Papers

Use of red cell distribution width and erythrocyte zinc protoporphyrin in differential diagnosis of α and β thalassaemia and iron deficiency

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Abstract

***Aims*—To determine the value of the red cell distribution width (RDW) and erythrocyte zinc protoporphyrin (ZPP) concentration in discriminating between iron deficiency, and β and α thalassaemia in a mixed urban Asian population.**

***Methods*—The RDW and ZPP concentrations were measured in 1412 subjects attending for outpatient phlebotomy, with classification into diagnostic groups on the basis of haemoglobin, mean cell haemoglobin, ferritin, HbA_2 and haemoglobin electrophoresis.**

***Results*—Non-parametric 95% reference ranges for RDW were 11·7–15·7% and for ZPP 38–104 μmol/mol haem. Both RDW and zinc protoporphyrin rose with increasing severity of iron deficiency, but were also raised in significant numbers of subjects with β and probable α thalassaemia.**

***Conclusions*—Measurements of RDW and ZPP do not differentiate between α or β thalassaemia trait and moderate degrees of iron deficiency (hypochromasia without anaemia). ZPP is a more accurate indicator of iron deficiency than RDW and concentrations above 150 μmol/mol haem strongly suggest iron deficiency, usually with anaemia, rather than thalassaemia.**

(*J Clin Pathol* 1994;47:205–208)

The red cell distribution width (RDW) and erythrocyte zinc protoporphyrin (ZPP) concentration have both been proposed as simple tests for identifying iron deficiency and distinguishing it from other causes of microcytosis, including α and β thalassaemia.[1–4] The RDW is the coefficient of variation of the mean cell volume (MCV)—that is, (SD/MCV) × 100—and is derived from the red cell histogram on Coulter automated cell counters. It reflects anisocytosis, and is increased in many types of anaemia, including iron deficiency. ZPP is formed in the final reaction in the biosynthetic pathway of haem when zinc is incorporated into protoporphyrin IX by the enzyme ferrochelatase, rather than the usual substrate iron. Concentrations of zinc protoporphyrin rise in iron deficiency and in other conditions where the transfer of iron to or within the bone marrow is limited, such as lead poisoning or the anaemia of chronic disease.

As part of an investigation into the prevalence of iron deficiency and haemoglobinopathies in a mixed Asian population,[5] we compared RDW and ZPP in normal subjects and those with iron deficiency of different grades and α and β thalassaemia, to determine the diagnostic value of these parameters, and their value as screening tests.

Methods

Subjects were referred by general practitioners, hospital outpatient, and antenatal clinics to the local phlebotomy service for routine blood tests. They were about 80% female, of whom one quarter were pregnant, and 20% male. Ages ranged from 14 to 85 years. The study was approved by the local ethics committee and informed consent obtained. An additional 5 ml of blood was taken when necessary.

A total of 1412 subjects (1256 South Asian and 156 Caucasian) were studied and 1233 subjects had both RDW and ZPP measured. Thirty eight subjects did not have the RDW measured, and are included only in the ZPP analysis, while none of the Caucasians, who were added as a small control group to the original survey, had ZPP measured, and these are included only in the RDW data.

All subjects had a full blood count (including haemoglobin, red blood count, mean red cell volume (MCV) and mean red cell haemoglobin (MCH) using a Coulter S + VI automated cell counter: when the MCH was less than 27·0 pg, HbA_2 was quantified using an inhouse column method. Haemoglobin electrophoresis was performed on cellulose acetate (pH 8·6) (Helena Laboratories): when a structural variant was found, electrophoresis was repeated at pH 6·0 on citrate agar (Beckman Paragon Gel). A sickle solubility test was performed when a band was identified in the S/D region. Plasma ferritin was measured using the Becton-Dickinson immunoradiometric assay. RDW was obtained from the red cell profile on the Coulter S + VI. ZPP was measured in a portable haematofluorometer using the Proto-

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Fluor Reagent System (Helena Laboratories). The analytical range of the haematofluorometer was 0–200 μmol/mol haem.

