

Immunohistological detection of *Legionella pneumophila* in lung sections

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SUMMARY Immunohistology was used for the detection of *Legionella pneumophila* serogroup I in necropsy tissue. Study of pneumonic lung from the recent Stafford outbreak has shown that this technique has a high sensitivity. A retrospective postmortem examination showed that *L pneumophila* serogroup I was an unusual cause of pneumonia in Oxfordshire during the study period. *L pneumophila* serogroup I can be successfully subgrouped, using a panel of monoclonal antibodies on formalin fixed paraffin embedded sections. Immunohistological methods have a potentially useful role in the diagnosis of Legionellosis at postmortem examination and in the epidemiological investigation of individual cases and outbreaks.

The recognition of the agents responsible for infectious disease usually depends on culture of the organism, followed by examination of its morphological and biochemical properties. Some organisms, however, are difficult to grow, and on occasions suitable material may not be available for culture. Immunohistological techniques permit the sensitive and specific detection of antigenic determinants and already have an established use in the identification of micro-organisms, both in fresh¹ and fixed tissue sections.^{2,3}

Legionella pneumophila is a cocco-bacillary bacterium that can cause pneumonia both sporadically and in epidemics.⁴ It may be difficult to culture from pathological specimens, and correct identification in tissue sections with the silver impregnation Dieterle stain is often impossible, especially in lung where carbon and other particulate material can cause confusion. Accordingly, its rapid diagnosis is now often made by immunofluorescence examination of bronchial washings or sputum.⁴ On occasion, however, (when the organism is not suspected), such fresh material is not available and the diagnosis cannot be confirmed. In view of this we investigated the possibility of detecting *L pneumophila* by immunohistology on formalin fixed necropsy lung sections to determine whether this technique may be a useful adjunct in the diagnosis of *Legionella* pneumonia. We assessed the sensitivity of the method by postmortem examination of lung sections from patients who died during

the recent *Legionella* outbreak at Stafford,⁵ and we also determined whether the organism could be subgrouped in tissue sections using a panel of monoclonal antibodies. In addition, we retrospectively investigated the incidence of *Legionella* pneumonia at postmortem examination using this technique.

Material and methods

Formalin fixed paraffin embedded lung sections were kindly provided from 28 necropsy cases of suspected *Legionella* pneumonia from the Stafford outbreak. Sections were also obtained from the files of this hospital from 70 consecutive necropsy cases of pneumonia, both hospital and community acquired, in which *L pneumophila* had not been suspected.

These were stained by an indirect immunoperoxidase technique⁶ after endogenous peroxidase activity was blocked with hydrogen peroxide and after trypsin treatment of the sections.

In the first stage a rabbit polyclonal anti-*L pneumophila* serogroup I antibody was used at a dilution of 1/200. This is the antiserum used routinely in the immunofluorescence examination of sputum and bronchial washings in Oxford (supplied by Dr AG Taylor, Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, London) and has proved reliable in clinical testing over seven years, detecting no organisms other than *L pneumophila*.

The appropriate test dilution used was determined by staining necropsy lung sections from three cases of known *L pneumophila* serogroup I pneumonia, using

serial dilutions of the antiserum. The three cases gave consistent and similar staining. A negative control was performed by substitution of the anti-*Legionella* antibody by non-immune rabbit serum or by omission of the first stage antibody.

Six of the Stafford cases were further studied. Paraffin sections were dewaxed, fixed in acetone for five minutes, and then treated for one hour at 37°C with 20 µl of one of the nine monoclonal antibodies that have been used to subgroup members of *L pneumophila* serogroup 1.⁷ After washing twice for five minutes in phosphate buffered saline (PBS) 20 µl of antimouse fluorescent conjugate was applied for 25 minutes. After further washing in PBS and drying each section was examined for organisms with a Leitz fluorescence microscope, using incident light illumination. The results of postmortem lung culture for *L pneumophila* from the Stafford patients were kindly made available by Dr JGP Hutchinson, Public Health Laboratory Service, Birmingham.

