One should not lose sight of the scale of the problem. We only found two cases in 1758 biopsy specimens where the diagnosis of papillary carcinoma led to resection. CD19 papillary non-nodular thyroid cancer is not a problem. We only found two cases in as many as 34% of adult thyroids when they are histologically examined in 2 to 3 mm steps. If those thyroids were thoroughly examined historically, it was estimated that about 300 microcarcinomas would have been found. The prevalence of thyroid cancer will increase still further with an increasing number of sections studied per gland. Not to mention the primary thyroid lesions where fine needle aspiration cytology, serum calcitonin levels or genetic studies helped to yield a diagnosis of an unsuspected medullary microcarcinoma where thyroidectomy should be advocated.

H RUBEN HARACH
Department of Haematopathology, Addenbrooke's Hospital, University of Cambridge, Hills Road, Cambridge CB2 2QQ


Leukaemia immunophenotyping: effect of antibody source and fluorescein on antigen detection

We read with interest the recent publication by Howard et al in which the authors highlight discrepant findings of myeloid antigen expression in cases of childhood acute lymphoblastic leukaemia (ALL). They concluded that the detection of antigens CD13 and/or CD33 may be dependent upon both the commercial source of antibody and the type of fluorochrome used. We wish to add support to their conclusions by reporting results from the United Kingdom National External Quality Assurance Scheme (UK NEQAS) for leucocyte immunophenotyping, in addition to data from our own investigations.

Results from UK NEQAS surveys have frequently shown variability in antigen detection attributable, in part, to the use of different commercial monoclonal antibodies. In survey 935, for example (acute biphenotypic leukaemia), the following mean values for CD13 expression were obtained for each reagent: Becton Dickinson (LeuM7) 15% (n = 15), Dako Cytomation (clone 2F9) 40% (n = 25), Coulter 48-25% (n = 8), Serelab 3% (n = 2), Ortho 0-5% (n = 2), and Serotec 89% (n = 1). In addition, the scheme has consistently shown statistically significant differences between samples analysed with fluorescein isothiocyanate (FITC) conjugated antibodies for the following antigens: CD3, CD5, CD13, CD14, CD19, and CD33. In survey 923, investigating CD13 detection in a case of acute myeloid leukaemia, eight of 14 laboratories using FITC conjugated antibodies obtained values less than 50% (overall mean 58%), of which three were negative results, as defined as less than 20%.

In contrast, all 12 laboratories using PE conjugated antibodies obtained results greater than 50% (mean 77%). This variation may be as a result of PE having a higher quantum yield than FITC, thus potentially increasing sensitivity.

In a parallel study to that of Howard and colleagues we have recently determined the expression of myeloid antigens in B cell chronic lymphocytic leukaemia (B-CLL). As with childhood ALL such "aberrant myeloid" expression has been reported to be of prognostic significance. To confirm these findings we examined 53 cases of B-CLL (stages 0 to IV), using Becton Dickinson PE conjugated anti-CD13 and anti-CD33 (clones L138 and P6-7, respectively), by whole blood lysis and triple colour staining. In 51 cases fewer than 4% of B cells expressed either CD13 or CD33 (6% in two cases) when compared with isotype matched controls. Mean fluorescein staining intensity (MFI) for both CD13 and CD33 expression was significantly higher than from the negative controls. Previous studies, reporting positive myeloid antigen expression, predominantly used Coulter anti-CD13 (MY7) and anti-CD33 (MY9) thus raising the possibility that these discrepant findings may relate to antibody source.

To confirm this hypothesis we re-examined 15 of the B-CLL cases with PE conjugated MY7 (CD13) and MY9 (CD33). Of these, nine expressed the CD33 antigen on > 10% of the leukaemic B cells, with five cases being regarded as positive (>20% expression); results in agreement with previous studies. The MFI values showed a significant increase when compared with controls (p < 0.001). No sample expressed less than 20% of the leukaemic B cells (one had 12%) although the values were significantly raised when compared with those obtained using Becton Dickinson antibodies (p < 0.001). We feel, therefore, that antibody source and also the fluorochrome used should be taken into account when comparing reports studying "aberrant" myeloid antigen expression.

Data from UK NEQAS, together with our own studies in acute leukaemia, do not support the conclusions of Howard and colleagues and raises several important issues. Firstly, which result is right? This question may only be answered if all commercially available reagents are standardized and validated. Considerations regarding the study of leukaemia immunophenotypic data in multicentre trials, particularly if meaningful diagnostic and prognostic information is to be obtained, will require the development of newer and more sensitive fluorochromes, coupled with multiparameter technology, will increase the dilemma as to what should be regarded as positive. The simplistic approach using an arbitrary cut off point, as suggested in the recent BSCH guidelines, will probably not be applicable in the future. Data analysis procedures which currently employ the placement of a cursor at the boundary of the negative population are likely to be inappropriate. More biologically relevant procedures, such as antigen density quantification using calibrated flow cytometers, may yield more meaningful data. Finally, despite the experience of a number of quality control schemes worldwide, there is no consensus as to the best antibody within a CD group for diagnostic use. Such evaluations would require the production of reference materials which are far from practical.


discussion

Several criticisms of Howard's paper are germane. Firstly, the authors did not attempt to standardize or validate a flow cytometric laboratory. Secondly, the analysis did not include a fluorochrome control test of antibody expression. Finally, the results were not stratified according to the type of fluorochrome used, nor other possible sources of variation such as antibody source or the method of detection.

In summary, we believe that the results presented by Howard et al. are likely to be unrepeatable in centres with different antibody sources or fluorochromes and we would like to see the results reproduced in studies with different sets of antibodies and fluorochromes.


Recurrent thrombotic occlusions of arteries and veins caused by intravascular metastatic adenocarcinoma

I refer to the case reported by Levi et al. of a young woman with recurrent vascular occlusions found at necropsy to be caused by microscopic metastatic adenocarcinoma. They rightly suspected the presence of malignant disease during life, but despite widespread invasive, radiological and laboratory investigations were unable to confirm their clinical suspicion. In their last sentence, the authors speculate that pathological examination of the blood, using specific markers for malignant cells, might have detected the adenocarcinoma cells; this may have been so, but I wonder if they performed bone marrow trephine biopsy as it is not mentioned in their list of investigations. They have not mentioned bone marrow trephine or biopsies in their paper. Similarly, no mention was made of bone marrow studies carried out on post-mortem tissues.

It is thought that one large trephine biopsy or bilateral biopsies can provide a detection rate of metastatic disease somewhere in the region of 60%. Certainly, it is a worthwhile investigation in the type of case reported by Levi et al. if performed might have resulted in the detection of occult metastases.