A comparison of 13 guinea pig and human anti-tissue transglutaminase antibody ELISA kits

R C W Wong, R J Wilson, R H Steele, G Radford-Smith, S Adelstein

Aims: Tissue transglutaminase (tTG) is a major autoantigen recognised by IgA anti-endomysial antibodies (IgA EMA). Enzyme linked immunosorbent assays (ELISA) for IgA anti-tissue transglutaminase antibodies (IgA tTG) have therefore been developed as an alternative serological screening test to IgA EMA for coeliac disease (CD). The use of human tTG (h-tTG), as opposed to guinea pig liver tTG (gpl-tTG), in these assays has been reported to produce superior results. This study compared 13 commercial IgA tTG ELISA kits to ascertain their performance characteristics in the diagnosis of CD in patients with biopsy confirmed disease compared with controls. All patients and controls were adults aged 21 years or older.

Methods: Sera from the following groups of patients were tested in each kit: (1) 49 patients with CD confirmed on small bowel biopsies (all IgA EMA positive); (2) 34 patients with small bowel biopsies that were not consistent with CD; and (3) 30 patients with biopsy confirmed inflammatory bowel disease. All controls were negative for IgA EMA and were not IgA deficient. Sensitivities and specificities were determined using both the manufacturers’ recommended cut off points and receiver operating characteristic (ROC) analysis derived decision thresholds. The area under the curve (AUC) for each ROC plot was also calculated and compared between kits.

Results: In general, the h-tTG based IgA tTG ELISA kits demonstrated superior performance (especially specificity) compared with the gpl-tTG based kits, although 100% sensitivity and specificity (comparable to the IgA EMA assay) was obtained in only one recombinant h-tTG based kit.

Conclusions: The use of h-tTG in IgA tTG ELISA kits is generally, but not universally, associated with superior performance. Factors other than antigen source are important in determining kit performance.

Although many studies have concluded that the IgA tTG assay has comparable performance to the IgA EMA IIF assay, several have reported false negative IgA tTG results in subjects with IgA EMA positive untreated CD.4 10–16 19–21 25 26 28–30 32 33 34 36 38 and false positive IgA tTG results in the absence of IgA EMA and CD.13–15 23 24 29 35 38 40 However, most of these studies used gpl-tTG, which has only about 81% homology with h-tTG.4 In contrast, the use of h-tTG has been reported to be associated with fewer false negative and false positive results, and an overall performance closely comparable or equal to the “gold standard” IgA EMA IIF assay.15–17 21 24 29 35 38 However, because none of these studies has compared gpl-tTG based ELISAs with two or more h-tTG-based ELISAs, it is unclear whether the use of h-tTG alone results in superior performance to the gpl-tTG-based assays.

We compared 13 commercial IgA tTG ELISA kits, seven gpl-tTG based and six h-tTG based (four recombinant h-tTG), in 49 IgA EMA positive adult patients with CD and 64 adult disease controls to establish the sensitivity and specificity of each
Comparison of anti-tTG ELISA kits

Table 1

<table>
<thead>
<tr>
<th>Manufacturer/Site</th>
<th>Source of tTG</th>
<th>Calcium activated</th>
<th>Source of NS antibody</th>
<th>Calcium activated</th>
<th>Serum dilution</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>AESKULISA/Aesku.Lab Diagnostica (Wendelsheim, Germany)</td>
<td>Recombinant human</td>
<td>Yes</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>The Binding Site (Birmingham, UK)</td>
<td>Recombinant human</td>
<td>Yes</td>
<td>Guinea pig liver</td>
<td>Yes</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>Eurospital S.p.A (Trieste, Italy)</td>
<td>Recombinant human</td>
<td>No</td>
<td>Sheep</td>
<td>No</td>
<td>1/100</td>
<td>PNPP</td>
</tr>
<tr>
<td>Genesis Diagnostics (Littleport, UK)</td>
<td>Recombinant human</td>
<td>No</td>
<td>Guinea pig liver</td>
<td>No</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>Immunopharmacology Research Diagnostics (Catania, Italy)</td>
<td>Recombinant human</td>
<td>No</td>
<td>Guinea pig liver</td>
<td>No</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>QUANTA Lite/Inova (Diagnostics Inc, San Diego, California, USA)</td>
<td>Recombinant human</td>
<td>No</td>
<td>Guinea pig liver</td>
<td>No</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>Medizyme/Medipan Diagnostica GmbH (Selchow, Germany)</td>
<td>Recombinant human</td>
<td>No</td>
<td>Guinea pig liver</td>
<td>No</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>Orgentec Diagnostika GmbH (Mainz, Germany)</td>
<td>Recombinant human</td>
<td>No</td>
<td>Guinea pig liver</td>
<td>No</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>Varelisa/Pharmacia &amp; Upjohn Diagnostics GmbH &amp; Co (Freiburg, Germany)</td>
<td>Recombinant human</td>
<td>No</td>
<td>Guinea pig liver</td>
<td>No</td>
<td>1/100</td>
<td>TMB</td>
</tr>
<tr>
<td>†In the manufacturer’s kit insert, alkaline phosphatase was mentioned under “Principle”, but HRP was mentioned under “Reagent supplied.”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HRP, horseradish peroxidase; NS, not stated; PNPP, paranitrophenyl phosphate; TMB, 3,3′,5,5′-tetramethylbenzidine; tTG, tissue transglutaminase.