Results

Eleven of the 28 cases from Stafford showed positive staining with the rabbit anti-*Legionella* serum (figs 1 2, and 3). Several patterns were seen. Firstly, organisms could be seen within neutrophil polymorphs and macrophages, either as single bacteria or as small clusters. Secondly, a large globular area of strong staining could be seen in scattered macrophages, which, on oil immersion microscopy, showed a coarse granular pattern, probably representing a colony of intracellular bacteria. Thirdly, in the strongest staining case there was a diffuse, weak staining of the alveolar exudate and cytoplasm of phagocytes. This may represent specific staining of bacterial anti-

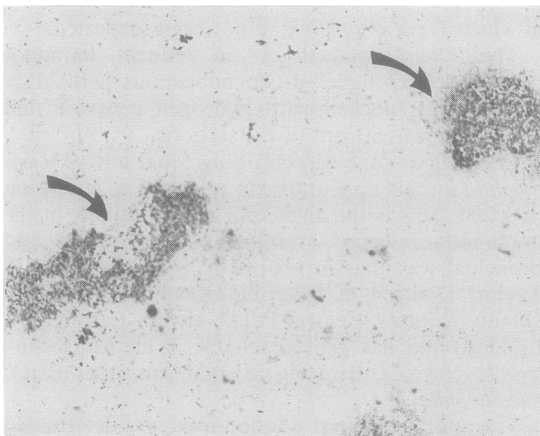
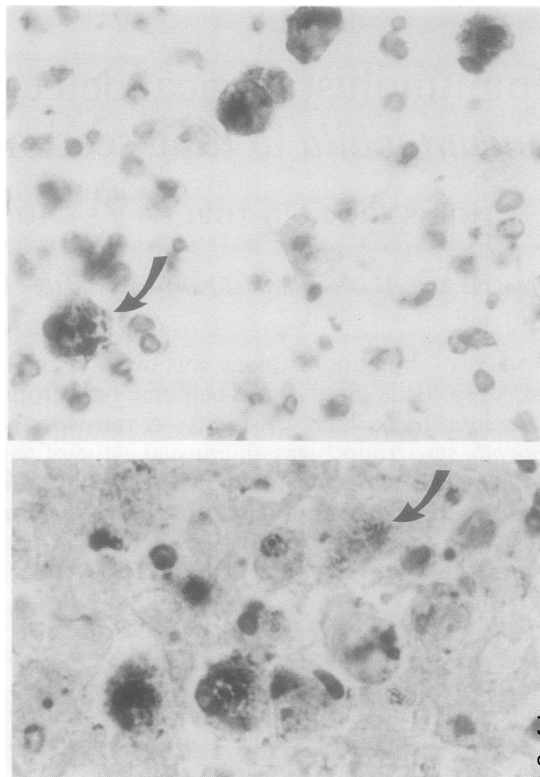


Fig 1 Low power view of pneumonic lung showing patchy staining for *Legionella* (arrows) (Peroxidase.) × 50.



Figs 2 and 3 Clusters (arrows) and larger colonies of organisms present mainly within macrophages. (Peroxidase.) × 1200.

genic debris. In all cases, however, staining was patchy, with a few cases having only one or several small detectable foci.

In all cases no staining was noted with the negative control, and there was no difficulty distinguishing the positive staining from background carbon, haemosiderin, or other particulate debris.

The table shows the relation between *Legionella* staining and the histological features seen in the stained sections, and also indicates, for comparison, the results of culture. Most cases of acute pneumonia (eight of 11), and of organising pneumonia (three of six) were positive on immunohistology. By contrast, sections of lung tissue from the 11 cases, which showed severe changes, but which were not predominantly pneumonic (shock lung, infarction, alveolar haemorrhage, thromboembolism), were negative on immunohistology.

Of the nine culture positive cases, three were negative by immunohistology on the available lung sections. Of these three cases, one showed the histological features of shock lung, one organisation of pneumonia, and only one showed acute pneumonic

Table Immunoperoxidase staining of necropsy lung sections from Stafford for *Legionella pneumophila* serogroup 1*

Dominant histological features	Total	No positive for <i>Legionella</i>	No negative for <i>Legionella</i>
Severe pneumonia, early or minor organisation	11 (6)	8 (5)	3 (1)
Predominantly organisation with focal active pneumonia	6 (2)	3 (1)	3 (1)
Major pathology other than pneumonia	11 (1)	0	11 (1)
	28 (9)	11 (6)	17 (3)

*Numbers in parentheses represent cases confirmed by culture.

consolidation. Conversely, immunohistology detected five cases which showed *Legionella* staining, but which were negative on culture.

