

Wash off liver cytology: a complementary diagnostic tool to liver biopsy

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SUMMARY Parenchymal and non-parenchymal cells were harvested by washing the liver tissue core and needle after percutaneous biopsy. The cytological material obtained was suitable for morphological analysis, including showing the presence of surface and cytoplasmic antigens using labelled antibody techniques. This technique provides a combined cytological and histological approach to the diagnosis of liver disease.

Histological examination of liver biopsy material still provides the most important diagnostic information in liver disease.¹ The processing of the biopsy material for histology, however, can damage or mask antigens and reduces the possible information that may be obtained by histochemical and immunological techniques. Although the use of frozen sections overcomes this drawback to some extent, the information present on the cell surface may then be reduced by the cutting process.

In contrast, cytology provides an excellent technique for the study of cell surface markers, because whole cells can be processed either without or after only mild fixation.² In addition, the mononuclear cells present in the specimen can be defined by morphological or cytochemical means.³ In this paper we describe a method by which material may be obtained for cytological analysis by harvesting cells obtained from washing the liver biopsy needle and biopsy tissue core.

Material and methods

Biopsies were carried out as a routine diagnostic procedure, as previously described,¹ using either a Tru-Cut or a Menghini needle. These needles give biopsy specimens with a core length ranging from 2.5 cm (Tru-Cut) to 8 cm (large Menghini needle 1.9 mm × 120 mm, Steriseal). Using the large Menghini needle without an internal stopping device, the biopsy cylinder was drawn into 5 ml of sodium chloride 0.9%. The biopsy specimen and the wash fluid were then transferred into a sterile flat bottomed 5 ml plastic

tube (Sterilin) by flushing gently through the needle. Alternatively, the Tru-Cut needle carrying the core was dipped into a tube containing 5 ml of sodium chloride (0.9%), and the biopsy was removed from the needle by gentle agitation. The biopsy core was taken out of the wash fluid and fixed in 10% formol saline for routine histology.

Hepatocytes were pelleted by spinning the wash fluid at 100 × g for five minutes. The remaining non-parenchymal cells in the supernatant were then pelleted by spinning at 800 × g for five minutes. Contaminating red blood cells were lysed by incubation with 0.83% ammonium chloride 0.17 M Tris buffer (pH 7.2) for five minutes at 37°C, followed by three washes in Dulbeccos phosphate buffered saline (PBS) containing 1% bovine serum albumin (fraction V, Sigma). After counting with a haemocytometer the cells were spun at 400 rpm for 15 minutes in a Shandon cytocentrifuge on to albumin coated slides. The slides were then allowed to air dry before fixing for 20 seconds in ice cold acetone. The specimens were either processed immediately or stored at -20°C.

After blocking of endogenous peroxidase the specimens were incubated for one hour at 37°C with either a monoclonal antibody against the transferrin receptor at 1/1000 dilution (OKT 9, Ortho Diagnostics) or against an Ia-Antigen at 1/100 dilution (anti-MHC major histocompatibility complex) class I, kindly provided by Dr P Lydyard, Middlesex Hospital, London). An antibody against human liver ferritin was raised and characterised, as described previously,⁴ and used as above at a dilution of 1/40. These primary antibodies were visualised, using either an indirect antibody-peroxidase or an antibody-alkaline-phosphatase method,⁵ using the antibodies at recommended dilutions (Dakopatts). The peroxidase reaction was developed using diaminobenzidine (Sigma)

