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Fine-needle aspiration biopsy in the monitoring of liver allografts

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Abstract. The diagnosis of acute liver allograft rejection is difficult, as clinical signs or liver function tests are too unspecific. The diagnosis is mainly based on biopsy histology. However, the liver core biopsy may be associated with complications. The fine-needle aspiration biopsy (FNAB) method, originally developed for the monitoring of renal transplants, is a reliable and atraumatic technique to diagnose acute cellular rejection of liver allografts. FNAB makes it possible to quantity the inflammation associated with rejection, and to monitor the response to anti-rejection therapy. Additional information is received from changes in liver parenchymal cells indicating tissue damage and/or possible hepatotoxic effects of the drugs used. In addition, FNAB may be helpful in differential diagnosis of infections, cholestasis or other complications. A good correlation between FNAB findings of acute liver rejection and histology has been reported. However, histological examination is needed to diagnose chronic rejection. Several liver transplant centres now use FNAB technology as a routine diagnostic tool.

Key words: Liver transplantation, fine-needle aspiration biopsy – Biopsy, fine-needle, liver

The histology of acute liver allograft rejection has been described in several animal models and in clinical transplantation [26, 29, 38]. The hallmarks of acute cellular rejection are mononuclear cell infiltration of portal areas, some degree of inflammation around central veins together with oedema and parenchymal lesions. At advanced stages of rejection, parenchymal necrosis occurs, lymphocyte response in portal areas becomes less prominent and the cellular infiltration becomes dominated by neutrophils and macrophages.

The diagnosis of acute liver rejection is difficult to establish [3]. Clinical signs or biochemical findings of liver dysfunction are rather nonspecific and may also be caused by, for example, cholangitis, infections or cholestasis. Until recent years, the only reliable method to diagnose liver rejection was histological examination. However, the conventional core needle biopsy (NB) always carries some risk of complications such as bleeding or infections.

The fine-needle aspiration biopsy (FNAB) technique, originally developed for renal allografts [10], is a reliable and atraumatic method to monitor renal transplants in situ. FNAB makes it possible to diagnose acute cellular rejection, to quantity the inflammation associated with rejection and to visualize the response to anti-rejection therapy [11]. After 10 years' experience of FNAB in renal transplantation in Helsinki, the method is now used in more than 50 transplantation centres around the world. In 1982, FNAB was applied to liver transplantation in humans [20] on the basis of experimental findings in the pig [19]. The following report is a short overview on the use of FNAB in liver transplantation.

Methods

The current FNAB method, originally developed in Helsinki for clinical monitoring of renal allografts, is employed [10]. Liver allografts are monitored by FNAB from the day of transplantation at 1–3 day intervals. The technique of obtaining and processing FNABs and corresponding blood specimens is similar to that used with kidney grafts [10]. In short, 10–20 µl aspiration specimens are obtained from liver allografts without local anaesthesia, and blood specimens of similar size are taken from the fingertip. Both specimens are drawn into a syringe containing Hepes buffered tissue culture medium. The specimens are cytocentrifuged onto microscope slides and the preparations are stained with May–Grünwald–Giemsa (MGG) for reading.

The inflammatory infiltrate is evaluated from the cell smears by the increment method [10]. To quantity the intensity of inflammation from the differential count, various correction factors are used according to the diagnostic value of the inflammatory cells involved in acute cellular rejection [11]. Lymphoid blasts, plasma cells, have the monoblasts and macrophages highest correction factor of 1.0. Lymphocytes carry a correction factor of 0.1, activated lymphocytes 0.5, large granular lymphocytes (LGL) 0.2, polymorphonuclear cells 0.1 and monocytes 0.2. The intensity of inflammation is expressed in corrected increment units (CIU). The total corrected increment is the sum of corrected increment values of the aspirate differential

