Use of Polymerase Chain Reaction to Diagnose Tubercular Arthritis From Joint Tissues and Synovial Fluid

To the Editor.—Titov et al\(^1\) used Mycobacterium tuberculosis MPB64 gene-specific primers to amplify \(M\) tuberculosis genetic sequences in joint and bone tissues from their 14 patients with bone and joint disorders. Conventional polymerase chain reaction (PCR) on the arthroscopically obtained tissue biopsy tissues was diagnostic and resulted in only 2 false positives among patients who had suffered from tuberculosis in the past. Nevertheless, to achieve maximum PCR diagnostic utility for clinicians, it would be imperative to carry out extended investigations among those who had been offered bacille Calmette-Guérin (BCG) vaccine either as a prophylactic or a therapeutic agent. A significant proportion of false positives would vitiate the excellent data obtained by Titov et al.\(^1\)

An in situ rather than conventional PCR on arthroscopically obtained bone or joint tissues would be intriguing and more informative. That was illustrated with the fixed lung cancer tissues at the First Military Medical University, Guangzhou, China. A sensitive and specific indirect in situ nested PCR was used to identify and localize tubercular DNA in 15 formalin-fixed, paraffin-embedded lung cancer tissue specimens, which had been demonstrated to be positive for \(M\) tuberculosis DNA by the conventional PCR. Positive, brown granules of \(M\) tuberculosis were found mainly in the cytoplasm of the alveolar epithelial cells, pulmonary macrophages, inflammatory cells, and a few tumor cells within lung cancer tissues.\(^2\)

Funds should be allocated to standardize an in situ PCR format for \(M\) tuberculosis on different surgically excised or aspirated tissues. Last but not least, the magnitude of any false positives attributable to BCG or other environmental mycobacterium species should be evaluated in different geographic areas. Mycobacterium ulcerans causing Buruli ulcer is an environmental mycobacterium responsible for an infectious necrotizing panniculitis.\(^3\) This disabling disease is strongly linked to the aquatic ecosystem. Occurring mainly in children, it is an emergent public health threat in many humid rural tropical areas. Certainly, it would be important to assess any \(M\) tuberculosis PCR false positives on arthroscopically obtained bone and joint tissues.\(^1\)

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In Reply.—Bacillus Calmette-Guérin (BCG) is a live, attenuated Mycobacterium tuberculosis strain, which remains latent in vaccinated individuals. It may later become activated and is a known cause of, for example, osteomyelitis in children.\(^1\) Presence of this strain in damaged tissue often means that it has been disseminated and has become a causative agent of the disease.\(^2\) The Department of Paediatric Bone and Joint Tuberculosis of the St Petersburg Institute of Physio-pulmonology specializes in the treatment of tuberculosis caused by Mycobacterium bovis BCG. All polymerase chain reaction (PCR)-positive specimens from children are also tested with BCG-specific primers\(^3\) to verify the diagnosis. Results have shown that about 16% of tuberculotic osteomyelitis is caused by the BCG strain. False-positive PCR of biopsy specimens from children with non-tuberculotic diseases was observed in no more than 1% of cases. Therefore, it can be concluded that \(M\) bovis BCG may be a cause of clinically evident tuberculosis.

In principle, \(M\) bovis BCG might lead to false-positive reactions in PCR-based diagnosis of tuberculosis. Primers used for PCR diagnosis of \(M\) tuberculosis are often designed so that they only recognize the wild-type \(M\) tuberculosis, but do not recognize the attenuated strain used in BCG vaccination. In practical work, conventional PCR primers have been found to be good and relatively specific for the diagnosis of tuberculosis. Such primers are used, for example, in the well-known PCR test Amplicor MTB (Hoffman La Roche, Basel, Switzerland), in which the primers recognize all species of the \(M\) tuberculosis complex, including \(M\) bovis BCG. The sensitivity and specificity of the Amplitude kit (Litech, Moscow, Russia) used for PCR diagnosis of \(M\) tuberculosis in our work are close to those of Amplicor MTB. This kit has been licensed for commercial use in Russia. In accordance with the rules of the Russian Ministry of Health, before a license is granted for any diagnostic test, several independent experts must validate the test using hundreds of known positive and negative samples. The proportion of false positives among patients with nontuberculotic diseases in these tests did not exceed 0.5%.\(^4\) As almost the whole population of Russia has been vaccinated with BCG, these test results clearly validate the use of this test.
Letters to the Editor

