ERG IMMUNOHISTOCHEMISTRY IN THE DIAGNOSIS OF ACUTE MYELOID LEUKAEMIA: A SINGLE CENTRE RETROSPECTIVE AUDIT

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OUTLINE

• The importance and aims of our project
• Methods and Materials
• Results
• How this will help clinicians
INTRODUCTION

• AML with a dry bone marrow aspirate
  – IHC CD34, CD117 negative can be a diagnostic dilemma

• Erythroblastosis transformation specific (ETS) regulated gene 1 (ERG) is a transcription factor involved in cell proliferation, differentiation, angiogenesis

• Anti-ERG used for prostate cancer since 2011

• Subset of AML have t(16:21) ERG:FUS fusion
ANTI-ERG IN MYELOID MALIGNANCY

• 2016: useful marker in the diagnosis of leukaemia cutis (LC).
  – 32 biopsies, 16 with LC, and 16 with reactive infiltrates.
  – ERG positivity 13/16 LC cases. Negative in all reactive cases
• 2020: Looking for concordance with MPO (6 Normal, 12 AML, 6 MPN)
  – ERG is concordant with myeloperoxidase (MPO) IHC,
  – 7/12 AML cases the ERG staining identified the myeloblast population
• 2022: 207 cases of immature and mature haematolymphoid lesions
  – ERG immunopositivity in 15 of 16 (94%) acute myeloid leukemias/myeloid sarcomas
  – 4 of 5 (80%) CD34-negative/CD117-negative acute myeloid leukemias/myeloid sarcomas
  – Positive 9 cases of B-lymphoblastic and T-lymphoblastic leukemia/lymphoma
  – Negative in 148 B and T Cell Lymphomas
  • 2 High Grade Lymphomas, and 2 Blastoid Mantle Cell tumors

Koo M, Natkunam Y. ERG Immunoreactivity in Blastic Hematolymphoid Neoplasms: Diagnostic Pitfall in the Workup of Undifferentiated Malignant Neoplasms
ANTI-ERG IS A NUCLEAR STAIN

- IHC has inherent limitations.
- Membrane and cytoplasmic stains are subject to significant inter and intra-observer variability when compared to nuclear staining.
AIMS

(a) Validate ERG immunohistochemistry staining in bone marrow trephine samples in our centre,
(b) Quantify ERG IHC positivity in an AML cohort, and correlate concordance with CD34 and CD117 immunostaining, when available,
(c) To see whether ERG is a useful adjunct in the diagnosis of cases of AML where the blast cell population has proven difficult to identify by other means.
MATERIALS AND METHODS

• Retrospective audit of all new and relapsed cases of acute myeloid leukaemia (AML) between November the 1st 2021, and November 1st 2022
• Demographic data, immunophenotype (by flow cytometry), cytogenetics, myeloid NGS panel data collected secure spreadsheet
• For IHC validation we followed the College of American Pathologists Pathology and Laboratory Quality Centre guideline
• Statistics completed using STATA V16 was used to analyse results
PROJECT WORKFLOW

29 Cases of AML/Relapsed in Canterbury Nov 21- Nov 22

18 Cases 'Local Trephine' AML diagnosis

IHC reviewed by 3 SMO’s and 1 RMO

H&E review (Blasts)

1 removed – diagnostic uncertainty on re-review

17 CD34
17 ERG
9 CD117
17 Reticulin

Reasons for exclusion
- 3 Out of town diagnostics
- 7 No trephine
- 1 Peripheral Blood Relapse
**RESULTS- PARTICIPANT CHARACTERISTICS**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No of Participants (N=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>NZ European</td>
<td>15 (83.3%)</td>
</tr>
<tr>
<td>Maori</td>
<td>1 (5.6%)</td>
</tr>
<tr>
<td>African</td>
<td>2 (11.2%)</td>
</tr>
<tr>
<td>Age (Years)</td>
<td></td>
</tr>
<tr>
<td>Mean: 60.9</td>
<td></td>
</tr>
<tr>
<td>Median: 64.5</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6 (33.3%)</td>
</tr>
<tr>
<td>Male</td>
<td>12 (66.6%)</td>
</tr>
<tr>
<td>Karyotype</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>11 (62.0%)</td>
</tr>
<tr>
<td>T(8:21)</td>
<td>1 (5.6%)</td>
</tr>
<tr>
<td>+8</td>
<td>1 (5.6%)</td>
</tr>
<tr>
<td>Complex (&gt;3 abnormalities)</td>
<td>4 (22.4%)</td>
</tr>
<tr>
<td>Insufficient Sample</td>
<td>1 (5.6%)</td>
</tr>
</tbody>
</table>
**AGREEMENT**

