Correspondence

Central nervous system involvement by Mycoplasma pneumoniae

I read the article by Fink et al with interest. I am surprised by their study population and report, which do not, except for a single case of Guillain-Barré syndrome, include patients with encephalitis, which is considered to be the main type of the central nervous system (CNS) involvement by Mycoplasma pneumoniae in children. However, despite the fact that their subjects were mainly children. When discussing CNS involvement by M. pneumoniae, particularly in children, one cannot ignore cases with encephalitis, since the clinical and pathological features of these patients are examined as well.

Recently, my research group studied CNS involvement by M. pneumoniae using the polymerase chain reaction (PCR), and found that patients with encephalitis, in whom onset of neurological symptoms occurred within seven days of the onset of fever, exhibited a significantly higher incidence of neurological DNA in cerebrospinal fluid (CSF) than patients with later onset of fever.1 Fink et al stated that six of seven patients with confirmed M. pneumoniae infection reported a febrile illness or upper respiratory tract infection six to 14 days before the onset of neurological symptoms. Our data suggest that the onset of neurological symptoms in these patients mycoplasma DNA may not be detectable in the CSF. We are of the opinion that the presence of mycoplasma DNA in CSF is not evidence of a direct, invasive mechanism. Nevertheless, the clinical characteristics of illnesses involving the CNS or other factors, such as the interval between the onset of mycoplasma infection and the onset of neurological symptoms, should be taken into account before a conclusion is reached whether or not a direct invasive mechanism plays a role in CNS involvement by M. pneumoniae.

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Dr Fink and Sills comment:
We are pleased to receive Dr Narita’s interest in our paper. We have addressed the points that he has raised in chronological order for clarity.

Firstly, we agree that encephalitis is reported as the main CNS manifestation of M. pneumoniae infection, but some reports show meningoencephalitis to be associated in younger age groups. Although we have reported a role for PCR in a number of cases, we believe that most of the patients with clinical presentations suspected of being M. pneumoniae infection in the hospitals surveyed would have been referred to us. We suspect that the patients we reported are a true reflection of the clinical presentation but numbers are too small for any definitive comment. All patients with encephalitis during the study period were referred to our laboratory.

Secondly, in Dr Narita’s report of method of PCR for mycoplasma2 we understand that the system does not differentiate M. genitalium from M. pneumoniae. We believe that M. genitalium is a more toxic organism than M. pneumoniae. It may be that the two species have not been sufficiently studied in previous studies, so linking a larger number of encephalitis presentations with mycoplasma infection.

Thirdly, clinical onset of M. pneumoniae infection is very difficult to recognise because of its insidious nature. In our series and in Dr Narita’s series all of the patients had antibodies and this suggests that the patients had been infected more than 41 days.

Fourthly, our simplified PCR method was developed to facilitate earlier clinical diagnosis and provide further opportunity for studying the natural history of the disease. In Dr Narita’s paper, primary diagnosis seems to have been made on serology, using complement fixation (CF) and gel particle agglutination (GPA). In contrast, our series noted the very rapid disappearance of antibodies revealed by GPA.

We are concerned that this could be a “false” initial result; Kleemola and Kayhty2 demonstrated elevated titres and an increase in CF antibodies to M. pneumoniae in patients with proven bacterial meningitis.

Fifthly, we believe that M. pneumoniae is not usually a significant cellular agent. T cell energy is recognised in M. pneumoniae infections, thereby providing an opportunity for the organism to escape from the respiratory tract. This may be important for the development of neurological lesions.

Finally, there is still insufficient evidence to be sure of the mechanism for neurological lesions in M. pneumoniae infection. There is evidence in the literature both for an immune mediated mechanism and anecdotal reports of the recovery of organisms from CSF.

The relevance of neurological disease is conflicting. There is some evidence of a rapid reversal of neurological lesions in M. pneumoniae infection with the application of antibiotic therapy. In contrast, some neurological lesions are reported to resolve rapidly to plasmapheresis.