Subjects were assigned to a diagnostic group on the basis of haemoglobin, MCH, haemoglobin electrophoresis, HbA_2 concentration and plasma ferritin, as follows:

(1) *Iron deficiency—mild* (reduced iron stores): normal haemoglobin (≥130 g/l in men; ≥110 g/l in non-pregnant women; ≥100 g/l in pregnant women), normal MCH (≥27·0 pg), ferritin <20·0 μg/l.

(2) *Iron deficiency—moderate* (hypochromic red cells): normal haemoglobin, MCH <27·0 pg, ferritin <20·0 μg/l.

(3) *Iron deficiency—severe* (iron deficiency anaemia): reduced haemoglobin, MCH <27·0 pg, ferritin <20·0 μg/l.

(4) *Normal*: normal haemoglobin (as above), normal MCH (≥27·0 pg), ferritin ≥20·0 μg/l, normal haemoglobin electrophoresis.

(5) *β thalassaemia trait*: MCH <27·0 pg; HbA_2 >3·5%; ferritin ≥20·0 μg/l.

(6) *β thalassaemia trait plus iron deficiency*: MCH <27·0 pg; HbA_2 >3·5%; ferritin <20·0 μg/l.

(7) *Probable α thalassaemia trait*: MCH <27·0 pg; HbA_2 ≤3·5%; ferritin ≥20 μg/l; no structural variants on haemoglobin electrophoresis.

Subjects with structural haemoglobin variants, macrocytosis, and other miscellaneous abnormalities are not considered further in this paper.

Data were analysed using BMDP/386 (BMDP Statistical Software) and SYSTAT for Windows 5·2. Intergroup comparisons were made using the Kruskal-Wallis ANOVA with multiple comparisons of BMDP3S, and discriminant analysis using BMDP7M with prior probabilities estimated from the sample frequencies and log-transformed variables.

Table 1 MCV and MCH in different diagnostic groups

		MCV	MCH
Diagnosis	*n =*	*Mean (SD)*	*Mean (SD)*
Normal	664	88·4 (4·8)	30·0 (1·5)
Iron deficient			
Mild	366	87·1 (4·3)	29·5 (1·7)
Moderate	129	77·3 (3·8)	25·4 (1·4)
Severe	87	70·1 (6·8)	22·2 (2·7)
β thalassaemia trait (iron replete)	27	64·2 (3·0)	20·2 (1·0)
β thalassaemia trait (iron deficient)	9	65·0 (3·5)	20·7 (1·2)
α thalassaemia (probable)	68	76·7 (3·8)	25·3 (1·5)

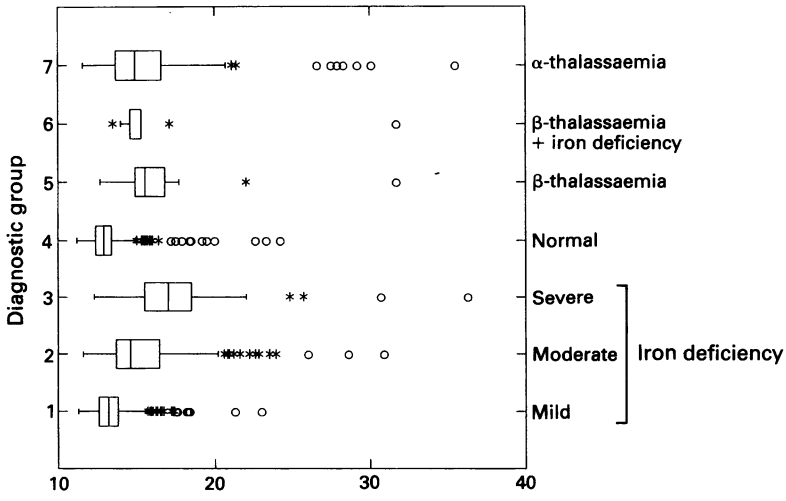

Figure 1 Box and whisker plots of RDW (%) in the different diagnostic groups. The box delimits the interquartile (50%) range or "Hspread" of each distribution and contains a vertical line which indicates the median. The whiskers show the ranges within 1·5 Hspreads of the ends of the boxes (the upper and lower hinges; (★) marks "outside" values > ±1·5 Hspreads from the box hinges; (o) marks "far outside" values >±3 Hspreads from the box hinges.

