

Material and methods

The Coulter Electronics S Plus IV D automated white cell differential is based on the size of cell residues after partial lysis of white cell cytoplasm.¹ The instrument classifies white cells into three populations—lymphocytes, mononuclears, and granulocytes. A histogram of leucocyte volume distribution is produced which incorporates a series of visual alarms. These alarms correspond to the separation valleys between the three cell populations. They are activated when there is an overlap between populations, indicating unclassified cells.

Eighty seven venous edetic acid blood samples containing blasts were processed between 30 minutes and four hours after collection. The samples were derived from 51 treated and untreated patients with a variety of malignant blood disorders (table).

Sensitivity of blast detection was assessed by comparing the automated differential with 100 cell microscopic differential counts in blood films stained by Wright's stain.

Table Haematological disorders included in study

Type of disease	No of patients
Acute myeloblastic leukaemia	26
Acute lymphoblastic leukaemia	9
Chronic myeloid leukaemia in transformation	2
Chronic myelomonocytic leukaemia	2
Other myelodysplastic disorders	8
Myelofibrosis	4

Results

Alarms indicating the presence of abnormal leucocytes occurred in 76 of the 87 samples (87%). The total white cell counts of patients showing flagged blasts ranged from $1.0-95.7 \times 10^9/l$, and those of unflagged blasts from $1.0-13.3 \times 10^9/l$. The percentage of blasts ranged from two to 94. Blasts were detected in all nine new untreated patients.

An analysis of 11 samples with blasts noted at microscopical examination and not generating blast associated alarms showed that in three of the samples the alarm associated with nucleated red cells was generated. This was confirmed by microscopical examination. The remaining eight unflagged samples were derived from patients receiving treatment. During treatment blasts may become reduced in size and therefore be included in the lymphocyte count.² Such blasts will not produce an alarm and will not be detected. Twelve per cent of

the patients had non-flagged blasts while receiving treatment. The sensitivity of regional alarms in identifying blasts was 87%. Blasts could be identified in all samples processed, either by the instrument's alarm system or by scrutiny of the blood count results and attention to proffered clinical details, including chemotherapy, resulting in microscopic review.

Discussion

The Coulter S Plus IV D automated differential does not specifically recognise blast cells and blasts may not be identified based on regional alarm criteria alone.³ The automated differential does not correlate with the microscopical differential when blasts are recognised. Our study indicates that the Coulter leucocyte volume analysis instrument has acceptable limits of operation for the detection of blasts. A microscopic examination of the blood film should be undertaken, however, when there are abnormal findings in the three part differential, in patients with blood disease receiving chemotherapy or when clinical diagnoses indicate a blood film examination is required.⁴ High detection rates for early and rapid diagnosis of malignant blood disorders must call into question the utility of further refined instruments with their attendant high capital cost.

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A tickle at the back of the throat

We report a case of a 15 year old boy who presented with a short history of nasal obstruction. He complained of frequent frontal headaches and had had several episodes of a sore throat in the past year. On

clinical examination both tonsils appeared pitted and unhealthy with the left tonsil being larger than the right. A tonsillectomy was performed without complication and both tonsils were submitted for histological examination.

Both tonsils showed reactive hyperplasia with large active germinal centres. Intraepithelial lymphocytes and neutrophils were present within the crypts. The features were therefore those of active chronic inflammation. In the left tonsil a hair and surrounding hair follicle was also present (figure).

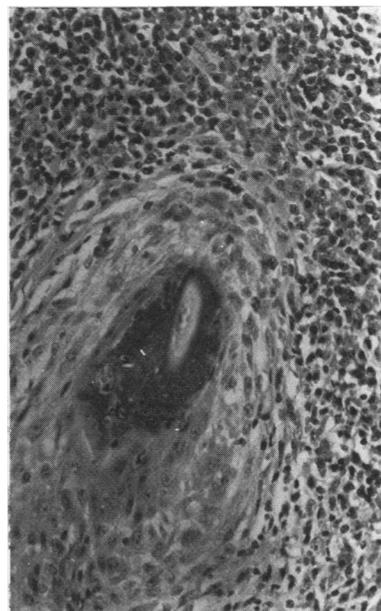


Figure A hair follicle is present within the lymphoid tissue, the hair is clearly seen in the centre. (Haematoxylin and eosin.)