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We agree with Dr Grant that legal issues regarding the acceptance of this methodology for routine proficiency testing in gynecologic cytopathology and reluctance from pathologists and cytopathologists to adopt newer computer-based technologies probably contributed to the ‘management and development’ problems briefly discussed in the letter. However, the issue is more complex, as there have been technological and economic challenges that needed to be overcome for virtual microscopy to become widely used for routine proficiency testing. I am not familiar with the technology used in Dr Grant’s work, but to my knowledge, ‘virtual slides’ that include the 2-dimensional images from an entire Papanicolaou test are usually digitized at a single focal plane with the commercial systems that are currently available, which are already considerably more advanced than the methods used in our study. Although the technology is available for the preparation of 3-dimensional virtual slides that allow for changes in focal planes (the so-called z-plane), this requires the use of very large files that are difficult to process and store with current personal computers. It is possible to construct reproducible proficiency tests with 2-dimensional images, but the method does not exactly match the daily practice of cytopathology. Indeed, although virtual images are usually of good quality, the inability to focus up and down on an image introduces an additional element of guessing into the diagnostic process. In addition, it has been our experience and probably that of other colleagues that it takes longer to screen a Papanicolaou test with a computer-based system than with a light microscope.

Additional developmental work is needed to overcome these technical challenges, raising the practical question about the availability of financial incentives to improve the technology. To my knowledge, there has been no large pot of gold waiting for the development of proficiency testing systems for cytopathology. The College of American Pathologists already has very successful proficiency testing educational programs for gynecologic and nongynecologic cytopathology using real glass slides. There have been, to my knowledge, limited

Virtual Microscopy as a Tool for Proficiency Testing in Cytopathology

To the Editor.—I am writing in response to the following statement made by Marchevsky et al in the article “Virtual Microscopy as a Tool for Proficiency Testing in Cytopathology”1: “However, to our knowledge there have been few attempts to design and validate a practical proficiency testing program for gynecologic pathology using virtual microscopy techniques.”

In 1996, a PhD dissertation was copyrighted and published under the title Development and Testing of an Alternative Proficiency Test for Cytopathologists Using Computer Technology.2 The study published in the ARCHIVES is that dissertation in miniature. The 1996 study was a validated and statistically significant study. The abstract for this study appeared in the September/October 1995 issue of Acta Cytologica.3 The author won the Cytopathologist Scientific Presentation Award at the American Society of Cytopathology annual meeting (1995) in New York and testified for the American Society of Clinical Pathologists at the Clinical Laboratory Improvement Advisory Committee meeting held at the Centers for Disease Control and Prevention in March 1996, regarding the future of computerized proficiency testing. Interestingly, the College of American Pathologists contacted the author to request a copy of the dissertation. The study addressed locator and interpretive skills, as the digitized images could be “screened” and “focused,” simulating the microscopic experience. The study demonstrated that the computer-based test was statistically better than the current glass slide examination.

Why didn’t computerized testing “catch on”? Partially because the law was not changed to incorporate the computer medium, but also because it was new. Management and development became the issues of the day; almost 10 years later they do not seem to be resolved.

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In Reply.—We thank Dr Grant for her interest in our article, and we are delighted to learn that it was the subject of a PhD dissertation in 1996. Her letter raises interesting questions regarding why computer-based proficiency testing did not “catch on.”


Financial Conflict of Interest?

To the Editor.—I am writing to express my concern over the article authored by Kroll, Styer, and Vasquez in the May 2004 issue. The conclusion was that “there was strong evidence linking performance on PT [proficiency testing] surveys with performance on LN [linearity] surveys.” This statement carries a clear implication that LN surveys with performance on PT surveys from the College of American Pathologists (CAP) and therefore the article carries marketing connotations for the CAP (and therefore the incomes of its employees), the absence of a proper financial disclosure statement needs correction. Because the article carries marketing connotations for the CAP (and therefore the incomes of its employees), the absence of a proper financial disclosure is not appropriate.

To the Editor.—The concentrations of therapeutic immunosuppressants in blood are essential for the management of patients before, during, and after organ transplantation. Most laboratories use immunossay methods that can be practically and economically applied to TDM, rapid specimen preparation for LCMSMS measurements, and an awareness of their overlapping responsibilities. We learned about the myriad complexities of patient care and that MS methods, being “home brews,” are not standardized. They differ in important ways about the myriad complexities of patient care and that MS methods, being “home brews,” are not standardized. They differ in important ways about the myriad complexities of patient care and that MS methods, being “home brews,” are not standardized. They differ in important ways about the myriad complexities of patient care and that MS methods, being “home brews,” are not standardized. 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noted in a recent College of Ameri-
can Pathologists proficiency testing
survey for immunosuppressive
drugs: CVs (15%–25%) of MS meth-
ods are poorer than CVs (<12%) of
immunoassay methods performed
under controlled conditions with re-
agents and calibrators provided by
the manufacturers of these kits. The
time has come for system manufac-
turers and users to undertake a col-
laborative effort to standardize the
otherwise superior MS technique for
immunosuppressants.

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1. College of American Pathologists. Proficiency
   Testing Survey Summary CSM-A. Northfield, Ill: Col-
   RJ. Lack of specificity of cyclosporine immunoas-
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