Results

Mean red cell volume (MCV) and mean red cell haemoglobin (MCH) in the various diagnostic groups are shown in table 1. Values in β thalassaemia trait were more severely microcytic than those in the α thalassaemia group. In this population the α^0-thalassaemia genotype is not found,[6,7] and our subjects with probable α thalassaemia were either heterozygotes or homozygotes for the mild α^+-thalassaemia trait.

The distribution of RDW in normal subjects was not Gaussian and although logarithmic transformation approximated a Gaussian distribution it still had a pronounced right skew. From the lognormal distribution curve our 95% range (± 2SD) was 11·0-15·4, which was close to the 2·5–97·5 percentile range of 11·7–15·7 (n = 654; 95% confidence limits for 2·5 and 97·5 percentiles were 11·6–11·7 and 15·6–15·9, respectively). There were small but significant differences ($p < 0·05$) among normal subjects, between pregnant women (median 13·2%), and both non-pregnant women (median 12·9%) and men (median 12·7%) but no significant difference between men and non-pregnant women. The distributions of results and summary statistics for all subjects in different diagnostic groups are shown in fig 1 and table 2. There was a clear progression in the median RDW with increasing severity of iron deficiency, but considerable overlap between groups. Values in all the abnormal diagnostic groups were significantly higher than control groups, but there were no significant differences between subjects with moderate or severe iron deficiency and those with either β or probable α thalassaemia (table 2). This was also true when pregnant women were excluded from the analysis.

The distribution of ZPP values in normal subjects approximated a lognormal distribution. From the lognormal distribution curve our 95% range (±2SD) was 34-94 μmol/mol haem; the 2·5-97·5 percentile range was 38–104 μmol/mol haem (n = 578; 95% confidence limits for 2·5 and 97·5 percentiles were 38–39 and 101–111, respectively). There was a significant difference ($p < 0·05$), among the normal subjects, between men (median 49 μmol/mol haem) and both pregnant (median 62 μmol/mol haem) and non-pregnant women (median 57 μmol/mol haem), but no significant difference between pregnant and non-pregnant women. The distributions of results and summary statistics for all subjects in different diagnostic groups are

shown in fig 2 and table 2. As with RDW, ZPP values rise with increasing severity of iron deficiency. Again, values in all the abnormal diagnostic groups were significantly higher than normal subjects and there were no significant differences between subjects with moderate or severe iron deficiency and those with β thalassaemia (table 2). Subjects with probable α thalassaemia, however, did have significantly lower values than those with moderate or severe iron deficiency and this still held true when men were excluded from the analysis.

Receiver-Operating Characteristic (ROC) analysis comparing the abnormal diagnostic groups with normal subjects showed ZPP to be more accurate than RDW in the diagnosis of all grades of iron deficiency (fig 3),[8] but RDW was more accurate in the diagnosis of α thalassaemia. Both were equally effective in the diagnosis of β thalassaemia (fig 4). The ROC curve for ZPP in severe iron deficiency (fig 3) was very close to the upper left hand corner of the plot and suggested that at some cutoff levels it may achieve a near perfect discrimination from normal values. A concentration of 100 μmol/mol haem gave the most efficient discrimination between normal subjects and those with severe iron deficiency (fig 5).

A discriminant analysis of the data in this study showed that RDW, ZPP, and RDW and ZPP combined, gave correct overall diagnostic classifications of only 50%, 55%, and 54%, respectively.

