Correspondence

Comparison of electron microscopy and immunocytochemistry in tumour diagnosis

Dar *et al*, in a careful retrospective review, compared the relative usefulness of electron microscopy and immunocytochemistry in tumour diagnosis.¹ The authors' material did not include small round cell tumours of children and young adults, in which electron microscopy is very useful in diagnosis and in the recognition of "new" entities.^{2,3} In our experience, electron microscopy may also be helpful in diagnosing primary or metastatic large cell undifferentiated tumours, as well as in other "fine print" situations.

I believe that the relative merits of electron microscopy and immunocytochemistry in tumour diagnosis are very difficult to assess, even retrospectively. They achieve similar results in most cases, albeit by very different means (identifying ultrastructural features characteristic of a normal cell or specific tumour cell type on the one hand, and typical antigenic expression on the other). Either technique, if used alone, may be non-diagnostic in a given case,⁴ although the combined ultrastructural findings and immunostaining pattern are frequently complementary and precise enough to make the diagnosis. In practice, most pathologists have no problem in deciding whether to use immunocytochemical "markers" or electron microscopy: they have no access to an electron microscope; and they have little experience in interpreting ultrastructural features. Furthermore, immunostaining is perceived as convenient, less time consuming, and less expensive.

Restricting the use of electron microscopy in tumour diagnosis (as opposed to its use in medical biopsies, identification of unusual infectious agents, evaluation of cilia, etc.) deprives both pathologists and pathologists-in-training of an important means of improving their diagnostic ability and understanding of basic morphology. Electron microscopy not only clarifies the light microscopy appearance, but also clearly illustrates—in black and white—what is staining brown in light microscopy sections.

Finally, I support the authors' statement that electron microscopy must be done judiciously, but it should also be done promptly, and not solely as a last resort on suboptimal material, when the submitting pathologist has been overwhelmed by "immunoconfusion".

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- 2 Haas JE, Palmer NF, Weinberg AG, Beckwith AG. Ultrastructure of malignant rhabdoid tumor of the kidney. A distinctive renal tumor of children. *Hum Pathol* 1981;12: 646-57.
- 3 Gerald WL, Miller HK, Battifora H, *et al*. Intra-abdominal desmoplastic small round-cell tumor. *Am J Surg Pathol* 1991;15: 499-513.

4 Gaffney EF, McCormick C, Sweeney EC. Nondiagnostic electron microscopy. *Proc Roy Microsc Soc* 1989;24 (Suppl):4-5.

Professor Underwood *et al* comment:

We welcome Dr Gaffney's support for the judicious use of electron microscopy in tumour diagnosis and for his advocacy of its utility. The purpose of our paper was not to encourage restricted use of electron microscopy, but to enable a more rational decision if meagre tissue samples or laboratory resources force a choice to be made between electron microscopy or immunostaining in an individual case.

A viscometric method of measuring plasma fibrinogen concentration

We have also studied the correlation of plasma fibrinogen concentration against the difference in viscosity between plasma and serum. We can confirm the work of Ernst *et al*¹; we obtained a similar correlation coefficient of $r = 0.824$ ($p < 0.001$) using samples with values that were not excessively raised. The relation, however, does not hold true for samples with raised serum viscosity as seen in myeloma or macroglobulinaemia²; when these were included in our correlation we obtained $r = 0.117$ ($p = \text{NS}$).

The relation of fibrinogen to serum viscosity is not simple linear arithmetic as suggested by Ernst *et al*. It is exponential. Using logarithmic transformation we obtained a correlation of $r = 0.927$ ($p < 0.001$). This included normal and abnormal samples with high fibrinogen as well as myeloma and macroglobulinaemia.

As a simple quick calculation the method of Ernst *et al* can be used in normal subjects but the limitations of this method in abnormal conditions must be borne in mind.

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- 1 Ernst E, Resch KL, Saredeth T, Maier A, Matrai A. A viscometric method of measuring plasma fibrinogen concentrations. *J Clin Pathol* 1992;45:534-5.
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Professor Ernst comments:

Recalculating our data we still find the linear approach superior ($r = 0.87$). The situation is different using the raw data of the Clauss method (seconds) instead of the derived mg/dl. The fact that here we have a quadratic correlation leads to a higher value of r in the logarithmic approach ($r = 0.85$) compared to the linear one ($r = 0.79$). So it should be clarified whether Lane and L'Anson calculated their correlation coefficient applying mg/ml to both methods.