Cut off values

Both the manufacturers’ recommended cut off values and decision thresholds determined by receiver operating characteristic (ROC) plots (see below) were used to calculate the sensitivity and specificity of each assay/kit. The IBD controls kit, and thus determine whether the h-tTG based kits consistently outperformed the gpl-tTG based kits, and produced comparable results to the IgA EMA IIF assay.

METHODS

Patients

One hundred and thirteen sera were selected from samples submitted to: Division of Immunology, Queensland Health Pathology Services, Royal Brisbane and Princess Alexandra Hospitals; Central Sydney Immunology Laboratory; and Department of Immunology, Sullivan Nicolaides Pathology. These comprised sera from the following patients who were aged 21 years or older: (1) 49 patients with typical histological changes of CD on small bowel biopsy, who had previously been found to have a positive IgA EMA, 38 of whom had never been on a gluten free diet, and 11 of whom were poorly compliant or non-compliant with the diet and had an abnormal small bowel biopsy close to the time of blood sampling; (2) 34 subjects who had been investigated with upper gastrointestinal fibroptic endoscopy and small bowel biopsy for possible CD and were found not to have histological changes consistent with CD (non-CD, with the following results on small bowel biopsy (no evidence of villous atrophy in all cases): normal duodenum (n = 27), duodenal ulcer (n = 3), dilated Brunner’s glands (n = 1), non-specific duodenitis (n = 1), fibrotic and thickened small bowel (n = 1), and gastric atrophy (n = 1)); and (3) 30 subjects with biopsy confirmed inflammatory bowel disease (IBD controls).

All sera were retested for IgA EMA at the start of the study to ensure that the sera from patients with CD had not degraded during storage at −70°C. Total serum IgA values were also measured in all 64 non-CD and IBD control sera by nephelometry (Behring Diagnostics, Frankfurt, Germany). All 64 controls had values within the normal range for adults (1.24–4.16 g/litre), thus excluding IgA deficiency as a potential cause for negative results.

IgA EMA IIF assay

The IgA EMA assay was performed by IIF using cryostat sections of monkey oesophagus (The Binding Site, Birmingham, UK), as described previously at a screening dilution of 1/4. All slides were viewed by two independent observers and a positive or negative result was determined by consensus.

IgA tTG ELISA

The manufacturer’s instructions (table 1) were followed for all 13 IgA tTG ELISA kits. All specimens were tested in duplicate.

Bovine serum albumin and gelatin coated ELISA plates

To investigate the possibility of IgA anti-bovine serum albumin (BSA) antibodies producing false positive IgA tTG results, ELISA plates (Costar, Corning Inc, New York, USA) were coated with 250 µl of 5% BSA (Sigma Chemical Co, St Louis, Missouri, USA) or 1% gelatin (Bio-Rad, Hercules, California, USA). Serum diluted 1/100 in Tween/phosphate buffered saline was incubated for one hour at room temperature. After three washes, horseradish peroxidase (HRP) labelled goat antihuman IgA (Silenus Labs, Melbourne, Australia), at a dilution of 1/500, was added and the plates were incubated for one hour (room temperature). ABTS (2,2’-azino-bis-3-ethylbenzthiazolin-6-sulphonic acid) substrate (Medical Innovations, Sydney, Australia) was added for 15 minutes, and absorbances read at 405 nm.
were not used in the calculation of specificity because some had not undergone small bowel biopsy to exclude CD.

