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 as they did not, except for a single case of Guillaume-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 children, encephalitic episodes 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 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 this is not always the case, as the patients mycoplasmal 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 the febrile illness and the 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 Sillis comment:
We are pleased to have 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 range of clinical presentations, we suspect 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 reported method of PCR for mycoplasmal infection 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 encephalitic presentations with mycoplasma infection. Thirdly, clinical onset of M pneumoniae infection is very difficult to diagnose 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 before the clinical onset.

Fourthly, our simplified PCR method was developed to facilitate earlier clinical diagnosis and provide further opportunity for studying the natural history of infection. 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, some serological tests demonstrated the very rapid disappearance of antibodies revealed by GPA. We are concerned that this could be a "false" initial result; Kleemola and Kayhty demonstrated elevated titers 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 primary pathogen, but a consistent T cell energy is recognised in M pneumoniae infections, thereby providing an opportunity for the organism to escape from the respiratory tract. This is crucial 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 relationship with neurological diseases are 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 respond to rapidly to plasmapheresis.

We are concerned that in any study using PCR for M pneumoniae diagnosis, the serological criteria for diagnosis may be supportive of the PCR evidence. This 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 to reveal 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.1 The authors discuss several methods for measuring cell proliferation and indicate some requirements for their application in routine pathology. Concerning the silver stained nucleolar organiser region (AgNORs) count, Barnes and Gillett conclude that "NORs are difficult to identify, time-consuming to count and do not have a consistently proven correlation with other measures of tumour activity or prognosis." I strongly disagree with this view.

AgNORs are defined nucleolar components (corresponding, at the electron microscopic level, to the fibrillar centers of the surrounding dense fibrillar components) which can be visualised selectively at the light microscopic level by applying the one-step silver staining method originally described by Derenzini et al.2 Under these staining conditions, NORs can be identified easily as black dots of different sizes, localised throughout the nuclear area.3 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 structures calculated using image analysis software.4 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.5

The correlation between AgNOR numbers and cell proliferation is well-established and is found widely in tumours by comparing the AgNOR values with kinetic data obtained by applying other well established proliferation markers. A significant correlation between AgNOR numbers and the percentage of cells in cycle, defined by Ki67 immunostaining, has been demonstrated in non-Hodgkin's lymphoma, breast carcinomas, gastric carcinomas, soft tissue sarcomas, and in a group of tumours of different origin.6 AgNOR numbers have also been related positively to tumour histological grade in gastric carcinomas, soft tissue sarcomas, and in a group of tumours of different origin.7 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.8

Over the past few years, many retrospective studies have been performed associating the predictors of survival to counting AgNORs in tumour pathology. In their article Barnes and Gillett quote two investigations which failed to demonstrate a prognostic value of 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 carcinoma the AgNOR parameter correlates with tumour size, node status, and degree of differentiation. Adenocarcinomas, the AgNOR variable has been found to be related significantly to patient survival and in multivariate analysis was an independent prognostic parameter.9

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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