Improving conventional prognosticators in diffuse large B cell lymphoma using marker ratios

Kim-Anh Lê CAO

NHMRC Career Development Fellow, Statistician

The University of Queensland Diamantina Institute
Translational Research Institute
Brisbane, Queensland, Australia
DIFFUSE LARGE B-CELL LYMPHOMA

• Incidence of lymphoma more than doubled in last 20 years
• 6th most common cancer
• Risk by age 85yo of Non Hodgkin’s Lymphoma (NHL) is 1 in 42
• DLBCL is most common subtype in adults (30-40% of all cases in Australia) and is the most aggressive form of NHL

Biggest risk factor (by far) is impaired immunity
TREATMENTS AND SURVIVAL

Of the majority of patients that die from DLBCL, 30-40% will do so within 2 yrs despite chemotherapy

- Addition of Rituximab to combination chemotherapy CHOP improves survival in DLBCL by 10-15%
- Exposure to R-CHOP induce chemo-resistance and toxicity in certain group of patients
- Need to more accurately predict response to R-CHOP to stratify patients and identify new immune target agents
WHY DO PRE TREATMENT PROGNOSTICATORS FAIL?

Cell of Origin (COO), international prognostic index (IPI) and i-PET/CT still fail to identify patients that will relapse

**Tumour microenvironment (TME)** contributes to tumour initiation, progression and responses to therapy

Three players in TME:

- Tumour Cell
- Immune effector cell
- Immune checkpoint

→ need to understand **interaction of malignant B cell and anti-tumour immune response** using **gene expression**
Immune effectors drive potent anti-tumour responses

Tumour cells produce host-checkpoint responses: dampen immune-effectors, induce immunosuppressive microenvironment

In solid-organ cancers, tumor biopsies display ‘adaptive resistance’.

Is it also the case in DLBCL?

Creative idea from Prof. Maher Gandhi
A FINE BALANCE BETWEEN IMMUNE EFFECTORS AND CHECKPOINTS TO PREDICT OUTCOME?

Hypothesis:

The relative composition of antagonist checkpoints and immune effectors is prognostic to DLBCL

Does the ratio of Immune Effector(s) / Checkpoint(s) predict outcome?
DATA

Nanostring™ digital multiplex gene expression (Discovery data)
- 158 de novo DLBCL treated with R-CHOP from 2 Australian centres (Brisbane, Canberra)
- Formalin-fixed paraffin embedded tissue (FFPET) + survival outcome
- Median follow up: 4 yrs; Median age: 62 (27-86)

Affymetrix gene expression (Validation data)
- 233 patients treated with R-CHOP
- Fresh-frozen samples + survival outcome
- Median follow up: 2.8 yrs;
- Publicly available data (GSE10846*)

GENE EXPRESSION RATIOS

Investigating a fine balance between immune effectors and check points

• Immune-Effectors in **numerator**: CD4, CD8, CD56 and CD137
• Immune-Checkpoints in **denominator**: CD163, CD68, **M2 (CD163/CD68)**, PD1 and **PDL1**

Various combinations of gene expression ratios assessed:
1. Individual gene markers (total: 10)
2. Simple ratio (1 gene / 1 gene, total: 12 ratios)
3. All possible combinations of numerator and denominator (total: 44 ratios)
ANALYTICAL CHALLENGES

Use of gene ratios to stratify survival outcome has been successful in some cancer studies but limitations

- Define optimal cut-off ratio to segregate good and poor outcomes: median is not satisfying as we expect ~30% mortality
- Assess all possible ratio combinations in an efficient way

Our aims

- Identify the most robust gene ratio among our gene candidates
- Determine optimal cut-off with appropriate statistical method
- Validate results in external cohort

SEE THE FOREST FOR THE TREES

Tree-based survival models

- **Input**: gene expression ratio and survival outcome
- **Output**: stratify high vs. low risk patients with optimal ratio cut-off (‘best split’) based on entropy criterion

\[ p < 0.001 \]

\[ \text{CD4xCD8xM2xPD-L1} \]

\[ p < 0.001 \]

\[ \text{Node 2 (n = 65)} \]

\[ \text{Node 3 (n = 93)} \]
BEST SPLIT IN SURVIVAL TREE

Segregation rule based on patient partitioning for each ratio

- If ratio value ≤ best split, then patient classified as ‘high risk’ (LHS of tree)
- If ratio value > best split, then patient classified as ‘low risk’ (RHS of tree)

Discovery cohort (Nanostring)

- log rank test to assess differences btw the two survival curves obtained from tree + correction for multiple testing.

Test cohort (Affymetrix)

- Apply same split as in discovery data set, log rank test + correction for multiple testing.

RESULTS

ASSESSING VARIOUS COMBINATIONS

Single markers
- e.g. CD163, CD68, \( p = 1 \)

Simple ratio
- e.g. \( M2 = \frac{CD163}{CD68} \), \( p = 0.0056 \)

Best combination identified with tree-based survival model
- \( \frac{(CD4 \times CD8)}{(M2 \times PD-L1)} \), \( p < 0.0001 \)
- best split = -0.279
RESULTS

OUR IMMUNE RATIO ADDS TO CONVENTIONAL PROGNOSTICATORS

Cell of origin (COO) classifies
- germinal centre B-cell (GCB)
- non-GCB

40% of patients in high risk identified
VALIDATION IN EXTERNAL COHORT

Affymetrix gene expression study

1. Calculate our immune ratio \((CD4 \times CD8)/(M2 \times PD-L1)\)
2. Set up our best split -0.279
3. Apply our segregation rule
   - \(~60\%\) of patients assigned to high risk
   - Multivariate Cox regression confirms immune ratio is independent from prognosticators COO and IPI
# What if we had used a median cut-off?

<table>
<thead>
<tr>
<th></th>
<th># ratios with $p \leq 0.05$ (log rank test)</th>
<th>Median cut-off</th>
<th>Survival tree cut-off</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discovery Nanostring</td>
<td>21</td>
<td></td>
<td>54</td>
</tr>
<tr>
<td>Validation Affymetrix</td>
<td>0</td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

**Further work / Discussion**
- fresh vs. frozen samples have an influence on validation
- improve results with a confidence interval for best split
- not shown: experimental validation successful in on a 3rd cohort ($n = 160$, flow cytometry and i-PET/CT outcome)
CONCLUSIONS

Our immune ratio

- Based on biological hypothesis
- Complements conventional prognosticators
- Identifies 40% of patients with poor outcome
- Validation in two external cohorts
- May help identify better target drugs (e.g. PDL-1) for patients not responding to R-CHOP. Clinical trial? (patent submitted).

Ratios for big data? and my (statistician) point of view

- Address the issue of platform variability
- Combination of genes vs. single gene biomarker approach is beneficial
- Could be expanded to other cancer studies genomewide
ACKNOWLEDGMENTS

Prof M Gandhi lab incl. & Lê Cao group
• Maher Gandhi
• Colm Keane
• Erica Han
• Dipti Talaulikar

Statisticians
• Florian Rohart
• Ian Hughes
• *insert_your_name_here*

PhD students
• Ralph Patrick
• Chao Liu
• Jasmin Straube
• Amrit Singh
• Thom Cuddihy
• Aimee Hanson

Bioinf Masters student
• Vanessa Lakis

*we are hiring! UQjobs #498081