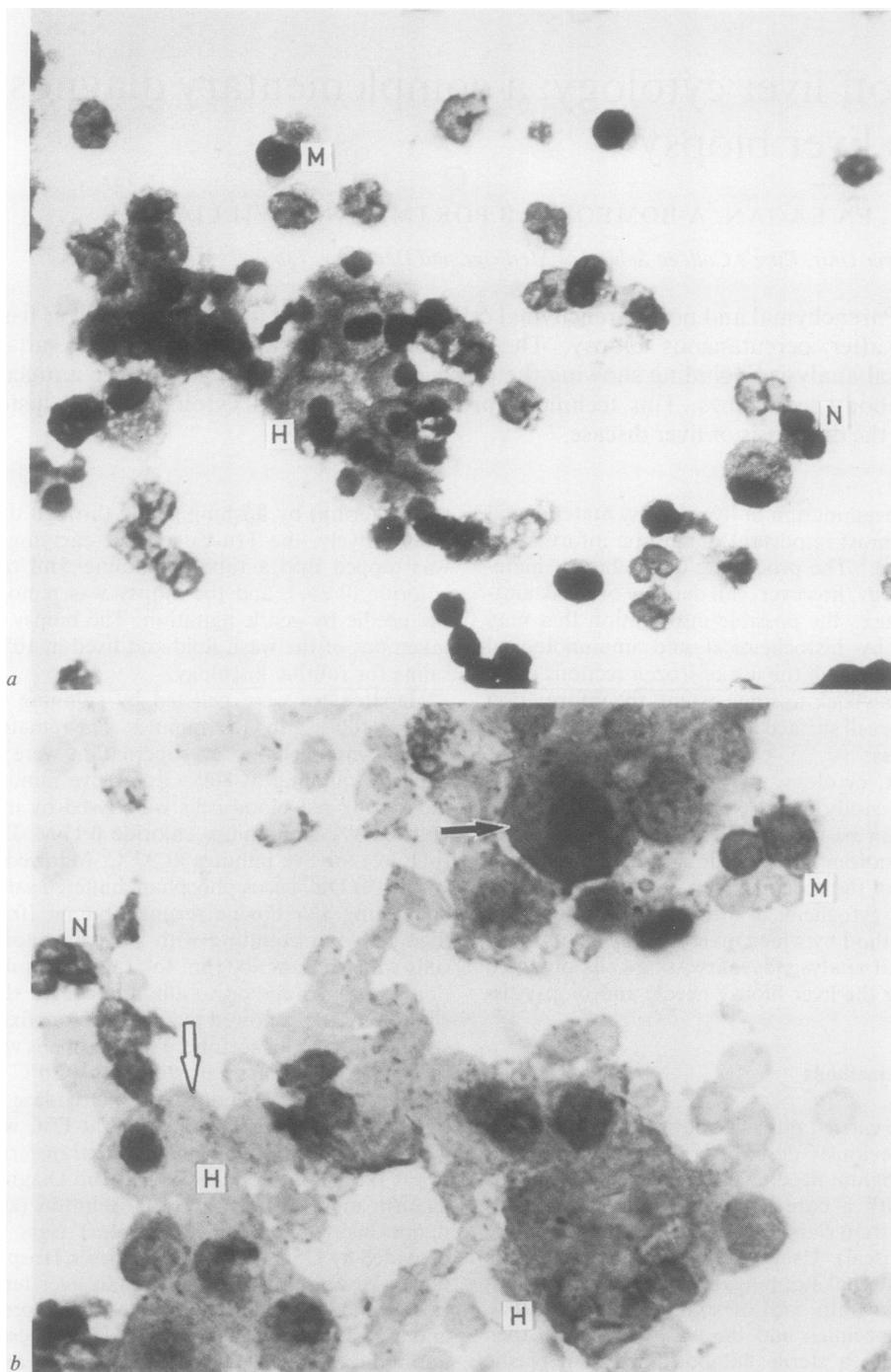


Fig. 1a Preparation from liver transplantation patient. Parenchymal and non-parenchymal cells show intense staining for major histocompatibility complex class I antigens (Immunoperoxidase.) $\times 140$.

Fig. 1b Preparation from a patient with cirrhosis and superimposed alcoholic hepatitis. There is varying intensity of staining for transferrin receptors (solid arrow—brown immunoperoxidase reaction) and ferritin (open arrow—red alkaline phosphatase reaction) on hepatocytes. (Double immunoenzyme staining.) $\times 380$. H = hepatocytes; M = macrophages; N = neutrophils.

Table Cell number obtained by wash off cytology in liver disease

Histological diagnosis	No	Mean (SEM) parenchymal cells $\times 10^3/cm$	Mean (SEM) non-parenchymal cells $\times 10^3/cm$
Normal	2	25 (15)	10 (12)
Acute hepatitis	5	56 (23)	80 (32)
Cirrhosis or fibrosis	5	10 (3)	8 (2)

