Correspondence

Central nervous system involvement by Mycoplasma pneumoniae

I read the article by Fink et al with interest. I am surprised that their study population did 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, despite the fact that their subjects were mainly children. When discussing CNS involvement by M. pneumoniae, particularly in chronic forms, which are associated with encephalitic episodes examined as well. Recently, my research group conducted a study of children with 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 mycoplasmal DNA in cerebrospinal fluid (CSF) than patients with later onset of fever. 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 in children, CNS involvement by M. pneumoniae DNA may not be detectable in the CSF. We are of the opinion that the presence of mycoplasmal 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 fever and the appearance 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.

M. NARITA
Department of Pediatrics,
Hokkaido University School of Medicine,
N15 W7 Kitaku, Sapporo 060, Japan


Dr Fink and Sillis comment:
We appreciate 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 meningitis to be associated in the younger age groups. Although we have reported a rare case of meningitis, and 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 mycoplasma we understand that the system developed 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 previously 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 recognize 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 for at least 14 days. Forthfourly, 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 report, primary diagnosis seems to have been made on serology, using complement fixation (CF) and gel particle agglutination (GPA). In contrast, some serological tests report the very rapid disappearance of antibodies revealed by GPA. We are concerned that this could be a “false” initial result; Kleemola and Kayhty’ve 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 part of a persistent T cell energy is recognized 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 meningitis. There is evidence in the literature both for an immune mediated mechanism and anecdotal reports of the recovery of organisms from CSF.

The relationship between neurological disease and meningitis is uncertain. There is evidence of a rapid reversal of neurological lesions in M. pneumoniae infection with the application of aggressive antibiotic therapy. In contrast, some neurological lesions are reported to respond rapidly to plasmapheresis.

We are concerned that in any study using PCR for M. pneumoniae diagnosis, the serological and clinical criteria to prove the PCR evidence is 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 Gillett. The authors discuss several methodological issues, as well as present the current data and indicate some requirements for their application in routine pathology. Concerning the silver stained nucleolar organizer region (NOR) scoring, the authors conclude that “NORs are difficult to identify, time-consuming to count and do not have a consistently 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 centers, the rER, as well as the bounding dense fibrillar components) which can be visualised selectively at the light microscopic level by applying the one-step silver staining method originally described by Fink et al. Under these staining conditions, NORs can be identified easily as black dots of different sizes, localised throughout the nuclear 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 enumeration of each silver stained dot per cell—and the morphometric method—the measurement of the area occupied by silver stained nucleolar organizer regions as related using image cytometry. While counting AgNORs is time-consuming and subjective, image analysis permits a rapid objective and reproducible quantification of NORs, as shown in a recent study in which the two methods were compared in the same series of breast carcinomas.

The correlation between AgNOR numbers and cell proliferation is highly significant and occurs widely in tumours by comparing the AgNOR values with kinetic data obtained by applying other well-established proliferation markers. A significant correlation was found between AgNOR counts and the number of cells in cycle, defined by Ki67 immunostaining, has been demonstrated in non-Hodgkin’s lymphoma, breast carcinoma, malignant melanoma, gastric carcinomas, soft tissue sarcomas, and in a group of tumours of different origin. AgNOR numbers have also been related to prognosis in various malignancies. AgNOR counts were correlated by both DNA flow cytometry (in non-Hodgkin’s lymphoma, breast and gastric carcinomas) and bromodeoxyuridine incorporation (in meningioma and peripheral neuroepithelial carcinomas and in a group of tumours of different origin). Moreover, in a series of experiments carried out on human cultured cancer cells line a highly significant correlation between AgNOR numbers and the speed of cell replication has been found.

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 Gillett quote two investigations which failed to demonstrate a prognostic value for the AgNOR counts in breast carcinomas, but do not mention any of the numerous studies obtained in other human tumours showing a significant predictive value for the AgNOR parameter. In colorectal carcinomas, gastric carcinoma, multiple myeloma, pharyngeal carcinoma, acute lymphoblastic leukaemia, oesophageal carcinoma, and stage I endometrial adenocarcinoma, the AgNOR variable has been found to be related significantly to patient survival and in multivariate analysis was an independent prognostic factor.

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

D. TRES G
Centro di Patologia Celulare,
Dipartimento di Patologia Sperimentale,
Università degli Studi di Bologna, Italy

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