We can find no reference to hair follicles in the tonsil in standard texts.^{1,2} Apart from being a novel discovery it has led us to conjecture about the correlation between the pathology and clinical data. There are no data to support the fact that the patient was a "headbanger" and that the repeated trauma had caused hairs in the frontal sinuses to give rise to headache or "cerebral jeep drivers disease". Obviously the hair could have caused a tickle in the throat, resulting in a focus of inflammation. As soon as pathology laboratories are privatised we think that all tonsils should be submitted for histological examination—the entire structure blocked, cut, and serially sectioned to look for hairs. If no hair is found in the tonsil then we advise a

routine oesophagoscopy, gastro-duodenoscopy, and colonoscopy to search for follicles in the upper and lower gastrointestinal tract. We have recently seen a hair within the wall of an intrahepatic abscess and this raises the advisability of biliary tree investigations for further hairs. If the result is positive it may be best, if the patient has any remaining money, to advise reference to a trichologist. As histopathologists and surgeons, we are loathe to actually advise a selective hair depiling agent though we have already bought shares in cocoa butter. The future for histology looks bright.

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Other correspondence

Choice of control patients in studies of local cervical immunology

We welcome the paper by Hughes *et al*¹ because it adds further weight to the evidence for the role of a local immunodeficiency state in the aetiology of cervical neoplasia, previously described by our unit.² The method of selection of control patients used in their study, however, requires comment.

It was stated that one group of controls had been drawn from women referred because of "clinical concern about the appearance of the cervix" or postcoital bleeding. We are not told whether any of these women had a current cervical infection, although this seems likely from our experience. The effects of infections on the distribution of Langerhans' cells in the cervical epithelium are poorly understood, but there is evidence that *Chlamydia trachomatis* may be immunosuppressive³ and that herpes simplex virus infection can increase the Langerhans' cell count in the skin.⁴ Until these effects are further investigated it would seem prudent to screen for cervical infections, especially in such high risk groups.

A second source of controls was taken from women undergoing hysterectomy who had had normal cytological smears. In view of the known false negative rate of cytology alone,⁵ it would have been appropriate if these women had undergone colposcopic examination to check further on the normality of their cervix.

Furthermore, the biopsy specimens of colposcopically visible abnormalities in the study group were exposed to acetic acid before collection; the hysterectomy specimens were not. We investigated the effects of acetic acid on three normal cervixes from fresh hysterectomy specimens of three premenopausal women. After hemisection one half of each cervix was soaked in 5% acetic acid for one minute before freezing. Each half was then similarly processed for immunohistochemical staining using DAKO-T6, as previously described.² There was no detectable effect of the acetic acid on the immunocytochemical staining. Measurements of the width of the cervical epithelium were made at five points on each biopsy specimen using a microscope attached to a computerised digitising tablet. These were performed independently by two separate observers, using only measurements through the same numbers of cell layers for comparison.

The mean width of the cervical epithelium was greater in all the acetic acid treated specimens, the difference ranging from 10 to 45%. Such a difference could lead to an artefactual increase in the measured area of an epithelium with an apparent decrease in the calculated cell count. The cause of this apparent tissue swelling is unknown, but may be related to the osmotic changes which have been reported to be the cause of aceto-whiteness in abnormal epithelium.⁶ Only controls from colposcopically normal cervixes after acetic acid application should be used to minimise such potential sources of error.

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Drs Hughes, Norval, and Howie comment

None of our first group of controls had clinical evidence of cervical infection, and no cytological or histological evidence of any specific infection. They were not screened for asymptomatic carriage of cervical pathogens, but to our knowledge there is no published work to suggest that infection with agents other than the human papilloma virus (HPV) affects Langerhans' cell counts in the cervical transformation zone. According to the paper of Levitt and Barol cited above, chlamydial infection may lead to cyclical systemic immunosuppression and stimulation but no evidence is presented to suggest that Chlamydia cause local immunosuppression. While Chlamydia have been shown to infect epithelial cells, fibroblasts, monocytes and granulocytes, we are not aware of any data on their interaction with Langerhans' cells or their effect on Langerhans' cell numbers in local epidermal sites. Braathen *et al* showed that HLA-DR positive epidermal cells, presumed to be Langerhans' cells, were able to present herpes simplex virus antigen to T cells but (contrary to the claim made above), did not even investigate whether herpes simplex virus infection had any effect on Langerhans' cell counts in skin.² Indeed, the association of herpes simplex virus with Langerhans' cells has not been established.

We are aware of the false negative rate of cervical cytology alone and it would, perhaps, have been appropriate for our second group of controls to undergo colposcopic examination. The published work from Dr Barton's own unit can be criticised on exactly the same grounds, as their six control patients did not undergo colposcopic examination at the time of cervical biopsy