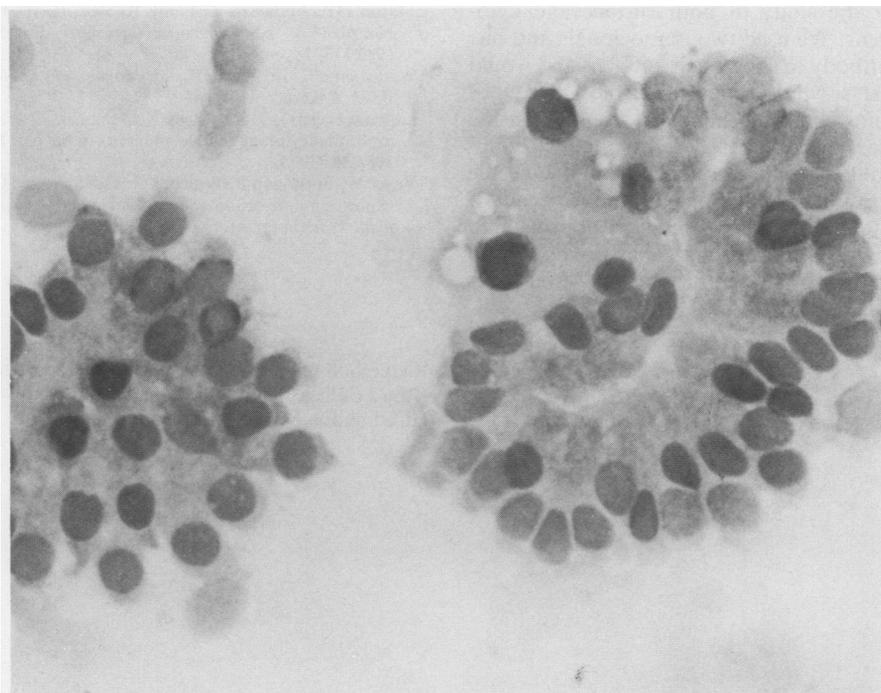


Fig. 2 Cell preparation from a patient with primary biliary cirrhosis. Bile ductular cells exhibit diffuse cytoplasmic staining for transferrin receptors. (Immunoperoxidase.) $\times 300$.

and the alkaline-phosphatase reaction with fast red salt (Sigma) dissolved in naphthol-AS-BI-phosphate containing 1 mM levamisole (Sigma) to block endogenous enzyme. The specimens were counterstained with haematoxylin.

Results

The number of cells obtained per cm of biopsy core depended on the underlying liver pathology. The lowest yield of hepatocytes was obtained from cirrhotic or fibrotic livers, whereas up to six times more hepatocytes were obtained from histologically normal or acutely damaged livers. Furthermore, livers with acute inflammatory cell infiltrates gave a good yield of non-parenchymal cells (Table). The viability of hepatocytes as judged by trypan blue exclusion did not exceed 60% ($n = 10$), although the cells seemed to

be intact. In contrast, the non-parenchymal liver cells and leucocytes maintained high viability ($> 90\%$).

The morphology of the hepatocytes was well preserved and showed the typical features of cells obtained by fine needle aspiration biopsy (Fig. 1a).⁶

Non-parenchymal cells were identified on morphological grounds as epithelial cells from bile ductules displaying the features of a secretory type cell (Fig. 2), or as cells such as macrophages, lymphocytes, or neutrophils (Fig. 1a). The accessibility of cell surface antigens was shown by two monoclonal antibodies recognising the transferrin receptor and major histocompatibility complex class I antigens, respectively. As shown in Figs 1a and b, these antigens are intact and accessible. Furthermore, simultaneous confirmation of surface and cytoplasmic antigens was possible, using a double indirect immunoenzyme sandwich technique.⁴ Transferrin

receptors on human liver cells were visualised by a brown colour (peroxidase reaction) and liver ferritin a red colour (alkaline phosphatase reaction) (Fig. 1b).

Conclusion

Cytological material obtained from washing liver biopsy needle and core provides an excellent source of liver cells for the study of both surface and cytoplasmic antigens. We used two monoclonals and one polyclonal antibody to show such antigens and would anticipate that a whole range of surface and cytoplasmic proteins could be shown on liver cells using this technique. The number of cells obtained compared favourably with that obtained from fine needle aspiration biopsies.⁷ Finally, this technique permits a combined cytological and histological approach to liver disease, which, in our hands, has proved useful for the diagnosis of allograft rejection after liver transplantation.⁸

References

- ¹ Hegarty JC, Williams R. Liver biopsy: techniques, clinical applications and complications. *Br Med J* 1984;**288**:1254-6.

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- ² Walker WS, Beller RHJ, Buckley PJ, Melvin SL, Shing-Erh Y. Some fixation reagents reduce or abolish the detectability of Ia-Antigen and HLA-DR on cells. *J Immunol Methods* 1984;**67**:89-99.
- ³ Vogel W, Margreiter R, Schmalzl F, Judmaier G. Preliminary results with fine-needle aspiration biopsy in liver grafts. *Transplant Proc* 1984;**16**:1240-3.
- ⁴ Bomford A, Conlon-Hollingshead C, Munro HN. Adaptive response of rat tissue isoferritins to iron administration. *J Biol Chem* 1980;**256**:948-55.
- ⁵ Mason DY, Abdulaziz Z, Falini B, Stein H. In: Polak JM, Van Noorden S, eds. *Immunocytochemistry*. Bristol: J Wright, 1983:113-28.
- ⁶ Wasastjerna C. In: Wied GL, ed. *Monographs in clinical cytology*. Basel: S Karger, 1979:167-93.
- ⁷ Lautenschlager I, Höckerstedt K, Taskinen E, *et al*. Fine-needle aspiration cytology of liver allografts in the pig. *Transplantation* 1984;**38**:330-4.
- ⁸ Vogel W, Portmann B, Williams R. Difference of OKT-9 antigen expression in rejection and viral infection of liver grafts. *Transplant Proc* (in press).

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