Further sections from six of the 11 positive cases were studied by immunofluorescence using a panel of nine monoclonal anti-*Legionella* antibodies. In all cases bacteria were stained by the same two antibodies alone, identifying them as subgroup Pontiac 1a, the same subgroup as the isolates from Stafford.⁵

Retrospective immunohistological examination of lung tissue from 70 consecutive cases shown to have pneumonia at postmortem examination was negative. One of these was a known case of *L. bozemanii* pneumonia, but this was negative to testing with the antibody to *L. pneumophila* serogroup I, although it proved positive using a rabbit polyclonal antibody against *L. bozemanii*.

Discussion

Immunofluorescence examination of bronchial washings, sputum, or homogenised lung tissue is a well established method for the diagnosis of *Legionella*.⁴ Indeed, this is generally more sensitive than culture, possibly due to difficulties with overgrowth of other bacteria, or loss of viability, either due to drug treatment or during storage and handling. Furthermore, these immunofluorescence methods offer the advantage of a more rapid diagnosis than culture. Therefore, it has been recommended that culture should be supplemented by immunofluorescence when the diagnosis of legionellosis is considered.⁸

Although the detection by immunofluorescence of the organism in formalin fixed paraffin embedded lung sections has been successful,⁹ it is well recognised that immunohistochemical techniques have several advantages compared with immunofluorescence, including direct visualisation of the stained antigenic material and the morphological features of the tissue. Accordingly, we investigated the use of an indirect immunoperoxidase technique in the diagnosis of *Legionella* pneumonia. The method gave clear and reproducible staining of colonies and single bacteria, and, on occasion, a more diffuse antigenic background.

The study of the lung tissue from the Stafford outbreak permitted an assessment of the sensitivity of

this technique. We detected *Legionella* by positive staining in 11 of 28 cases compared with nine culture positive cases. There was a close correlation with the histopathological nature of the lung lesion, as eight of the 11 cases with acute pneumonic consolidation showed positive staining for *Legionella* compared with six by culture. Only one case of immunohistologically negative acute pneumonia was positive on culture. Of the six cases of organising pneumonia examined, we detected staining for *Legionella* in three. As staining was strikingly patchy in some cases, investigation of additional blocks or of further levels might have increased the detection rate. Not surprisingly, none of the 11 cases, in which the lung section showed changes that may have complicated the severe pneumonic illness, but not pneumonia itself, was positive for *Legionella*. From these results we suggest that immunohistochemical identification of *Legionella* is at least as sensitive as culture, particularly if multiple blocks from the areas of greatest pneumonic consolidation are investigated.

This technique has the same advantages as immunofluorescence compared with culture—that is, equal or greater sensitivity, and, potentially a more rapid diagnosis. It also, however, has the unique advantage of permitting the retrospective detection of *Legionella*, if fresh lung tissue had not been sent for culture and if formalised lung tissue is no longer available for direct immunofluorescence on lung scrapings. Furthermore, the method could also be applied to lung biopsy specimens, in cases showing pneumonia of undetermined cause.

It has recently been shown that *L. pneumophila* serogroup 1 can be reliably further subgrouped using a panel of monoclonal antibodies against a range of surface antigens.^{7,10} In the present study we have shown that this method of subgrouping can be successfully performed on formalin fixed paraffin embedded lung sections. This could have considerable epidemiological importance in tracing the environmental source of the infecting *Legionella*, especially if several potential sources with different *Legionella* subgroups have been identified, and no isolates of *Legionella* have been obtained from the patients.

As the reported incidence of *Legionella* as a cause of pneumonia ranges from 1 to 20%¹¹ (depending on the population examined, methods used, and the

the population examined, methods used, and the region studied), we applied this technique to 70 unselected and consecutive cases of pneumonia detected at postmortem examination. We failed to detect *L pneumophila* serogroup 1 in any. As our study of the Stafford cases has shown that this technique has a high sensitivity, especially in cases of acute pneumonic consolidation, we conclude that *L pneumophila* serogroup 1 (the commonest serogroup causing *Legionella* pneumonia¹²) has been an unusual cause of sporadic pneumonia, both in hospital and community acquired cases in the Oxford region.

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Theaker, Tobin, Jones, Kirkpatrick, Vina, Fleming

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