**RESULTS**

The IgA tTG values of the patients with CD and the non-CD and IBD controls measured with the 13 kits are shown in fig 1 (gpl-tTG based kits) and fig 2 (h-tTG based kits) with corresponding ROC curves and AUC estimations. The numbers of sera from patients with CD, and the non-CD and IBD controls that were positive in each assay, using both the manufacturers’ and ROC analysis derived decision thresholds, are shown in table 2 (gpl-tTG based kits) and table 3 (h-tTG based kits), with corresponding sensitivities and specificities. Table 4 shows the AUC comparisons between kits, with a significant difference denoted by a p value of < 0.05.

**Figure 1** IgA anti-tissue transglutaminase (tTG) antibody values of the patients with coeliac disease (CD), and the non-CD (SBX) and inflammatory bowel disease (IBD) controls in the seven purified guinea pig liver tTG based enzyme linked immunosorbent assay (ELISA) kits, with corresponding ROC curve and area under curve (AUC) estimations. The solid lines represent the manufacturers’ recommended cut off values and the broken lines represent the ROC plot derived decision thresholds. (A) The Binding Site, (B) Eurospital, (C) Genesis Diagnostics, (D) Immunopharmacology Research Diagnostics, (E) QUANTA Lite (Inova), (F) Medizyme (Medipan Diagnostica), (G) Immulisa (Immco).
purified erythrocyte h-tTG based QUANTA Lite (Inova Diagnostic Inc, San Diego, California, USA) kits performed best, with sensitivities of 100% and 98%, specificities of 100% and 100% (using the manufacturers’ cut off values), and AUC estimations of 1.000 and 1.000, respectively (fig 2; table 3).

Of the seven gpl-tTG based kits (fig 1; table 2), the QUANTA Lite kit performed best, with 86% sensitivity and 100% specificity using the manufacturer’s cut off value of 20 arbitrary units/ml, and an AUC of 0.987. Applying the ROC analysis derived decision threshold of 14.1 arbitrary units/ml improved sensitivity to 92% but reduced specificity to 97%.

**Figure 2** IgA anti-tissue transglutaminase (tTG) antibody values of the patients with coeliac disease (CD), and the non-CD (SBX) and inflammatory bowel disease (IBD) controls in the six human tTG based enzyme linked immunosorbent assay (ELISA) kits, with corresponding receiver operating characteristic (ROC) curves and area under curve (AUC) estimations. The solid lines represent the manufacturers’ recommended cut off values and the broken lines represent the ROC plot analysis derived decision thresholds. (A) AESKULISA (Aesku.Lab), (B) The Binding Site, (C) Eurospital, (D) QUANTA Lite (Inova), (E) Orgentec, (F) Varelisa (Pharmacia & Upjohn).

**Table 2** IgA EMA and IgA tTG results in patients with CD and controls using the manufacturers’ cut off points and ROC plot analysis derived decision thresholds for the seven guinea pig liver tTG based ELISA kits

<table>
<thead>
<tr>
<th>Assay type/Manufacturer</th>
<th>Manufacturer’s cut off point</th>
<th>ROC plot analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cut off</td>
<td>CD (sensitivity)</td>
</tr>
<tr>
<td>IgA EMA IIF/The Binding Site</td>
<td>1/4</td>
<td>49/49 (100%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/The Binding Site</td>
<td>4 U/ml</td>
<td>43/49 (88%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Eurospital</td>
<td>5 AU</td>
<td>48/49 (98%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Genesis Diagnostics</td>
<td>10 U/ml</td>
<td>47/49 (96%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/ImmuLisa</td>
<td>20 EU/ml</td>
<td>45/49 (92%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Immunopharmacology Research Diagnostics</td>
<td>25 AU</td>
<td>49/49 (100%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/QUANTA Lite</td>
<td>20 units/ml</td>
<td>42/49 (86%)</td>
</tr>
<tr>
<td>Guinea pig liver tTG based ELISA/Medizyme</td>
<td>25 U/ml</td>
<td>48/49 (98%)</td>
</tr>
</tbody>
</table>