We are concerned that in any study using PCR for M. pneumoniae diagnosis, the serological criteria and the PCR evidence are both sensitive and specific and very strictly interpreted. It is also critically important to ensure that the PCR system is specific for M. pneumoniae.


Quantitative analysis of silver stained nucleolar organizer regions as a reliable marker of cell proliferation and a promising prognostic parameter in tumour pathology

I read with interest the review article by Barnes and Gilliet. The authors discuss several methods to measure cell proliferation, and indicate some requirements for their application in routine pathology. Concerning the silver stained nucleolar organizer region (AgNOR) method, the authors state that “NORs are difficult to identify, time-consuming to count and do not have a consistent proven correlation with other measures of proliferative activity or prognosis.” I strongly disagree with this view.

NORs are defined nucleolar components (corresponding, at the electron microscopic level, to the fibrillar centres and to the round dense fibrillar components) which can be visualised selectively at the light microscopic level by applying the one-step silver staining method originated by Adnet et al.2 Under these staining conditions, NORs can be identified easily as black dots of different sizes, localised throughout the nucleolar area. I have never had any difficulty recognising these structures in cytological or histological samples after appropriate silver staining.

Two methods can be used to quantify AgNORs: the counting method—the examination of each silver stained dot per cell—and the morphometric method—the measurement of the area occupied by silver stained nucleolar organizer regions (AgNORs) obtained using image analysis. While counting AgNORs is time-consuming and subjective, image analysis permits a rapid objective and reproducible quantification of AgNORs, as shown in a recent study in which the two methods were compared in the same series of breast carcinomas.4

The correlation between AgNOR numbers and cell proliferation has been widely accepted in tumours as well as in normal epithelial cells.5–10 AgNOR numbers have also been related positively to the number of mitotic figures and the number of cells in cycle, defined by Ki67 immunostaining, has been demonstrated in non-Hodgkin’s lymphoma, breast carcinoma, gastrointestinal carcinomas, and soft tissue sarcomas, and in a group of tumours of different origin.11 AgNOR numbers have also been related positively to the number of cells detected by both DNA flow cytometry (in non-Hodgkin’s lymphoma, breast and gastric carcinomas) and bromodeoxyuridine incorporation (in meningiomas, transitional and gastric carcinomas and in a group of tumours of different origin). Moreover, in a series of experiments carried out on human cultured cancer cell lines a highly significant correlation between AgNOR numbers and the speed of cell replication has been found.12

Over the past few years, many retrospective studies have been performed assessing the predictive value of counting AgNORs in tumour pathology. In their article Barnes and Gilliet quote two investigations which failed to demonstrate a prognostic value in lung cancer, where the AgNOR counts in breast carcinomas, but do not mention any of the numerous studies obtained in other human tumours showing a significant predictive value13–15 for the AgNOR parameter in squamous carcinomas of the head and neck, stomach, broncus and liver, carcinomas of the breast or uterus and in meningiomas. In the large scale study by Adnet et al.16 over 1000 squamous carcinomas of the head and neck have demonstrated a highly significant correlation between AgNOR numbers and patient survival in a large scale analysis.

These data demonstrate that the AgNOR parameter actually reflects the proliferative activity of cancer cells and represents a promising prognostic indicator in tumour pathology.

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This book is a comprehensive and detailed guide to the principles and practices of gene expression analysis. It is divided into several sections, each focusing on a different aspect of gene expression, such as RNA isolation, RT-PCR, and Northern blotting. The book is written in a clear and concise manner, making it accessible to both beginners and experienced researchers. It includes numerous illustrations and experimental protocols, which are valuable for understanding and applying the concepts discussed. Overall, this book is an essential resource for anyone involved in gene expression studies, providing a solid foundation for further research.


This book provides a comprehensive guide to PCR in situ hybridization (PCR-ISH), a powerful technique for the detection of specific DNA sequences within cells. It covers the fundamentals of PCR-ISH, including primer design, probe synthesis, and signal detection methods. The book also includes detailed protocols for various applications, such as the detection of viral and bacterial pathogens. It is an important resource for researchers in the fields of molecular pathology and microbiology, offering a practical and up-to-date overview of the latest techniques and their applications.