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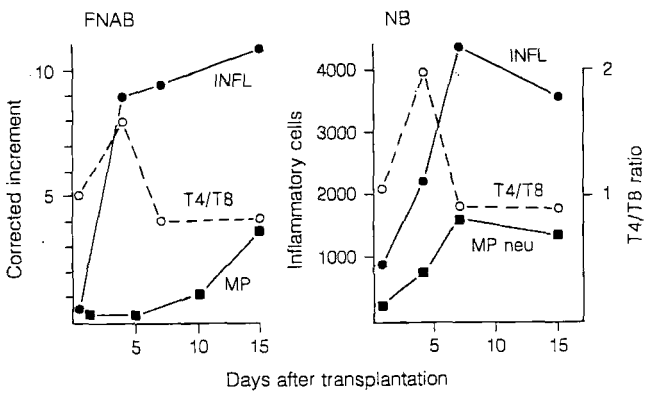


Fig. 1. Monitoring of pig liver allografts by serial *FNAB* and core *NB* during acute irreversible rejection. *INFL* Inflammation; *MP* frequency of macrophages; *T4/T8* ratio of T4 to T8 lymphocytes in the inflammatory infiltrate. The intensity of inflammation was quantified in the FNAB as corrected increment units, and in the NB as the number of inflammatory cells per unit area. For details, see Lautenschlager et al. [21]

after subtracting the blood background. Specimens containing liver parenchymal cells are considered representative according to the same criteria as used for kidney grafts [35].

In addition, immunoperoxidase staining techniques and monoclonal antibodies may by used to analyse inflammatory subsets and/or activation markers associated with rejection. These investigations are, however, not necessary in the routine FNAB monitoring of liver allografts.

FNAB in experimental liver allografts

Based on experience with FNAB in the monitoring of kidney allografts, an attempt was made to apply the method in liver transplantation. In an experimental pig hepatic allograft model, frequent FNABs were obtained to analyse the inflammatory changes associated with rejection in non-immunosuppressed and cyclosporin-A-treated (CyA-treated) animals [19]. The inflammatory changes recorded by FNAB were correlated with biopsy histology [19, 21].

In non-immunosuppressed piglets an inflammatory episode of rejection occurred promptly, peaked on day 4–7 after transplantation, and subsided thereafter in the grafts that were accepted. One-third of the grafts underwent irreversible rejection. At the beginning of the episode the inflammatory infiltrate consisted mainly of lymphoid cells, including blasts and plasma cells with minor involvement of monocytes. In piglets that died of rejection, the inflammation peaked earlier, was more prominent and the number of mononuclear phagocytes increased significantly during the course of the episode [19]. In CyA-treated recipients, the inflammation was significantly suppressed, and all animals survived. Biopsy histology from NB closely correlated with the FNAB findings [19].

In another series of piglet FNABs, we studied the subpopulations of inflammatory cells infiltrating the liver graft during rejection and correlated the FNAB findings with protocol biopsies and immunohistochemistry [21]. With FNAB, the inflammatory episode of rejection was detected on day 4 after transplantation, with lymphoid blast and lymphocyte infiltration, and infiltration of graft also by monocytes and macrophages. Maximal intensity of inflammation was recorded on day 14. An increase of both T4 and T8 cells in the FNABs during rejection was demonstrated by an immunoperoxidase technique. The T4/T8 ratio was low before rejection, increased at the beginning of the episode on day 4, and decreased again on days 7–14. The number of B cells in the graft was also elevated during rejection. Core biopsy immunohistology of frozen sections correlated well with the FNAB results, demonstrating a T4 predominance in the portal area on day 4 and a T8 predominance on days 7–14. In addition, granulocytes and macrophages infiltrated the portal area and were also seen scattered in the parenchyma. The inflammatory changes in the FNABs and NBs were thus entirely similar, and the time-related changes of the cellular infiltrate correlated well even in the subpopulation analysis in FNABs and vice versa (Fig. 1).

Technical aspects of FNAB in the clinical monitoring of liver transplants

A liver FNAB is obtained with a spinal needle as described previously in detail [12]. The skin is preferably punctured at the medial part of the right costal margin (Fig. 2). In some patients, the liver is best found through the right medial axillary line, especially if the graft is small. If the specimen is not representative, i.e. it contains < 7 hepatocytes per 100 inflammatory cells, the FNAB may be repeated during the same day. In less than 2% of cases, ultrasound or CT guidance is needed. The technique of obtaining a FNAB in liver transplant patients is summarized in Table 1, and the representativeness of liver FNABs is demonstrated in Table 2.

