bact	PCP-CMV	PCP TB-CMV	Pneum PCP-CMV	CMV-Bact PCP	Bact-CMV	PCP-CMV	TB Pneum-CMV	TB Bact-CMV	TB CMV-Pneum	Pneum-PCP	TB Pneum	TB	TB Pneum	TB Pneum

Abbreviations: MycoBact, mycobacterial infections; TB, tuberculosis; MAI, *Mycobacterium avium-intracellulare* infection; Pneum, pneumonia; CMV, *Cytomegalovirus* infection; HSV, *Herpes Simplex Virus* infection; PCP, pneumocystic pneumonia; Asperg, aspergillosis; Cryptoc, cryptococcosis; Cand, candidiasis; Histoplasm, histoplasmosis; Toxo, toxoplasmosis; Cryptosp, cryptosporidiosis; KS, Kaposi's sarcoma; NHL, non-Hodgkin's lymphoma; Bact, bacterial infections

Similarly, bacterial infections in the same way as tuberculosis showed a marked rise in the last decades and represent an important cause of mortality in AIDS patients. Bacterial pneumonias were identified in 21-36% of cases from low- and middle-income countries (Ansari et al., 2002; Garcia-Jardon et al., 2010; Lanjewar, 2011; Soeiro et al., 2008). Early American sets often not mentioned pneumonias apart from pyogenic infections like sepsis, because they were included in the list of AIDS criteria subsequently. HIV patients are prone to nosocomial pneumonias caused by bacterial associations which demonstrate relapsing course with complications such as abscess formation and pleural effusion (Parkhomenko et al., 2003).

The prevalence of *Cytomegalovirus* infection ranges from 13-19% in African, Indian and Brasilian series to 46-69% cases from USA and Europe (Jellinger et al., 2000; Klatt et al., 1994; Lyon et al., 1996; Masliah et al., 2000; Pillay et al. 1993; Walewska-Zielecka et al., 1996). The highest rates of 74-76% were described in Japan and Australia (Dore et al., 1995; Ohtomo et al., 2000). Among invasive fungal infections pneumocystosis exhibits most significant decline due to effective prophylaxis and introduction of highly active anti-retroviral therapy (HAART). Early reports described high prevalence of pneumocystic pneumonias in more than half of all patients (Klatt et al., 1994), however recent studies could reveal *Pneumocystis jirovecii* pneumonia in less than 10% cases (Parkhomenko et al., 2003). Low levels of pneumocystosis are also specific for African countries regardless time period (Garcia-Jardon et al., 2010; Lucas et al., 1993; Nelson et al., 1993). A large retrospective study of an Italian cohort showed that the prevalence of opportunistic mycoses decreased over time, owing mainly to a significant decrease in pneumocystosis and cryptococcosis, whereas the prevalence of aspergillosis and histoplasmosis remained relatively stable while that of candidiasis tended to increase in the last years (Antinori et al., 2009). Rates of toxoplasmosis showed no significant variation for decades and comprise 1-10% of cases (Cury et al., 2003; Guerra et al., 2001; Sehonanda et al., 1996). The levels of HIV-related neoplasms seem to be decreasing over time, which was demonstrated for both non-Hodgkin's lymphomas and Kaposi's sarcomas in reviewed series (Jones et al., 1999; Launay & Guillevin, 2003).

The main patterns of organ/system involvement in AIDS are pulmonary, generalized or system isolated (CNS, digestive system). In most autopsy series of HIV-infected patients, pulmonary pattern was the most common with an incidence of 60–88%, followed by the CNS (60–80%), and the gastrointestinal tract (Concepcion et al., 1996; Cox et al., 2010; Hofman et al., 1999; Jellinger et al., 2000; Mohar et al., 1992; Sehonanda et al., 1996). Opportunistic monoinfection was observed on the highest level only in 40% cases, while most postmortem studies detected several microbial agents (Rana et al., 2000; Soeiro et al., 2008).

Cases with advanced CNS alterations have a high frequency of opportunistic infections and neoplasms (Masliah et al., 2000). A generalized pattern may be caused by virtually all opportunistic agents, but the most disseminating microorganism today is *Mycobacterium tuberculosis* (Parkhomenko et al., 2003).

Not all deaths of AIDS patients are related to HIV. The percentage of deaths from AIDS-related diseases has decreased, especially in those countries where highly active antiretroviral therapy is widely available. Non-AIDS causes in high-income countries account for at least a third of deaths, and include non-natural causes such as drug overdose, suicide, and violence along with various somatic diseases (Kohli et al., 2005; Krentz et al., 2005). Important non-HIV-related complications contributing to mortality of AIDS patients