We did not include haematological diseases (myeloma, macroglobulinemia, etc.), where interactions between fibrinogen and pathological proteins are not unlikely to have a major role. There might indeed be some other diseases, where the viscometric

method fails and future studies will have to address this.

Lane and L'Anson did not mention the number of cases their results are based on. If the number is small, the constellation they report could be influenced by hazard. We keep on collecting data and will hopefully be able to present a recalculation of our formula based on several hundred additional cases in a while.

It would be very interesting to establish contact with Lane and L'Anson and discuss the remaining open question in more detail.

Bone marrow trephine biopsy in lymphoproliferative disease

Schmid and Isaacson present their experience of the pattern of marrow disease for various types of non-Hodgkin's lymphoma.¹ In their experience malignant lymphoma centroblastic/centrocytic (ML cb/cc) most commonly gives rise to a nodular pattern of marrow disease while malignant lymphoma centrocytic (ML cc) most frequently manifests in the marrow in a paratrabecular distribution. This is at variance with our observations, particularly with regard to the paratrabecular distribution and has led us to examine the published finding. The latter is surprisingly inconsistent and a comparison of the various studies is, of course, confounded by the multiplicity of classifications which have been applied to malignant lymphoma.

An early study,² using the Rappaport classification, states that both diffuse, well differentiated, and nodular poorly differentiated lymphomas tend to show a focal pattern and that in many of the patients in whom the pattern is focal, there seemed to be a distinct paratrabecular distribution. A subsequent paper from the same department³ studied nodular lymphoma specifically and, where the degree of marrow disease was limited, found the distribution to be usually paratrabecular; this became less easily recognised as the extent of marrow disease increased. A third paper from this group⁴ used the Lukes-Collins classification. Of 87 cases of malignant lymphoma, follicle centre cell, small cleaved, 31 (64%) showed a focal paratrabecular pattern. The paper also states that 76 of these 87 cases (87%) showed a follicular pattern in the affected lymph nodes. The study by Conlan⁵ uses the Working Formulation and therefore cannot be interpreted in detail, but a paratrabecular pattern was seen in a range of lymphoma types of low and intermediate grade, both B and T cell. Bartl *et al*⁶ have published a very large series in which bone marrow biopsy specimens from 3229 patients with lymphoproliferative disorders were studied; a paratrabecular pattern was uncommon (2% of affected trephines) but correlated strongly with MLcc, while a nodular pattern was more common (9% of affected trephines) and was seen with ML cb/cc and immunocytoma. However, a subsequent paper by Bartl *et al*⁷ is illustrated with two cases of ML cb/cc showing paratrabecular disease.

We have therefore studied the pattern of marrow disease in trephines from our archival material, and have reviewed the lymph nodes from these cases to verify the lymphoma typing. Forty six trephines from cases of ML cb/cc and ML cc were examined without knowledge of the lymph node morphology and an attempt was made to

classify them according to the patterns of infiltration proposed by Schmid and Isaacson. This proved surprisingly difficult because many of the infiltrates showed a combination of two or more patterns. In particular, "dense" is not a pattern and a paratrabecular distribution may still be discerned even at an advanced stage of marrow replacement. If one considers specifically whether the infiltrate showed application to the bone trabeculae in a laminar manner, 15 of 34 trephines from cases of ML cb/cc showed a degree of paratrabecularity, compared with two of 12 ML cc. None of the infiltrates was convincingly nodular. Our experience is therefore consistent with the published findings from the University of Minnesota^{2,4} and contrary to those of both Bartl *et al*⁶ and Schmid and Isaacson.¹

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Glutathione S-transferase expression in primary biliary cirrhosis supports concept of "ductular metaplasia" of hepatocytes

Bile duct proliferation is a feature of many cholestatic diseases. The mechanism of this phenomenon is unknown but there is some support for the concept of "ductular metaplasia". For example, Van Eyken *et al*¹ used an immunocytochemical approach to show, in a variety of cholestatic diseases, that hepatocytes can express cytokeratins which in the normal liver are restricted to the bile duct cells.