Our patients are closely monitored for eventual pain, bleeding or abscess, but no complications have been seen in a series of more than 1000 liver FNABs. Similar results have also been reported by others [16]. This finding com-

Fig. 2. a The liver is punctured at the medial part of the right costal margin. **b** The syringe is attached to a biopsy needle, and full vacuum is applied by means of a biopsy pistol

Table 1. Technique of FNAB in liver transplant patients

1. Use a 0.5 mm OD spinal needle (25-gauge 3.5 inch) and puncture the skin of the abdomen at the medial part of the right costal margin. No local anaesthetic needed.
2. Direct needle 30° upwards and 30° to the right. Instruct the patient to take a normal breath and hold it, while inserting the needle into the liver.
3. Remove the mandrin, attach the syringe containing culture medium to the needle and aspirate a specimen while making short 1-cm to-and-fro movements in the graft.
4. Release negative pressure in the syringe and remove it, rinse the aspirated sample twice in an injection needle holder (cuvette) and aspirate the sample into the syringe.
5. Transfer a blood drop from the tip of a finger to a smaller syringe containing the same culture medium.

Table 2. Representativeness of 1000 FNABs of liver grafts

	Number	%
Successful	868	86.8
Not representative	132	13.2
bloody	57	5.7
scanty	34	3.4
intraperitoneal contamination	36	3.6
bacterial infection	3	0.3
intestinal contamination	2	0.2

pares favourably with the complications reported in connection with normal core NB. These include liver haematoma, sepsis, lung empyema and even death of the patient [1, 27].

We have not hesitated to take FNAB specimens even when the patient is connected to a ventilator or cannot hold his breath for some other reasons. Specimen are also taken from patients with severe liver insufficiency and blood platelet count of < 20 000/l, and a coagulation factor *V* at < 15%. Especially in these very ill patients, a diagnosis, or exclusion of acute rejection is most important when planning the treatment.

Applications to human liver allografts

The inflammatory profile of liver allograft rejection is very similar to that reported for kidney allografts [34]. Figure 3 summarizes 22 characteristic inflammatory profiles of reversible rejection monitored with FNAB. The hallmark of acute liver rejection is the appearance of lymphoid blast and lymphocytes in the graft [22]. The first day of inflammation with ⩾3.0 CIU and presence of lymphoid blast cells in the FNAB is considered as the onset of immune activation [22]. Practically no blast cells or activated lymphocytes are seen in the corresponding blood specimens [22]. At advanced stages of rejection the blastogenic response subsides and the cellular infiltrate becomes dominated by mononuclear phagocytes. Appearance of large numbers of macrophages is usually associated with irreversible rejection.

The clinical diagnosis of liver rejection is based not only on the FNAB findings indicating immune activation but also on other laboratory parameters and clinical signs.