Table 2 RDW(%) and ZPP in different diagnostic groups

	RDW(%)			ZPP		
Diagnosis	*n =*	*Median*	*95% range*	*n =*	*Median*	*95% range*
Normal	654	12·9	11·7–15·7	578	54	38–104
Iron deficient						
Mild	363	13·2	11·8–17·2	315	65	44–113
Moderate	126	14·6	12·8–23·5	128	99	52– >200
Severe	91	16·9	12·6–25·7	86	159	90– >200
β thalassaemia trait (iron replete)	25	15·6	12·9–31·7	27	98	62–152
β thalassaemia trait (iron deficient)	9	14·6	14·0–31·7	9	119	77–147
α thalassaemia (probable)	64	15·0	12·6–29·2	68	78	38–>200

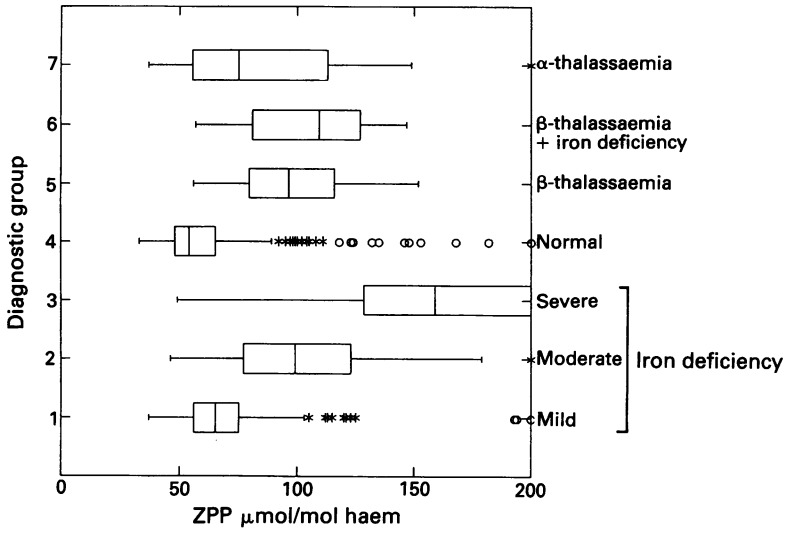

Figure 2 Box and whisker plots of ZPP in the different diagnostic groups.

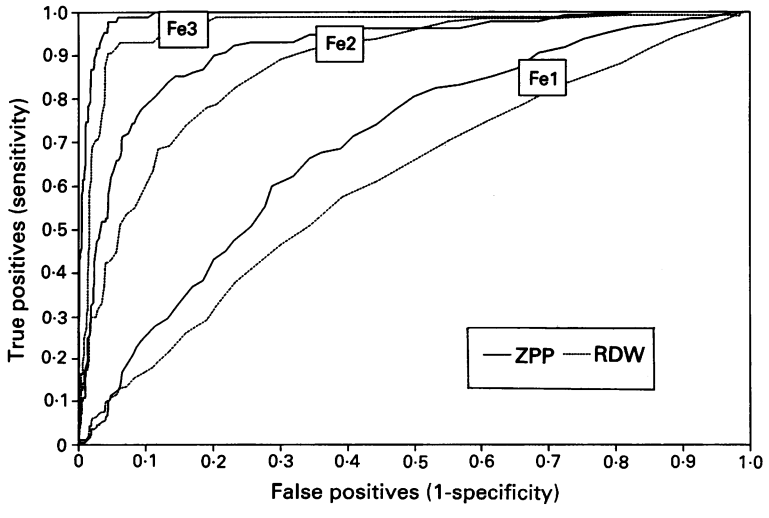

Figure 3 ROC plot of RDW and ZPP in mild (Fe1), moderate (Fe2), and severe (Fe3) iron deficiency.

