De waarde van moleculaire testen bij maligne lymfoom

Han van Krieken
Nijmegen, the Netherlands

Introductie

• Diagnostiek lymfomen moeilijk
• Toenemend relevant
• >30 entiteiten
• Clonaliteit
• