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Correspondence

Central nervous system involvement by *Mycoplasma pneumoniae*

I read the article by Fink *et al*[1] 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,[2] despite the fact that their subjects were mainly children. When discussing CNS involvement by *M pneumoniae*, particularly in children, it is vital that patients with encephalitic episodes are examined as well.

Recently, my research group studied CNS involvement by *M pneumoniae* using the polymerase chain reaction (PCR),[3] 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 mycoplasma DNA in cerebrospinal fluid (CSF) than patients with later onset of fever.[4] 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 most, but by no means all, of their 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 fever and the onset of 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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Drs 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[1] show meningitis to be associated in the younger age groups. Although we have reported a relatively small 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 reported method of PCR for mycoplasma[2] we understand that the system described would 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 distinguished 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 for at least 14 days.

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 one patient in his series we note the very rapid disappearance of antibodies revealed by GPA. We are concerned that this could be a "false" initial result; Kleemola and Kayhty[3] 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 invasive, but a transient T cell anergy is recognised in *M pneumoniae* infections, thereby providing an opportunity for the organism to escape from the respiratory tract. This may be a mechanism 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 reports of *M pneumoniae* neurological disease are conflicting. There is some 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 plasmapharesis.

We are concerned that in any study using PCR for *M pneumoniae* diagnosis, the serological criteria for supporting 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*.

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Quantitative analysis of silver stained nucleolar organiser regions: 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 of assessing cell proliferation and indicate some requirements for their application in routine pathology. Concerning the silver stained nucleolar organiser region (AgNOR) method, they 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 centres and 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 Ploton *et al.*[2] Under these staining conditions, NORs can be identified easily as black dots of different sizes, localised throughout the nucleolar 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 cytometry. While counting AgNORs is time-consuming and subjective, image analysis permits a rapid objective and reproducible quantification AgNOR, 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 investigated 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, gliomas, brain tumours, gastric carcinomas, soft tissue sarcomas, and in a group of tumours of different origin.[3] AgNOR numbers have also been related positively to the percentage of S-phase cells evaluated by both DNA flow cytometry (in non-Hodgkin's lymphoma, and breast and gastric carcinomas) and bromodeoxyuridine incorporation (in meningiomas, hepatocellular rat carcinomas and in a group of tumours of different origin).[3] 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.[5]

Over the past few years, many retrospective studies have been performed to ascertain the predictive value of counting AgNORs in tumour pathology. In their article Barnes and Gillett[1] quote two investigations which failed to demonstrate a prognostic relevance 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 of the AgNOR parameter. In colorectal carcinoma soft tissue sarcoma, renal cell carcinoma, 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 of independent prognostic value.[6]

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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