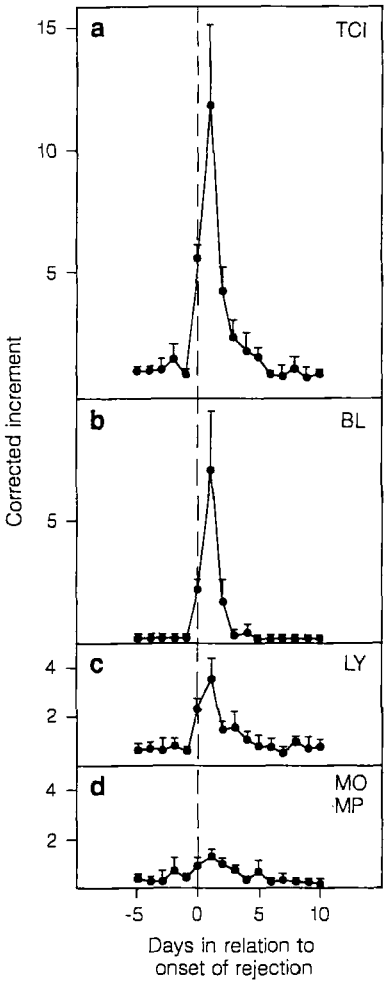
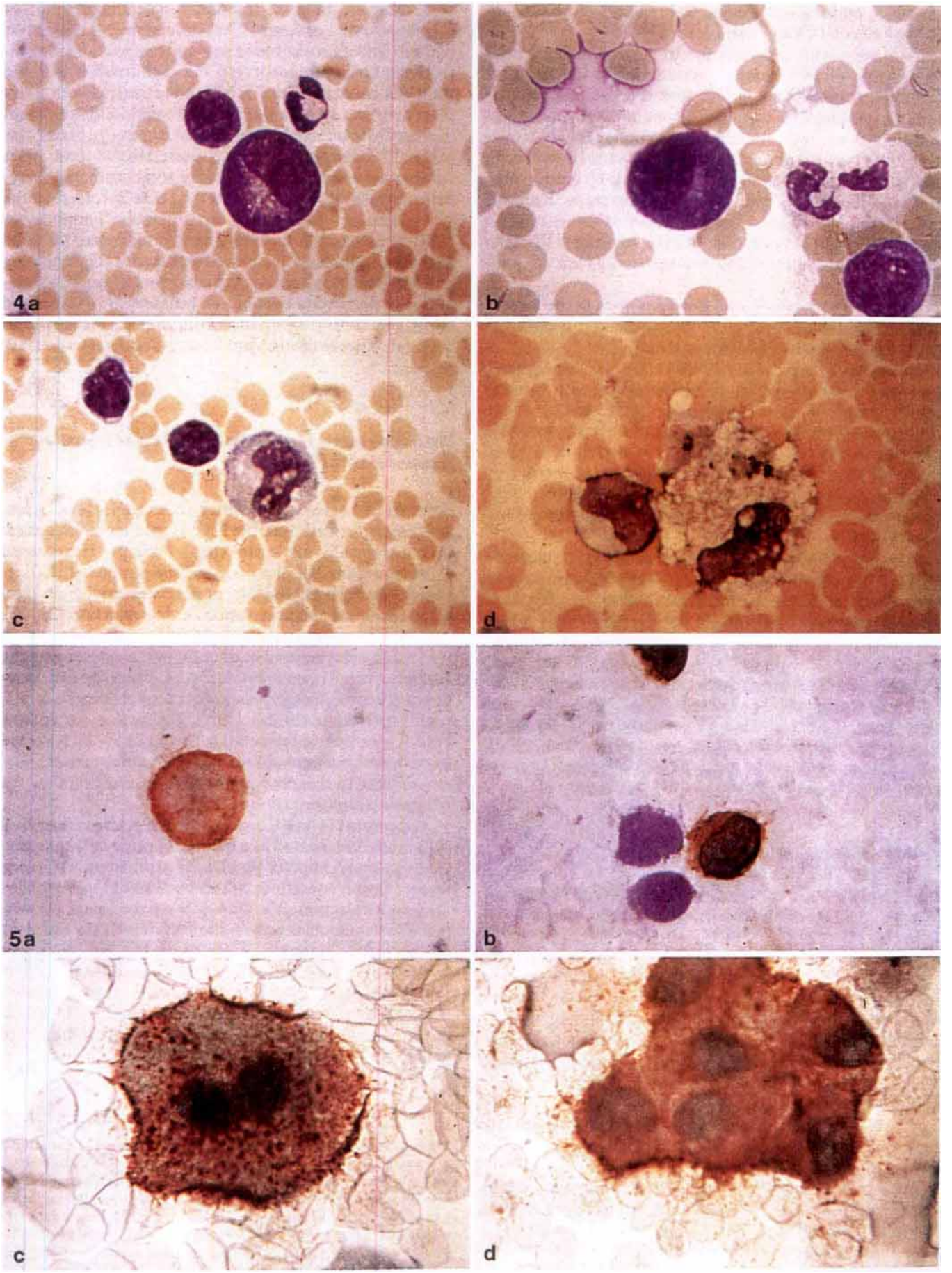