The glutathione-S-transferases (GST; EC 2.5.1.18) are a multigene enzyme family that catalyse the conjugation of reduced glutathione with a variety of electrophiles. By immunological and catalytic criteria, the major cytosolic isoenzymes can be categorised into four classes, α , μ , θ and π .^{2,3} Time and tissue specific changes in the expression of these GST classes has been described in developing human tissues and these data suggest that changes in GST expression are related to changes in cell phenotype.^{4,6}

We now describe observations of glutathione-S-transferase expression in primary biliary cirrhosis that provide further evidence for the concept of "ductular metaplasia".

Expression of GST classes in cells with intermediate phenotype

	α	μ	π
Hepatocytes	+	+	-
Bile duct epithelium	-	-	+
"Ductular metaplastic" cells	-	-	+

Samples of liver were obtained from 10 patients who had undergone liver transplantation at the Liver and Hepatobiliary Unit, Queen Elizabeth Hospital, Birmingham, England. A diagnosis of end stage cirrhosis as result of primary biliary cirrhosis was confirmed biochemically, serologically, and histologically. Paraffin wax sections (4 μ m) were cut, dewaxed, and the expression of α , μ , and π classes of GST demonstrated by immunocytochemical localisation as described before.^{4,6}

In all cases the cirrhotic nodules of hepatocytes were positive for α GST. At the periphery of these nodules, cells that might represent an intermediate phenotype undergoing transformation to bile duct type cells were negative for α GST as were bile duct epithelial cells.

The polymorphic GSTM1 gene encodes the predominant μ class isoenzyme in human liver. In those who expressed this gene, GSTM1 isoforms demonstrated strong positivity in hepatocytes but were not expressed in bile duct epithelium or those cells putatively undergoing metaplasia.

In general, μ class expression was restricted to the bile ducts and "ductular metaplastic" cells, these both strongly positive. In some cases a weak diffuse positivity was also observed in occasional regenerating nodules of hepatocytes.

As shown in the table cells with an intermediate phenotype undergoing "ductular metaplasia" from hepatocyte to bile duct-type cells expressed π class GST but not the α or μ class isoforms. Although such cells were seen only on the periphery of the cirrhotic nodules, in many instances they were clearly continuous with surrounding hepatocytes. These "metaplastic" cells, therefore, did not show the typical hepatocyte pattern of strong positivity for α and μ class isoforms but, rather, similar GST expression to bile duct epithelial cells. This observation is consistent with the concept of "ductular metaplasia" and complements recent data in patients with extrahepatic biliary atresia showing altered expression of π GST in hepatocytes.⁷

It was interesting to note diffuse positivity for π class GST in some regenerating nodules, though it is not usually expressed by adult hepatocytes. Expression of π class GST by hepatocytes is normally seen only in fetal liver and may therefore be an indication of increased cell proliferation.⁵

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Drs Matthew and Burt Comment:

We have read the letter of Dr Hiley *et al* with interest. Their observations of π class GST in cells with "an intermediate phenotype" between that of hepatocytes and bile duct epithelium in primary biliary cirrhosis are in keeping with the results of a previous study.¹ As the authors point out it is likely that this represents a similar phenomenon to that found in our cases of neonatal cholestatic liver disease.²

We agree that these findings provide further evidence that under certain circumstances hepatocytes may undergo a form of transdifferentiation. It is pertinent to note, however, that this so-called ductular metaplasia may not be the only mechanism by which increased numbers of ductular structures develop in the cholestatic liver. There is strong experimental evidence that in acute biliary obstruction, proliferation of true bile duct epithelial cells has a crucial (if not exclusive) role.^{3,4} Although this may be less important in the "atypical ductular proliferation" seen in chronic biliary disease,⁵ a significant contribution cannot be discounted. Furthermore, it is conceivable that the ductular structures may be derived from proliferation and differentiation of a putative stem cell population within periportal zones. Although controversial,⁶ there is growing acceptance of the concept that the mammalian liver contains a stem cell compartment at this site.⁷

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