Results equal to or greater than the cut off/threshold were considered positive. Specificity was calculated using only the non-CD controls (see text). AU, arbitrary units; CD, coeliac disease; ELISA, enzyme linked immunosorbent assay; IBD, inflammatory bowel disease; IgA EMA, IgA anti-endomysial antibody; IgA tTG, IgA anti-tissue transglutaminase antibody; IIF, indirect immunofluorescence; NA, not applicable; tTG, tissue transglutaminase; ROC, receiver operating characteristic.
DISCUSSION

In this comparison of 13 commercial IgA tTG ELISA kits, we found that the human tTG based kits tested generally demonstrated superior performance (especially specificity) to the gpl-tTG based kits in IgA EMA positive samples. However, three of the six h-tTG based kits (two recombinant h-tTG based) evaluated also produced false positive results in the absence of IgA EMA and CD, as previously reported. Therefore, other explanations for false positive results are required.

The methods used to extract and purify tissue derived tissue transglutaminase (tTG), produce and process recombinant tTG, and then coat tTG on to ELISA wells may lead to alterations in the tertiary structure of tTG.

More false positive IgA tTG results were detected in the non-CD controls compared with the IBD controls. A possible explanation may be the presence of IgA anti-BSA antibodies in some of the non-CD control sera, reacting with the BSA used as a blocking agent in some kits. However, Lock and colleagues did not detect significant IgA anti-BSA antibodies in two disease controls tested, and significant IgA anti-BSA antibodies were not demonstrated in our non-CD controls.

False negative results were found in six of the seven gpl based and five of the six h-tTG based kits in IgA EMA positive samples. These findings raise the important issue of contaminants in gpl-tTG, which may contain other hepatic proteins. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the gpl-tTG extract (Sigma T5398; Sigma Chemical Co) used in several gpl-tTG based ELISAs contains multiple bands in addition to the 82–83 kDa tTG band, which only accounted for about 30% of the total protein. This may be partially overcome by further purification steps, and should be less of an issue with recombinant h-tTG.

Results greater than or equal to the cut-off/threshold are considered positive. Specificity was calculated using only the non-CD controls (see text).

### Table 3: IgA EMA and IgA tTG results in patients with CD and controls using manufacturers’ cut-off points and ROC plot analysis derived decision thresholds for the six human tTG based ELISA kits

<table>
<thead>
<tr>
<th>Manufacturer’s cut off point</th>
<th>ROC plot analysis</th>
<th>Cut off</th>
<th>CD (sensitivity)</th>
<th>Non-CD controls (specificity)</th>
<th>IBD controls</th>
<th>Threshold</th>
<th>CD (sensitivity)</th>
<th>Non-CD controls (specificity)</th>
<th>IBD controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA EMA IIF/The Binding Site</td>
<td>Cut off</td>
<td>1/4</td>
<td>49/49 (100%)</td>
<td>0/34 (100%)</td>
<td>0/30</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Human tTG based ELISA/Aesku Lab</td>
<td>15 U/ml</td>
<td>35/49 (71%)</td>
<td>0/34 (100%)</td>
<td>0/30</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Human tTG based ELISA/The Binding Site</td>
<td>4 U/ml</td>
<td>48/49 (98%)</td>
<td>3/34 (91%)</td>
<td>1/30</td>
<td>6 U/ml</td>
<td>47/49 (96%)</td>
<td>1/34 (97%)</td>
<td>0/30</td>
<td></td>
</tr>
<tr>
<td>Human tTG based ELISA/Eurospital</td>
<td>7 AU</td>
<td>47/49 (96%)</td>
<td>4/34 (88%)</td>
<td>0/30</td>
<td>9 AU</td>
<td>47/49 (96%)</td>
<td>1/34 (97%)</td>
<td>0/30</td>
<td></td>
</tr>
<tr>
<td>Human tTG based ELISA/QUANTA Lite</td>
<td>20 U/ml</td>
<td>48/49 (98%)</td>
<td>0/34 (100%)</td>
<td>0/30</td>
<td>16 U/ml</td>
<td>48/49 (98%)</td>
<td>0/34 (100%)</td>
<td>0/30</td>
<td></td>
</tr>
<tr>
<td>Human tTG based ELISA/Orgentec</td>
<td>10 U/ml</td>
<td>49/49 (100%)</td>
<td>5/34 (85%)</td>
<td>1/30</td>
<td>11 U/ml</td>
<td>48/49 (98%)</td>
<td>2/34 (94%)</td>
<td>0/30</td>
<td></td>
</tr>
<tr>
<td>Human tTG based ELISA/Varelisa</td>
<td>5 U/ml</td>
<td>49/49 (100%)</td>
<td>0/34 (100%)</td>
<td>0/30</td>
<td>4 U/ml</td>
<td>49/49 (100%)</td>
<td>0/34 (100%)</td>
<td>0/30</td>
<td></td>
</tr>
</tbody>
</table>