Discussion

In the population studied iron deficiency and both α and β thalassaemia are common.[5] Of the South Asian subjects studied in this paper, 27·5% were hypochromic (MCH of < 27 pg). Distinguishing between the various possible causes of hypochromasia in such a population requires relatively expensive and labour intensive investigations. A simple and inexpensive screening test which would reliably distinguish iron deficiency from other causes of hypochromasia would be extremely useful. Both RDW and zinc protoporphyrin, as well as various discriminant functions based on red cell parameters, have been proposed.[9–11]

Bessman *et al*[1] reported a clear distinction between RDW in normal subjects (mean 13·4 (1·2)) and iron deficient subjects (mean 16·3 (1·8)), and found concentrations in thalassaemic subjects to be normal (mean 13·5 (1·5) in α thalassaemia; 13·7 (1·6) in β thalassaemia). Flynn *et al*[12] found that only 55% of thalassaemic subjects, overall, had a normal RDW. Our normal range was considerably wider than that of Bessman *et al*, and this may reflect the fact that our "normals" are defined as such by haematological parameters alone, and were patients attending for blood tests in routine clinical practice, whereas those in Bessman's study were students. We found only 44% of subjects with β thalassaemia and 61% of those with α thalassaemia to have normal RDW values, and no significant difference between the iron deficient and either β or α thalassaemic group. These results are similar to those of Flynn *et al*. We conclude that RDW values in iron deficiency and thalassaemia overlap, and do not distinguish between causes of microcytosis.

In some studies ZPP correlates well with

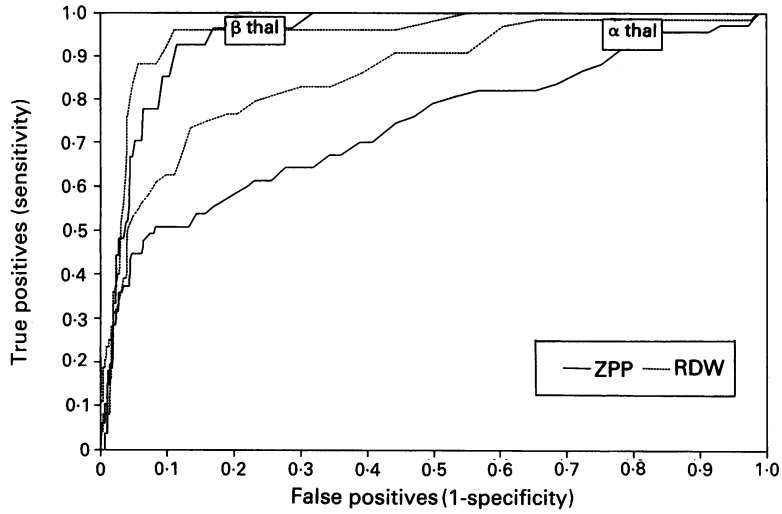

Figure 4 ROC plot of RDW and ZPP in β thalassaemia (Bthal) and α thalassaemia (α thal).