Fig. 3. a Inflammatory profiles of 22 episodes of rejection expressed in *corrected increment* units. **b–d** The major inflammatory cell components: **b** lymphoid blasts, **c** lymphocytes, **d** monocytes and macrophages. The onset of inflammation *(day 0)* is the first day with ⩾3.0 CI units and blast cells in the aspirate

The total increment score of ⩾3.0 CIU, together with lymphoid blasts in the FNAB, is only one way to express numerically the beginning of immune activation in the graft; it is not a 'cut off' point between rejection and no rejection. Usually, in specimens obtained before rejection, the TCI is low (< 2.0 CIU), and it peaks rapidly, even in only a few hours, to a significant level (> 3.0 CIU) when the blast response of lymphoid activation begins in the graft. Others have reported that a TCI of 3.5 CIU correlated with the clinical situation and NB findings in differentiation between rejection and no rejection [15].

Fig. 4a–d. Inflammatory cells in MGG-stained cytocentrifuge preparations of FNAB specimens. **a** A lymphoid blast, a lymphocyte and a granulocyte; **b** a plasma cell, a lymphocyte and a granulocyte; **c** two lymphocytes and a monocyte; **d** a macrophage and a lymphocyte

Fig. 5a–d. An IL-2-receptor-positive lymphoid blast **a**, a class II positive lymphocyte **b**, a class-II-expressing hepatocyte **c**, and a group of strongly class-II-positive bile duct cells **d** in immunoperoxidase-stained FNAB specimens of acute rejection


4a
b
c
d
5a
b
c
d

However, anti-rejection therapy should never be started on the basis of TCI score alone.

With successful anti-rejection treatment an acute episode of rejection is usually discontinued at the stage of lymphoid response, i.e. before severe tissue damage occurs, as indicated by damaged tissue parenchymal cells, and an increase in the number of macrophages [22]. However, the presence of macrophages does not always indicate irreversible rejection in this context [31]. Under normal conditions, the liver tissue macrophages, Kupffer cells, do not affect the corrected increment values significantly, although they are sometimes recognized in FNABs [18]. The most important inflammatory cells are demonstrated in Fig. 4.

In the blood, eosinophilia is usually seen at the beginning of lymphoid activation in the graft and it is possibly an IL-5-mediated non-specific reaction [2].

Most experience of liver FNAB is based on recipients receiving conventional immunosuppression with various combinations of azathioprine, CyA and steroids. The characteristic cellular pattern of acute rejection and the response to anti-rejection therapy is easy to follow in these cases. However, other immunosuppressive regimens, e.g. when monoclonal antibodies are used, may have additional still unknown effects on the cellular picture of inflammation.

Also preservation and perfusion of the graft, particularly if it has effect on the passenger cells of donor origin, may affect the initiation of immune response. Appearance of lymphoid blast cells in the liver graft only a few days after transplantation, without clinical signs of rejection, has been described [25, 29]. This type of immune activation subsides without additional immunosuppression [25] and may represent a GVH reaction initiated by donor cells. Thus, the FNAB findings, as with the NB findings, should always be evaluated and correlated with the clinical picture and other laboratory parameters before a comprehensive diagnosis of rejection can be made.

FNAB and infections

In general, systemic infections have no effect on inflammation, as quantitied by the corrected increment, of liver FNABs [14]. The total inflammation of liver recipients undergoing either bacteraemia or viral infections seldom exceeds 3.0 CIU [14]. However, any infection located in the liver graft itself may result in cellular changes in the FNAB. Presence of large numbers of granulocytes together with bacteria in the FNAB specimens indicates a bacterial infection in the graft, an abscess or contamination from a wound infection. Large numbers of neutrophils, often with hypersegmented nuclei, and macrophages are usually associated with bacterial infection, haematoma or local necrosis in the graft. In bacteraemia, a prominent neutrophilia is seen in corresponding blood specimens.

