Growth of Adenocarcinoma on Routine Microbiological Media Inoculated with Fluid from a Pleural Effusion in an 82-Year-Old Female

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Received 3 May 2008/Returned for modification 17 June 2008/Accepted 7 January 2009

We report the first documented case of adenocarcinoma cell growth on routine microbiological media. Pleural fluid culture from an 82-year-old female showed colonies with fried egg appearance on routine microbiological media that were negative for bacterial microorganisms. Stains of the colonies demonstrated clusters of viable neoplastic cells.

CASE REPORT

The patient was an 82-year-old female who presented to the Emergency Department with lightheadedness and urinary incontinence. She denied loss of consciousness, fever, chills, nausea, vomiting, abdominal pain, or recent falls. She had a past medical history of osteoporosis and a left arm fracture. On admission she had a temperature of 98.2°F, a pulse of 87, blood pressure 156/86, respiratory rate of 18, and an oxygen saturation of 97% on room air. Auscultation of the lung fields demonstrated decreased breath sounds over the right lung base. The remainder of the physical examination was normal. A chest roentgenogram was remarkable for a right lower lobe infiltrate and possibly a left lower lobe infiltrate. Initial laboratory studies showed a complete blood count as follows (parentheses indicate normal values): white blood cell count of 7,600 cells per microliter (4,000 to 10,000 cells per microliter), 69% neutrophils (38 to 81%), 22% lymphocytes (14 to 46%), 7% monocytes (2 to 15%), 1% eosinophils (0 to 5%), and a hematocrit of 24.4% (37 to 47%).

The patient received intravenous hydration and 2 units of packed red cells, and a repeat chest roentgenogram demonstrated a large right pleural effusion. Thoracentesis was performed, with samples sent for cytologic analysis and routine culture. In the clinical microbiology laboratory, Cytospin preparations and a Gram stain were made of 200 microliters of concentrated specimen and incubated at 37°C in a 5% CO2 atmosphere. The concentrated pleural fluid was also inoculated onto 5% sheep’s blood agar, chocolate agar, and colistin-nalidixic acid blood agar and incubated at 37°C in a 5% CO2 atmosphere. MacConkey agar was inoculated with 10 microliters of concentrated pleural fluid and incubated at 37°C in ambient air. Anaerobic blood agar (CDC formulation) plates were inoculated with 10 microliters of concentrated specimen and incubated at 37°C (Remel Inc., Lenexa, KS). Plates were examined daily for growth. Colonies detected on blood agar were subcultured on 5% sheep blood agar and ureaplasma/mycoplasma agar (Remel Inc., Lenexa, KS), incubated at 37°C in a 5% CO2 environment, and examined daily for growth. Turbidity and microcolonies were observed in the thioglycolate broth culture, and 400 microliters was subsequently centrifuged at 14,000 rpm. Multiple colonies were swabbed from the primary aerobic blood agar plate, added to 200 microliters of fresh thioglycolate broth, and centrifuged at 14,000 rpm. Both pellets were smeared onto glass slides and stained with acridine orange stain and Gram stain and examined under a light microscope.

Pleural fluid sent to hematology for cell counts demonstrated 12,500 red cells per microliter and 1,670 nucleated cells per microliter, with 2% granulocytes, 73% lymphocytes, and 25% tissue cells or other. The Cytospin preparation Gram stain of the original pleural specimen was negative for microorganisms. Culture of the pleural fluid showed small colonies with fried egg appearance on sheep’s blood agar, chocolate agar, and anaerobic blood agar after 2 days of incubation (Fig. 1A). The thioglycolate broth and blood agar plate were negative for organisms by both Gram and acridine orange stains. Subculture of the isolated colonies on ureaplasma/mycoplasma agar did not show growth. Subculture of the isolated colonies on blood agar showed growth after 2 days of incubation. Subculture on other media was not performed.