Results greater than or equal to the cut-off/threshold are considered positive. Specificity was calculated using only the non-CD controls (see text).

### Table 4: Comparisons between AUC estimations

<table>
<thead>
<tr>
<th>Assay type/Manufacturer</th>
<th>Assay type/Manufacturer</th>
<th>Assay type/Manufacturer</th>
<th>Cut off</th>
<th>CD (sensitivity)</th>
<th>Non-CD controls (specificity)</th>
<th>IBD controls</th>
<th>Threshold</th>
<th>CD (sensitivity)</th>
<th>Non-CD controls (specificity)</th>
<th>IBD controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guinea pig liver tTG</td>
<td>Binding Site</td>
<td>0.406</td>
<td>0.494</td>
<td>0.314</td>
<td>0.128</td>
<td>0.014*</td>
<td>0.026*</td>
<td>0.008*</td>
<td>0.198</td>
<td>0.027*</td>
</tr>
<tr>
<td>Human tTG</td>
<td>0.071</td>
<td>0.054</td>
<td>0.026</td>
<td>0.006*</td>
<td>0.019</td>
<td>0.005*</td>
<td>0.022*</td>
<td>0.006*</td>
<td>0.012</td>
<td>0.003*</td>
</tr>
<tr>
<td>Human tTG</td>
<td>0.056</td>
<td>0.071</td>
<td>0.022*</td>
<td>0.006*</td>
<td>0.012</td>
<td>0.003*</td>
<td>0.027*</td>
<td>0.006*</td>
<td>0.012</td>
<td>0.003*</td>
</tr>
<tr>
<td>Human tTG</td>
<td>0.033*</td>
<td>0.089</td>
<td>0.047*</td>
<td>0.006*</td>
<td>0.027</td>
<td>0.006*</td>
<td>0.025*</td>
<td>0.006*</td>
<td>0.016</td>
<td>0.003*</td>
</tr>
<tr>
<td>Human tTG</td>
<td>0.023*</td>
<td>0.057</td>
<td>0.023*</td>
<td>0.006*</td>
<td>0.016</td>
<td>0.006*</td>
<td>0.027*</td>
<td>0.006*</td>
<td>0.012</td>
<td>0.003*</td>
</tr>
</tbody>
</table>

*p<0.05

AUC, area under curve; tTG, tissue transglutaminase.
patients with CD (figs 1, 2; tables 2, 3), in agreement with previous reports.4–10,16–19,21,25,26,28–30,32–34,36,38 The methods used to extract and purify tissue derived tTG, produce and process recombinant tTG, and then coat tTG on to ELISA wells may lead to alterations in the tertiary structure of tTG. Therefore, conformational epitopes may be lost or formed, with a loss leading to a reduced ability of tTG to bind IgA tTG, thus explaining some of the false negative results.19 Furthermore, the formation of conformational neoepitopes may also result in false positive results (see above).

The function and tertiary structure of tTG is also altered by the presence of ionised calcium.35 It has been suggested that antibody binding epitopes may be formed or hidden by the presence of ionised calcium in the coating buffer of the IgA tTG ELISA,36–46 Sulkainen and colleagues reported that the pretreatment of tTG with calcium (“calcium activation”) dramatically improved the separation between CD and non-CD sera in a gpl-tTG based ELISA, and also increased the binding affinity of IgG to CD sera. However, in our study, the two kits in which the use of “calcium activation” of tTG is recorded (Binding Site gpl-tTG kit and Genesis) did not clearly demonstrate superior performance to the other kits. Furthermore, Lock and colleagues found that the addition of calcium to the coating buffer increased both the signal and background values, and therefore produced no overall improvement in the performance of their in house gpl-tTG based IgA tTG ELISA. Nakachi and colleagues also reported that the autoantibody binding sites of tTG were formed in a manner that was essentially calcium independent. Finally, we found that the performances of most of the IgA tTG ELISA kits were significantly improved by adjusting the cut off values/decision thresholds via ROC plot analysis. These discrepancies between the ROC analysis derived decision thresholds and manufacturers’ recommended cut off values illustrate the importance of cut off point revalidation by laboratories, using appropriate samples from their referral population.