Viral hepatitis, CMV and other virus-induced inflammatory processes of the liver may, however, cause differential diagnostic problems with acute allograft rejection. Inflammation in FNABs, probably caused by viral infections, has been reported by others [28].

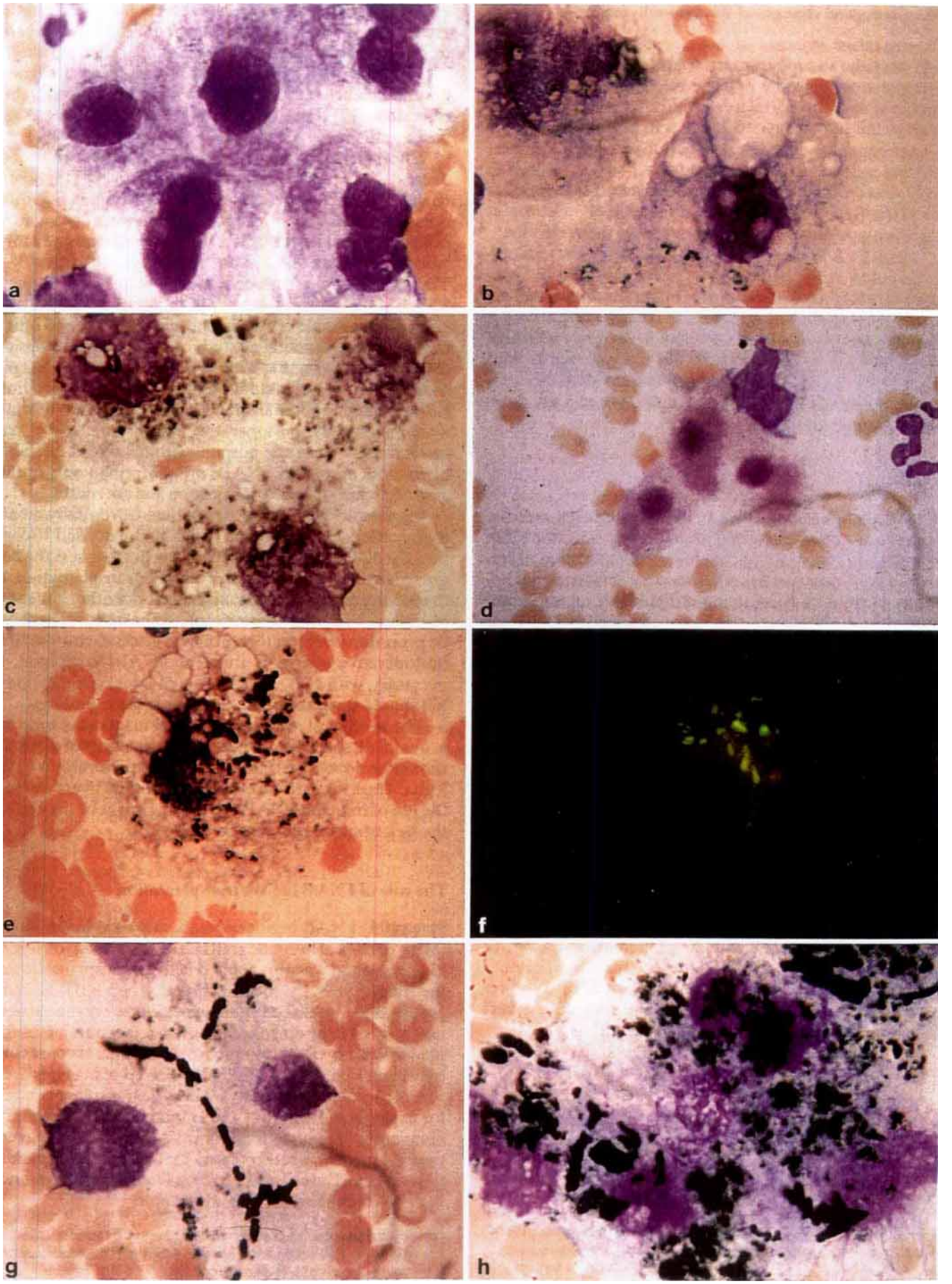
In a recent study, we compared the cellular picture of rejection to that of severe CMV infection [24]. Diagnosis of CMV infection was based on rapid antigen detection directly from blood leucocytes and confirmed with rapid shell vial culture. A mild lymphoid activation with a few blast cells was seen in both FNAB and blood specimens during CMV infection. This indicates that the blasts, few in the FNAB specimen, have clustered from blood. After subtracting blood background the total corrected increment remained low. Inflammatory cells involved in the CMV episode were mainly large granular lymphocytes (LGL cells), a phenomenon already well known [8]. Also slight eosinophilia in the blood appeared at the beginning of CMV infection and was associated with lymphoid activation in peripheral circulation. Inflammation associated with CMV subsided with successful antiviral therapy, indicating that it was due to viral infection and not to graft rejection.