Because of the negative microbial stain results from the growing colonies on the agar plates and thioglycolate broth, bacterial 16S rRNA gene sequencing of the first 500 bp was performed as described previously (8, 9, 13, 14). This technique has been successfully used in the clinical microbiology laboratory to identify Mycoplasma spp. (1, 5, 8, 11, 16). Four hundred microliters of thioglycolate broth was centrifuged at 14,000 rpm.
rpm, and the pellet was used in the PCR analysis. The 500-bp PCR product was present in the positive control (*Leuconostoc* sp.) and absent in the negative control. No PCR product was present in the thioglycolate broth (undiluted, 1:10 dilution, and 1:100 dilution) or the agar colonies (undiluted broth suspension), demonstrating that the colonies were not bacteria or *Ureaplasma/Mycoplasma* spp. and that PCR inhibition was unlikely.
Because of the presence of colonies, negative Gram stain reaction, negative acridine orange stain, and negative PCR results, hematoxylin and eosin, Kinyoun acid-fast, Wright’s, and Papanicolaou stains were performed and examined by light microscopy to assess the presence of human cells in the colonies. Material from the thioglycolate broth (Fig. 1B and C, Wright’s and Papanicolaou stains, respectively) and blood agar plate colonies (Fig. 1D and E, Wright’s and Papanicolaou stains, respectively) showed clusters of viable, large pleomorphic neoplastic cells with large nucleoli diagnostic of adenocarcinoma. In the cytopathology laboratory, adenocarcinoma was also definitively identified independent of the microbiology findings from a separate pleural fluid sample. Examination of both the microbiology samples and the cytology samples together by two pathologists was definitive for the diagnosis.

Diagnostic criteria for adenocarcinoma include several light microscopic, morphological features, all of which were present in our patient’s specimens (Fig. 1B to 1E) (4). Adenocarcinoma neoplastic cells classically are large and pleomorphic with vacuolated cytoplasm. The patient’s cancer cells were an average of 21 μm in size (normal red blood cells are 7 μm, and neutrophils are 12 μm). They have large pleomorphic nuclei (rather than regular, smaller circular nuclei) with an abnormally high nuclear to cytoplasmic ratio and large central (“owl’s eye”) nucleoli (not found in normal cells), and they are disorderly arranged in clusters or “balls.” No other cell types (specifically microorganisms) were present from colony Gram stains or other stains. These neoplastic cells were also positive for cytokeratin 7 and WT-1 by immunoperoxidase staining. They were negative for estrogen receptor, progesterone receptor, and cytokeratin 20. These findings suggest that possible primary sites include, but are not limited to, pancreaticobiliary and gynecologic tumors. Lung, breast, and colon tumors are less likely.

Following the diagnosis, the patient received supportive care only. Her cancer progressed to metastatic stage IV disease with metastases in the liver, peritoneal cavity, and internal chest wall.

To our knowledge, this is the first reported case of neoplastic cells growing as colonies on routine microbiological media. Further studies are necessary to examine the potential growth of adenocarcinoma cells on routine clinical microbiology media.

Variations of soft agar have been utilized for neoplastic cell culture and motility assays (2, 10, 12, 15), and it is conceivable that neoplastic cells with a high rate of growth could produce colonies on various microbiological agar and broth media. These media are supplemented with peptone, peptides, amino acids, vitamins, and, in the case of chocolate agar and thioglycolate broth, glucose, which would provide the necessary metabolites for cell growth (insert sheets for blood agar [tryptic soy agar with 5% sheep blood], chocolate agar, and thioglycolate broth with additives; technical manual of microbiological media package insert, Remel, Inc. Lenexa, KS, 2007). A previous experiment described in the literature by F. Gerlach in 1937 details the apparent isolation of “virus bodies” from tumors by growing the tumor cell filtrate on blood agar, with the demonstration of colonies (3, 6, 7, 17). These may have been the actual tumor cells growing in cell culture, as we have described in this article.

Because of the “fried egg” appearance of the colonies and their inability to stain by Gram stain, they were originally thought to be Mycoplasma spp. Mycoplasma spp. are occasionally cultivatable on routine media and have a trilaminar cell membrane without cell wall peptidoglycan; thus, they are negative by typical Gram stain (18). Mycoplasma hominis colonies are small, pinpoint, and translucent and have a fried egg appearance. Mycoplasma hominis is usually sensitive to clindamycin and tetracyclines. From a microbiological standpoint, making the distinction between neoplasia and Mycoplasma infection in our patient was important in that no antibiotic therapy was initiated, thus preventing potential resistance and unnecessary complications such as the antibiotic-associated Closstridium difficile colitis that is often seen with clindamycin treatment.

There is no risk to laboratory personnel when isolating cancer cells on primary media, but this case demonstrates an unusual colony morphology and identification problem that poses an interesting, as yet unforeseen diagnostic challenge to the clinical microbiologist.

REFERENCES
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