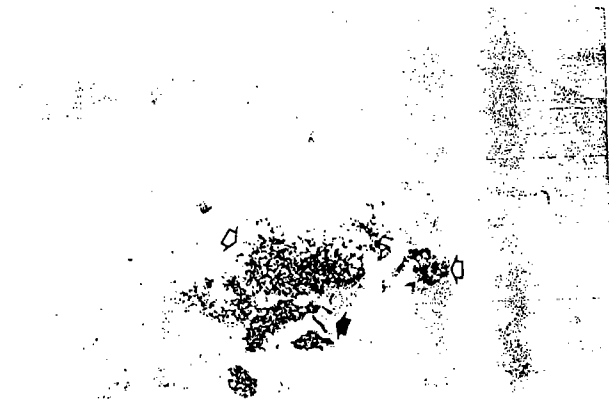


Fig. 1. Polymicrobial mandibular osteomyelitis. Bacterial colonies (empty arrow) and fungal hyphae (black arrow) within the intertrabecular space in intimate relationship with foci of necrosis, inflammation, and osteoclastic resorption.



described in relation with its long-term prophylactic use in HIV-infected patients [10].

In summary, we have documented a case of polymicrobial osteomyelitis caused by *Streptococcus salivarius* and *Candida albicans* that occurred via a mechanism of contiguity in a severely immunocompromised AIDS patient with neutropenia, who showed an excellent response to a combination of surgery and oral fluconazole and amoxicillin.

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Evaluation of an Indirect Immunofluorescence Assay and Two Cell Lines in the Detection of Influenza B Virus in Nasopharyngeal Samples

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Since Bartholoma and Forbes [1] described the greater efficacy of the shell-vial culture (SVC) compared with the tube culture in the isolation of the influenza B virus, the SVC seems to be the method recommended for rapid and specific diagnosis of influenza B virus infection. Of the different existing cell lines, the Madin-Darby canine kidney (MDCK) continuous cell line has shown the greatest efficiency in the isolation of the influenza B virus [2-4].

The routine use of a rapid system to detect influenza B virus antigen does not seem to be recommendable due

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to the low prevalence of this type of infection. However, when an epidemic outbreak of this infection is suspected, certain diagnostic benefits may be obtained from the use of rapid techniques. The indirect immunofluorescence assay (IIF) is one of the rapid techniques commercially available, but it has been found to have poor sensitivity [5, 6].

To assess the diagnostic accuracy of a commercial IIF assay for rapid diagnosis of lower respiratory tract infections caused by the influenza B virus, we compared it with the isolation of the virus in the MDCK and LLC-MK2 cell lines by the SVC method.

Between December 1996 and March 1997, 312 respiratory samples from patients with lower respiratory tract infections caused by influenza B virus were studied. Only those samples negative for respiratory syncytial virus in a rapid detection antigen assay (Directigen RSV; Becton & Dickinson, USA) were included in this study. The respiratory samples were obtained by nasopharyngeal aspiration. For the rapid detection of influenza B virus antigens, an IIF assay with a commercial anti-influenza B virus monoclonal antibody (clone 1B-82) (Monofluokit Influenza B; Diagnostics Pasteur, France) was used [7]. All samples that contained at least two epithelial cells with the presence of fluorescent cytoplasmic inclusions were considered positive. Samples were considered adequate for microscopic examination (IIF assay) if they contained more than 25 epithelial cells per slide.

At the same time, 200 µl of each sample was inoculated into one vial of the MDCK cell line and one vial of the LLC-MK2 cell line (Vircell, Spain). The vials were centrifuged at 700 × g for 45 min and, after an absorption period of 30 min at 36 °C, the maintenance medium was changed to minimum essential medium containing 2 µg/ml of trypsin. After incubation for 2 days at 36 °C, the vials were fixed with acetone at -20 °C for 10 min. The vials were stained with the same monoclonal antibody used for the direct examination of the samples. Vials showing evidence of cytopathic effects with specific fluorescence were considered positive.

Statistical analysis was carried out by performing the Student's *t* test on paired data. All *P* values are two-tailed and considered significant if <0.05.

Of the 312 nasopharyngeal aspirates analyzed, 53 (16.9%) contained less than 25 epithelial cells per slide and were considered inadequate for direct microscopic examination. Influenza B virus was isolated from 38 (12.1%) samples using the SVC, while the IIF assay detected only 20 (6.4%) of all 312 samples and 20 (52.6%) of the 38 positive samples (*P*=0.0001). Of the 38 SVC-positive samples, 30 (79%) were considered adequate and 8 (21%) inadequate for the IIF assay. If the inadequate samples are excluded, the sensitivity of

the IIF assay compared with the SVC increases to 66.6% (20/30) (*P*=0.001). When compared with the IIF assay, the SVC displayed a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 93.8%. No sample that was positive in the IIF assay was negative in the SVC.

Comparing the two cell lines, we found that the MDCK line permitted the isolation of all 38 influenza B viruses detected in this study, whereas the LLC-MK2 cell line permitted the isolation of only 31 (81.5%) isolates (*P*=0.001). In seven (18.5%) patients the influenza B virus was isolated only in the MDCK cell line.

In our study only 16.9% of the nasopharyngeal aspirates studied were considered adequate. This low percentage is due to the cytospin system used for the preparation of samples. As described previously in herpes infections, cyto centrifugation of the samples brings about a significant increase in the number of samples considered adequate [8].