Agreement between measurements refers to the degree of concordance between two (or more) sets of measurements.

<table>
<thead>
<tr>
<th>No</th>
<th>Scale</th>
<th>Kappa Agreement</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34</td>
<td>Pos, Weak, Neg</td>
<td>0.58</td>
</tr>
<tr>
<td>CD117</td>
<td>Pos, Weak, Neg</td>
<td>0.62</td>
</tr>
<tr>
<td>ERG</td>
<td>Pos, Weak, Neg</td>
<td><strong>0.76</strong></td>
</tr>
<tr>
<td>Retic</td>
<td>MF (0-1=Low, 2-3=High)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

**SENSITIVITY : WHEN COMPARED TO MORPHOLOGIC BLASTS**

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>CD34</th>
<th>0.59</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD117</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>ERG</td>
<td><strong>0.93</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Kappa statistic</th>
<th>Strength of agreement</th>
</tr>
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<tbody>
<tr>
<td>&lt;0.00</td>
<td>Poor</td>
</tr>
<tr>
<td>0.00–0.20</td>
<td>Slight</td>
</tr>
<tr>
<td>0.21–0.40</td>
<td>Fair</td>
</tr>
<tr>
<td>0.41–0.60</td>
<td>Moderate</td>
</tr>
<tr>
<td>0.61–0.80</td>
<td>Substantial</td>
</tr>
<tr>
<td>0.81–1.00</td>
<td>Almost perfect (very good)</td>
</tr>
</tbody>
</table>

High sensitivity: Few false negatives (blue)

Low specificity: Many false positives (red)

Failed test        Passed test
QUALITATIVE ANALYSIS

**ERG Study #13**

- One case negative for ERG
- OSTEOSCLEROTIC AML
- Two other cases weak
- Both had grade 2/3 MF reticulin
- Does reticulin impair staining?
Comments:

ERG Study #17

3 Reviewers thought it identified 5/6 CD34/CD117 negative cases.

1 Reviewer thought it identified 4/6 CD34/CD117 negative cases.

ERG was positive in all except the osteosclerotic AML case.

ERG Study #5

CD34 and CD117 negative cases

3 Reviewers thought it identified 5/6 CD34/CD117 negative cases.

1 Reviewer thought it identified 4/6 CD34/CD117 negative cases.

ERG was positive in all except the osteosclerotic AML case.
ASSESSING SPECIFICITY

**ERG Study #1**

- Very difficult to prove!
- One case demonstrated nice preservation of lymphoid nodules
- ERG stains the megakaryocytes
- We know from using it in other settings, useful quantifying M:E ratio so **not specific** for blasts

**ERG Study #8**
LIMITATIONS OF ERG

- Sensitive but not specific
- Limited validation worldwide of the stain for the setting of myeloid malignancies
CONCLUSIONS

- Anti ERG stains can be added to the AML IHC ‘tool kit’
- Particularly useful in CD117 and CD34 negative AML
- Highly sensitive
- Could this replace MPO? Is it just an immature marker?, cleaner nuclear stain
WHAT NEXT

• Dual staining IHC – Could a combined ERG, CD34 be useful?

• Flow cytometry, ERG-FITC flurochrome, can this identify the ERG on immature myeloid cells identified by flow?
ACKNOWLEDGEMENTS

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Dr Penny Wright
Dr Siobhan Cross
Dr Blake Hsu
Dr Catherine Neal
REFERENCES