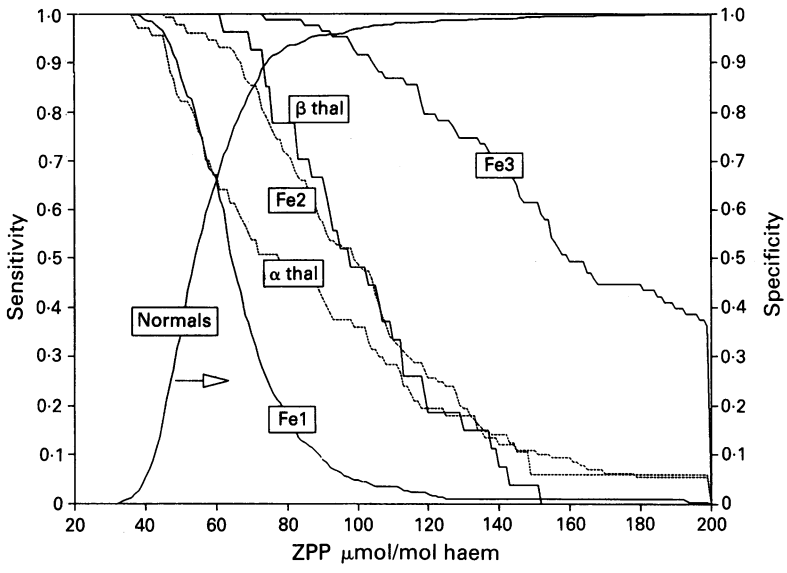

Figure 5 ZPP concentration and sensitivity or specificity. The arrow indicates the y-axis for normal subjects (specificity). For any chosen concentration of ZPP the graph indicates the proportion of true positive results (sensitivity) in each abnormal diagnostic group and true negative results in the normal group (specificity).

serum ferritin and rises in the relatively early stages of iron deficiency before the onset of hypochromasia.[13] The results in normal iron-replete subjects in our study show different ZPP concentrations in men and women who are either non-pregnant or pregnant. This may reflect relative lack of bone marrow iron availability in women, especially in pregnancy, even with plasma ferritin concentrations well within the normal range. We found significantly higher ZPP values in subjects with mild iron deficiency, without anaemia or hypochromasia, compared with normal subjects, and also between the different grades of iron deficiency. The overlap between normal and mild iron deficiency was too great to rely on ZPP as a very sensitive early screening test, 95% of those with mild iron deficiency falling within our normal range (fig 5).

We found raised ZPP values in 48% of the subjects with β thalassaemia even when coincident iron deficiency was excluded. Similar increases in FEP (free erythrocyte protoporphyrin) in β thalassaemia were found by Junca *et al*[14] and in ZPP by Han *et al.*[15] Additionally, we were not able to distinguish most cases of moderate or severe iron deficiency from β thalassaemia using the ZPP value. The α thalassaemia group did have significantly lower values than the moderate and severe iron deficiency groups, but with considerable overlap, and 37% were outside the normal range. We found that values greater than 150 μmol/mol haem were highly suggestive of iron deficiency rather than thalassaemia. The increased ZPP found in β, and to a lesser extent α, thalassaemia trait in subjects who are clearly not iron deficient is interesting and may suggest an intracellular disturbance of iron metabolism in these conditions.

We thank Helena Laboratories for the loan of the haematofluorometer and use of reagents.

1 Bessman JD, Gilmer PR, Gardner FH. Improved classification of anemias by MCV and RDW. *Am J Clin Pathol* 1983;**80**:322–6.
2 Bhambhani K, Aronow R. Lead poisoning and thalassaemia trait or iron deficiency. The value of the red cell distribution width. *Am J Dis Child* 1990;**144**:1231–3.
3 Hershko C, Konijn AM, Link G, Moreb J, Grauer F, Weissenberg E. Combined use of zinc protoporphyrin (ZPP), mean corpuscular volume and haemoglobin measurements for classifying microcytic RBC disorders in children and young adults. *Clin Lab Haematol* 1985;7:259–69.
4 Marsh WL, Nelson DP, Koenig HM. Free erythrocyte protoporphyrin (FEP) II. The FEP test is clinically useful in classifying microcytic disorders in adults. *Am J Clin Pathol* 1983;**79**:661–6.
5 Tillyer ML, Varawalla NY, Tillyer CR, Sandhu P, Modell B. Thalassaemia, abnormal haemoglobins and iron deficiency in a British Asian population. *Clin Lab Haematol* 1993;**15**:157–64.
6 Walford DM, Deacon R. Alpha-thalassaemia trait in various racial groups in the United Kingdom: characterization of a variant of alpha-thalassaemia in Indians. *Br J Haematol* 1976;**34**:193–206.
7 Kulozik AE, Kar BC, Serjeant GR, Serjeant BE, Weatherall DJ. The molecular basis of α-thalassaemia in India. Its interaction with the sickle cell gene. *Blood* 1988;**71**:467–72.
8 Zweig MH, Campbell G. Receiver-Operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clin Chem* 1993;**39**:561–77.
9 England JM, Fraser PM. Differentiation of iron deficiency from thalassaemia trait by routine blood count. *Lancet* 1973;**i**:449–52.
10 Mentzer WC. Differentiation of iron deficiency from thalassaemia trait. *Lancet* 1973;**i**:882.
11 Walford DM, McPherson K, Deacon R. Discrimination between iron deficiency and heterozygous thalassaemia. *Lancet* 1979;**i**:323.
12 Flynn MM, Reppun TS, Bhagavan NV. Limitations of red cell distribution width (RDW) in evaluation of microcytosis. *Am J Clin Pathol* 1986;**85**:445–9.
13 Labbe RF, Rettmer RL. Zinc protoporphyrin: a product of iron-deficient erythropoiesis. *Semin Hematol* 1989;**36**: 40–6.
14 Junca J, Flores A, Roy C, Alberti R, Milla F. Red cell distribution width, free erythrocyte protoporphyrin, and England-Fraser Index in the differential diagnosis of microcytosis due to iron deficiency or beta-thalassaemia trait. A study of 200 cases of microcytic anemia. *Hematol Pathol* 1991;**5**:33–66.
15 Han P, Kung KP, Teo CP, Tam LP. α-Thalassaemia and β-thalassaemia traits: biological difference based on red cell indices and zinc protoporphyrin. *Clin Lab Haematol* 1990;**12**:169–76.