As reported in the study of Rabalais et al. [6], our commercial IIF assay displayed a low sensitivity (52.6%) in the detection of influenza B virus antigens. In general, the sensitivity of the IIF assays depends on the quality of the sample and the monoclonal antibody used in the staining process [2, 3, 6]. In our study, when samples considered inadequate were eliminated, the IIF assay had a sensitivity of 66.6% compared with the SVC (100%), significantly inferior to that of the cell culture (*P*=0.001).

In the study of Frank et al. [3], the MDCK cell line was found to have a sensitivity of 73%, compared with the 62% of the LLC-MK2 cell line. Later studies have confirmed these results and provided evidence that the SVC system significantly increases the isolation capacity of the MDCK cell line [1, 5]. Our results show that the MDCK cell line is more sensitive than the LLC-MK2 line when using the SVC. The MDCK line enabled us to isolate all 38 influenza B viruses (100%), while only 31 viruses (81.5%) were isolated using the LLC-MK2 (*P*=0.001). These results are better than those reported recently by Brumback and Wade [9], who report a sensitivity of 79% for the MDCK line and 25% for the LLC-MK2 line. However, these authors did not use the SVC method, which might explain, in part, the lower sensitivity.

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Porphyria Cutanea Tarda and Hepatitis G and C Virus Infection

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Hepatitis G virus (HGV), a newly discovered human flavivirus distantly related to the hepatitis C virus (HCV), may be responsible for acute or chronic hepatitis and is thought to be involved in the pathogenesis of extrahepatic disease [1]. However, its clinical role still has to be determined, especially in view of the fact that the liver may not be the site of primary replication of HGV, at least not in HCV/HGV coinfecting patients [2]. Little information is available on its natural history or routes of infection [3]. The virus can be transmitted parenterally but also by other means [4]. In a Japanese study, HGV-RNA was found to be present in 3.1% of patients on haemodialysis and in about 0.9% of healthy blood donors [4]. The highest prevalence has been reported in liver transplant recipients (64%) [5] and in

patients with chronic δ - ν -related liver disease (40%) [6]. Although HGV-RNA is frequently associated with HCV, patients with mixed HCV/HGV infection do not have a worse disease outcome than those infected with HCV only [6].

Recently, a strong association between porphyria cutanea tarda (PCT), a metabolic disorder characterized by an abnormal porphyrin metabolism with typical cutaneous lesion, and HCV has been proposed [7]. In view of the high frequency of HGV in patients with HCV infection, we attempted to determine whether HGV or HGV/HCV coinfection could be a major triggering factor for the onset of PCT.

This study was conducted on sixty consecutively-observed patients. Thirty-one patients (mean age 54 years; 26 men and 5 women) affected by PCT, which was suspected on the basis of cutaneous lesions and confirmed by a high level of urinary porphyrins, were studied [8]. None of these patients had been exposed to known hepatotoxin compounds or drugs assumed to be potentially responsible for PCT. Twenty-nine patients (mean age 58 years; 23 men and 6 women) with chronic HCV infection served as the control group. HCV disease was diagnosed on the basis of alanine aminotransferase levels more than twice the normal limit, detection of antibodies to HCV in a third-generation enzyme immunoassay (Abbott), and liver biopsy findings compatible with chronic viral hepatitis. Results in all samples positive for HCV antibodies were confirmed in a third-generation RIBA-3 test (Ortho). All patients enrolled in the study were negative for hepatitis B virus infection.

Twenty-three of the 31 PCT patients with HCV antibodies were positive for HCV-RNA in a nested polymerase chain reaction (PCR) using primers in the highly conserved 5' non-coding region of the HCV genome [3]. Only one of these patients was positive for HGV-RNA in a nested PCR using primers from the 5' non-coding region of HGV [3]. Twenty-seven of the 29 control patients were HCV-RNA positive, and in six of these patients HGV-RNA was detected (Table 1). In the control group, the lower prevalence of HGV infection than in the PCT patients could be due to the route of infection, although most patients were not intravenous drug abusers. However, HGV-RNA was always found in the presence of HCV-RNA.

Shortly after the discovery of HCV, it was realised that this agent may also be the cause of extrahepatic disorders [7]. HCV infection may also affect non-hepatic tissues, and be associated with many unrelated diseases and with a plethora of immune and autoimmune disturbances [9]. In a study carried out in Italy, a high frequency of infection with HCV (76%) was observed in patients with PCT [8]. However, in patients infected by HCV, regardless of coinfection with the human

Table 1. Prevalence of hepatitis C and G virus in patients with porphyria cutanea tarda (PCT) and patients with chronic viral hepatitis

	No. of patients	No. (%) HCV-RNA positive	No. (%) HGV-RNA positive
PCT	31	23 (76)	1 (3)
Chronic viral hepatitis	29	27 (93)	6 (21)

immunodeficiency virus (HIV), it was not possible to demonstrate a direct effect of the virus on porphyrin metabolism because the clinical changes of PCT were not related to the virological findings, suggesting an indirect role of HCV or HIV infection [10]. This indicates that other viruses may also be implicated in the onset of PCT. Nevertheless, our results confirmed that HCV is closely associated with PCT and that HGV or HGV/HCV coinfection does not seem to be a risk factor, or co-factor, for the onset of this metabolic disorder. Similar data have been recently reported in patients with mixed cryoglobulinemia [11], another extrahepatic disorder related to HCV [7].

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