Activation markers and cellular findings of rejection monitored with FNAB

Activation marker analysis may, although it is not necessary, be performed from the FNAB specimens to confirm immunologically the cytological findings. Increase of class-II-positive lymphocytes and appearance of IL-2-receptor-expressing cells in the graft correlate with the blast response of immune activation during rejection [23, 36]. The IL-2 receptor molecule is usually demonstrated on the surface of lymphoid blast cells, but is also seen on the surface of small lymphocytes (Fig. 5). Expression of transferrin receptor on lymphocytes is also reported to be a useful activation marker [23, 32]. However, in routine FNAB monitoring the cellular findings of MGG-stained smears, without activation marker analysis, are informative enough to diagnose graft rejection and to follow the response of anti-rejection therapy.

Expession of class-II antigens on the graft parenchymal cells is also considered as a marker of acute cellular rejection. Although hepatocyte class-II expression is not seen under normal conditions, it has been found to be associated with rejection [30, 33, 40]. We have found class-II-positive hepatocytes only in the FNABs of very intensive inflammatory episodes of rejection, but bile duct cells were always strongly positive when analysed during rejection [23] (Fig. 5). The problem is that the class-II expression of parenchymal cells is also associated with viral infections, and cannot be considered as a specific marker of acute liver allograft rejection [24, 37, 41].

Fig. 6a–h. Liver parenchymal cells in cytocentrifuge preparations. **a** A group of normal hepatocytes; **b** vacuolation of hepatocytes indicating parenchymal damage during rejection; **c** degenerated hepatocytes during severe rejection; **d** necrotic parenchymal cells; **e** isometric vacuolation in a hepatocyte during high dose CyA treatment; **f** CyA accumulation in a hepatocyte visualized by indirect immunofluorescence; **g** accummulation of bile between or **h** in the hepatocytes, indicating cholestasis


a
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Morphological changes of the parenchymal cells

Degenerative changes of hepatocytes, mainly swelling and irregular vacuolation, are recorded in FNABs during inflammatory episodes of acute rejection [22]. Parenchymal degeneration correlates with the intensity of inflammation but lasts longer. Necrotic cells in FNABs indicate severe tissue damage. The degenerative changes of parenchymal cells are usually scored from 1 to 4, 1 indicating only minor changes and 4 necrosis (Fig. 6). Degeneration of parenchymal cells without inflammatory inflitrate in the liver is caused by complications other than rejection.

Upon CyA hepatotoxicity, a typical isometric vacuolation is seen in the hepatocytes [5, 22]. Deposits of CyA, demonstrated in the hepatocytes by immunofluorescence, correlate well with morphological changes (Fig. 6). CyA deposits and the isometric vacuolation correlate with high doses of CyA to some extent, but not necessarily with CyA blood levels [13, 22], indicating individual differences in sensitivity to CyA. Upon reduction of CyA dose, these changes disappear.