brief descriptions of geographical distribution, morphology, and life cycle.

Section 2 contains over 250 colour photographs covering a wide range of parasite morphology, pathology (including stained sections), and clinical pictures with captions on the facing page. Although the overall quality of the photographs is excellent, I was disappointed to see a lack of size markers on all but a handful. In the clinical laboratory size is of vital importance for identifying ova and cysts.

The third section contains black and white electron micrographs, radiographs, and other illustrations, separated from the colour section for reasons of economy. This does not detract from the atlas in any way, and indeed some of the scanning electron microscopic images are quite breathtaking. I would, however, like to have seen some indication of size on the photographs.

This atlas has a spacious and orderly feel to it, and I am impressed by the overall quality. Clinical microbiologists, particularly those in training, will find it useful.

AJ HAY

Atlas of Ovarian Tumors. L Deligdisch, A Altchek, CJ Cohen. (Pp 182; £94.) Igaku-Shoin. 1994. ISBN 0-89640-240-1.

This sumptuously produced atlas is subdivided into two main sections with three chapters devoted to "clinical aspects" and seven chapters allocated to "pathology". This strategy may be convenient in a multiauthor text, but it has resulted in a clinical section which is pathologically naive and a pathological section impoverished by the paucity of clinicopathological correlation.

The wide-ranging introductory chapter, which covers epidemiology, genetics, molecular biology, early diagnosis, and screening for ovarian cancer, provides a useful overview of the subject, although the emphasis placed on ultrasonography is excessive. The two ensuing chapters, both rather lengthy and repetitive, are devoted to management of ovarian carcinoma and nonepithelial tumours, respectively.

The pathology section comprises four chapters devoted to primary epithelial neoplasms including a whole chapter on the interesting but controversial subject of ovarian intraepithelial neoplasia. Other chapters deal with sex cord-stromal tumours, germ cell tumours, and metastatic tumours. This section is well illustrated with adequate photomicrographs and gross photographs of excellent quality. However, many entities are skimpily and uncritically described with no attempt to evaluate the taxonomic overenthusiasm displayed by recent authors in this field. On the other hand, many rare but well established entities are not included. An even more serious drawback for a book aimed at the practising histopathologist is the lack of consideration given to possible differential diagnoses.

In conclusion, this new atlas is unlikely to fulfil the need for a comprehensive, authoritative, and up to date reference text on ovarian neoplasms. It cannot be recommended as a bench book for the reporting room.