**ACKNOWLEDGEMENTS**

We acknowledge K Smithers (Central Sydney Immunology Laboratory, Royal Prince Alfred Hospital) for excellent technical assistance; the Australian distributors of the commercial IgA tTG kits for the generous provision of their kits for assessment in this study; and P Hobson (Department of Immunology, Sullivan Nicolaides Pathology, Brisbane, Australia) for providing some of the sera for the study and performing some of the assays.

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**REFERENCES**


18 Garlit HR, Freeman HJ. Comparison of IgA endomysium antibody and IgA tissue transglutaminase antibody in celiac disease. Can J Gastroenterol 2000;14:668–71.


42 Bomber D. The area above the ordinal dominance graph and the area below the receiver operating graph. Journal of Mathematical Psychology 1975;12:387–415.

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H Holzel, P van Diest
Acute erythremic myelosis (true erythroleukaemia): a variant of AML FAB-M6

Our group has been actively researching the acute erythroleukaemias since the late 1980s, during which time, we have developed and extensively published our classification:

• acute erythroleukaemia, M6a (traditional FAB-M6, DiGuglielmo’s syndrome, 1917)
• acute erythroleukaemia, M6b (pure erythroleukaemia, DiGuglielmo’s disease, 1926)
• acute erythroleukaemia, M6c (mixed erythroleukaemia).

We have also published abstracts and presented this classification at meetings of the International Academy of Pathology, International Society of Haematology, and the American Society of Haematology, and our recommendations have also been cited in the recent literature. Therefore, we are surprised to find the M6b subtype now “discovered” and re-named in the article by Hasserjian et al., and the M6c subtype completely overlooked. Although we suspect this incongruity to be an oversight, we believe it is important to set the record straight.

This established classification of the acute erythroleukaemias is based partly on the old FAB criteria and also upon morphological, cytochemical, and immunophenotypical criteria. All bone marrow aspirates demonstrate ≥ 50% erythroid precursors, with erythroid dysplasia. Dysplasia of the granulocytic and megakaryocytic cell lines may or may not be present. The M6a subtype is defined as ≥ 30% blasts of the non-erythroid component (FAB exclusion criteria); the M6b subtype is defined as ≥ 30% pronormoblasts of the erythrocytic elements; and the M6c subtype has ≥ 30% blasts and ≥ 30% pronormoblasts by the aforementioned exclusion criteria. Because the dysplastic changes may, at times, make definitive characterisation of the blasts as erythroid versus non-erythroid difficult, the morphological features must always be confirmed by cytochemical stains, immunohistochemical stains, and/or flow cytometric analysis.

These three separate subtypes must be distinguished from one another to provide useful prognostic information for the clinician and the patient. When treated with the standard myeloid protocol, the M6a and M6c subtypes demonstrate a very high remission rate, whereas most patients with the M6b subtype remain refractory to treatment. Notably, patients with the M6c subtype remain in remission for a significantly shorter time than the M6a group. Mean survival for these subtypes is: M6a, 31.4 (SD, 32) months; M6b, 3.13 (SD, 4.2) months; M6c, 10.3 (SD, 12.7) months.

The malignant clonal cell of origin manifesting as acute erythroleukaemia of any subtype appears to be a multipotential stem cell, which shows varying degrees of erythrocytic and granulocytic lineage maturation. Therefore, the three distinct subtypes of acute erythroleukaemia are not three separate diseases, but rather represent a spectrum of the same disease. The poor remission rate and short survival characteristic of this disorder are dependent upon:

1. a high pronormoblast to myeloblast ratio within diagnostic bone marrow aspirates,
2. a high proliferative index, (3) “unfavourable” cytogenetic aberrations, and (4) a high incidence of P-glycoprotein expression (the multidrug resistance phenotype).

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References

CORRECTIONS