Cholestasis

Bile droplets in hepatocytes, or between the cells, indicate cholestasis, often recorded also during rejection [22]. Cholestasis may also indicate biliary complication or impaired graft function for other reasons. Evidence of cholestasis without inflammation in FNABs is an important finding in the differentiation between rejection and other complications (Fig. 6). Cholestasis is also recorded as an elevation of serum bilirubin [13, 22].

Correlation between FNAB and biochemical parameters of rejection

The biochemical parameters reflecting hepatic dysfunction are non-specific, and increased levels may also be due to, for example, cholangitis, infection or cholestasis. A rapid increase in serum transaminases is usually recorded in association with an episode of acute rejection, but elevated values may still be seen for some time after surgery, and in early rejections the true meaning of the elevations may remain unclear. Serum bilirubin and alkaline phosphatase levels correlate with an inflammatory episode of acute rejection, but in FNABs the evidence of rejection is recorded 1–5 days earlier [13, 22]. After successful anti-rejection therapy elevated levels of biochemical markers last longer than the cellular findings [22].

Correlation between FNAB and NB

In our experimental model of acute liver rejection, a close correlation between FNAB and core NB was recorded [18, 20]. In human liver allografts, a good correlation between FNAB and NB has been reported by others [6, 16]. When FNAB and NB were performed in parallel and correlated with the clinical situation, the positive predictive value of cytological diagnosis was 86.3%, the sensitivity was 76.7% and the specificity was 86.9% [15]. Using FNAB we have observed acute rejection in 73% of liver grafts [12], which is of the same magnitude as found in centres using mainly core NB in the postoperative period [1, 27], although the Dallas group has reported a slightly lower incidence of rejection (60.6%) in their protocol biopsy study [17]. Thus, it is unlikely that we have overdiagnosed with the FNAB method.

The value of FNAB in the diagnosis of liver rejection decreasis after some months postoperatively [15] when changes of chronic rejection in the transplant begin to dominate. In chronic rejection, only a minor inflammatory infiltrate is seen in the graft, and the diagnosis is mainly based on vascular, bile duct and parenchymal changes [38]. These changes can be recorded only with NB. Although FNAB is very useful in the diagnosis of acute rejection, NB gives more reliable results in chronic rejection and other complications. In a detailed study of 1100 protocol biopsies (NB) it was shown that 95% of acute liver rejection episodes appear between days 4 and 21 after transplantation [17]. Thus, the optimal time to use FNAB monitoring would be the first postoperative month. In Helsinki, patients usually leave the hospital within 4 weeks of transplantation; thereafter FNABs are performed only in the case of clinical suspicion of rejection and not routinely.

In general, FNAB is used for frequent, even daily, monitoring of liver grafts, but a NB is obtained, if needed, to confirm the diagnosis of rejection, or in order to assess the degree of liver damage and the need for retransplantation. If there is any suspicion of chronic rejection or other late complications, NB should be performed. The optimal diagnostic procedure for monitoring of liver grafts during the first postoperative weeks, is frequent FNABs combined, if necessary, with NBs. In diagnosis of later complications, NB should always be obtained to confirm the findings.

The histologist responsible for NB diagnostics must be a qualified specialist; similarly, the reading of FNABs should be done by a trained specialist, preferably a cytologist. Thus, in centres transplanting several livers weekly, the use of frequent FNAB monitoring might be limited by the lack of such a full-time specialist.

The role of FNAB in liver transplantation

Since 1982 FNAB has been frequently used in the monitoring of clinical liver allografts [20]. In addition to Helsinki, the method was also applied in liver transplantation in the early 1980s by the Innsbruck group [31]. Several groups have published their experiences on the clinical use of FNAB in diagnosis of liver rejection and in the quantification on the response to immunosuppressive treatment [4, 6, 7, 9, 16, 25, 28, 39]. FNAB of liver has been accepted and found useful also in large transplant centres, for example in Dallas [7] and Hannover [25, 28] where a great number of hepatic allografts are transplanted every year. At present, a total of more than 20 liver transplantation units in Europe, USA, Canada and South America use FNAB as a routine diagnostic tool.

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