SM ISMAIL

Notices

Postgraduate course: Current concepts in surgical pathology

November 14–18 1994

Massachusetts General Hospital, Harvard Medical School

This course is designed for pathologists at resident and practitioner levels. It will provide an in-depth review of diagnostic surgical pathology with emphasis on morphological features, newly recognised entities, and new techniques, presented by the faculty of the Department of Pathology, Massachusetts General Hospital. Instruction will be primarily by lecture, but will also include discussion periods. Each participant will receive a comprehensive course syllabus.

The course has category 1 accreditation for about 35 hours CME credit by the American Medical Association. The fee for the course is $785·00 (residents and fellows $575·00).

For further information contact: Department of Continuing Education, Harvard Medical School, 25 Shattuck Street, Boston, MA 02115 USA (Tel: 0101 (617) 432 1525).

Update on Cerebrovascular Pathology

Thursday 8 December 1994 (one-day) to be held at The Royal College of Pathologists, 2 Carlton House Terrace, London SW1Y 5AF.

The meeting is open to members and non-members of the College. Further details and application forms can be obtained from the Scientific Meetings Officer, RCPath, 2 Carlton House Terrace, London SW1Y 5AF (Tel: 071 930 5862 ext: 24/26).

Cytopathology for histopathologists Northwick Park Hospital

30 January-3 February 1995

This is an intensive course in cytopathology suitable for candidates preparing for the MRCPath examination in histopathology, and for established histopathologists requiring revision. It is given by the Department of Cellular Pathology, Northwick Park Hospital (Dr Elizabeth A Hudson) and the Department of Cytopathology, St Mary's Hospital Medical School, University of London (Professor Dulcie Coleman).

The programme will consist of lectures, microscopy sessions, and discussions. Topics will include cytopathology of the cervix, urine, the respiratory tract, serous effusions and fine needle aspiration cytology of breast and other sites. The course is limited to 30 participants. The course fee is £300 excluding accommodation.

Applications and enquiries should be made to: Dr Elizabeth Hudson, Department of Cellular Pathology, Northwick Park Hospital, Harrow, Middlesex HA1 3UJ (Tel: 081–869 3312).

Corrections

J Clin Pathol 1994;47:205–8; Tillyer *et al.* The title of the correspondence should read "zinc protoporphyrin assays in patients with α and β thalassaemia trait." The title at present implies that zinc assays were performed which was not the case.

In paragraph 2, the second sentence should read ". . . not only were the drugs causing substantial interference extremely unlikely in the outpatient and general practice population we studied, . . .". Paragraph 3 second sentence should read ". . .Paul and Brumfitt's is 15 μmol/mol haem lower."

DR ML TILLYER

Estimation of haemoglobin concentrations using spectrophotometric tests. *J Clin Pathol* 1994;47:681.

The name of the author was given incorrectly as J Larner rather than AJ Larner.

ANDREW J LARNER

J Clin Pathol 1993;46:1116–9. (Darjee R, Gibb AP. Serological Investigation into the association between *Streptococcus bovis* and colonic cancer.) The methods section refers to "NCTC10449 (*Enterococcus fecalis*), but this should read ATCC19433 (*Enterococcus fecalis*). NCTC10449 is in fact the reference number of the type strain of *S mutans*.

AP GIBB

Increased pentane and carbon disulfide in the breath of patients with schizophrenia *J Clin Pathol* 1993;46:861–4. The concentrations of pentane and carbon disulfide were reported incorrectly. All values of pentane should be multiplied $\times 50$; all values of carbon disulfide $\times 0{\cdot}05$. The statistical analyses and conclusions of the paper are not affected by these corrections.

MICHAEL PHILLIPS

Chu CM, Liaw YF. Coexpression of intercellular adhesion molecules and class I major histocompatibility compression antigens on hepatocyte membrane in chronic viral hepatitis. *J Clin Pathol* 1993;46: 1004–8. The correct version of fig 2D is reproduced below.

CHIA-MING CHU