
Correspondence

Seasonal variation in the necropsy incidence of pulmonary thromboembolism

The two recent articles1,2 on this topic are interesting, and I wonder whether either set of authors has any views about possible aetiologies, which have not been proposed in their articles.

It is recognised that a number of respiratory infections have a propensity for being complicated by thrombotic phenomena of a minor or major type. The best known of these are Mycoplasma pneumoniae, psittacosis, Q fever, and the various rickettsial infections. In the UK and Hong Kong, the most likely infection common to both territories is M pneumoniae, and like other respiratory infections, it is likely to be present in increased incidence during the winter season. I offer this as one possible explanation for the interesting findings.

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Dr Chau, Yuen and Wong comment:
We have examined the clinical and pathological data of our patients with significant pulmonary thromboembolism (PTE) for any evidence of pulmonary infection, in particular atypical mycoplasma pneumonia, as suggested by Dr Boyd. The most significant associated medical conditions in these 130 patients are shown in the table. Most patients had major risk factors for thromboembolic diseases, as shown in groups 1 and 2. For patients in group 4, no other diseases could be identified clinically or at necropsy besides PTE. There was also no radiographic or histological evidence of atypical pneumonia. For group 3 patients, it is possible that PTE might be related to mycoplasma infection. The number of these patients, however, is small and the occurrence of infection is spread evenly over the year. Therefore, although it is impossible to prove or disprove the causal relationship between mycoplasma infection and PTE at this stage, mycoplasma infection probably does not play an important role in the seasonal variation of PTE in the Chinese patients in Hong Kong. Further prospective studies may be needed to substantiate this interesting hypothesis.

Should superheating be used for immunohistochemistry?

Numerous recent publications have advocated the use of superheating to lower the antigen detection threshold for many cellular markers.1,2 The intensity of immunostaining and visualisation of formerly undetectable antigens in paraffin wax processed tissues has made superheating a valuable tool for immunocytochemical techniques. However, these superheating methods have added more variables to those already influencing immunocytochemistry (fixation, processing, detection system, etc.). As with other immunocytochemical methods, there is no standardisation of heating times, cooling times, unmasking solutions, and mode of superheating.

A number of authors have advocated the use of pressure cookers instead of microwave ovens for superheating tissue sections.3 However, there are limitations associated with the use of pressure cookers which have not been highlighted. The hazards posed by the use of pressure cookers have not been mentioned—bringing solutions to the boil on the workbench, using pressurised containers, the weight of the pressure cooker when filled, the possibility of receiving a steam burn (3 litres of citrate buffer plus 110 slides as recommended by one author is a very heavy load to be lifting to a cooling sink of water). Certainly, in busy diagnostic laboratories there would be a substantial risk of accident. In addition, the pressure cooker has been recommended because of the need to steam-heat slides for only two minutes; however, the solution will take some time to come to the boil and will have to be monitored until this is achieved.

Microwave heating also has limitations, the most important being the presence of "hot-spots" which could generate inconsistent immunostaining results. If immunopositivity is to be quantified, in particular for hormone receptors, p53 protein or proliferation antigens, duplicate slides for each case should be immunostained if microwave heating is used for the very large spread-staining with S100 has been observed following superheating, false negative immunostaining for CD21 and CD23 and germinal centre immunopositivity with protein gene product (PGP) 9.5 and p53 antibodies.4,5

It is now vital that evaluation of the effect of superheating be undertaken on panels of normal and malignant tissues to establish whether there are altered specificitivities which might compromise tumour cell identification.

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Dr Norton comments:
I have read this letter with interest as superheating has become a routine method of antigen unmasking in our laboratory and has now been in use in our hospital for nearly two years. Many of the reservations given in this letter are based simply upon speculation. Provided adequate safety procedures are in place in the laboratory there is no risk of steam burns, especially from the use of pressure cookers. Indeed, during the period of active use of this method in our laboratory we have had no such accidents and from other laboratories where they are using pressure cookers I have not heard of any accidents occurring there either. I certainly share their concerns about alterations in thresholds of detection of antigens. However, they refer to it as false positives. This is in fact not the case and many of the results obtained with antibodies after heating are simply a manifestation of lowering the detection threshold. Proteins such as S100 and other members of the calcium binding family are very sensitive in nature and it is not surprising, therefore, that they should be detected after heat pretreatment in very many tissues. The staining with PGP 9.5 and related antibodies again reflects alterations in the threshold of detection of antigen. The paper cited actually shows that the antigen is present by western blotting and is therefore not a false positive result as the authors maintain. The abolition of staining with antibodies such as CD21 and CD23 is not a reason for not adopting the method. Some antibodies do not require a proteolytic digestion step and it now seems that some antibodies do not require a heat pretreatment step to reveal their antigen. The only part of the letter I am in agreement with is the last statement which would apply in an ideal world but is practically impossible given the thousands of reagents available on the market. We have not attempted to include a large range of tissues on which they might be applied. We are already on the steep part of the learning curve with heat pretreatment and the effects on antigen retrieval and I think this letter simply reflects unnecessary whistle blowing.

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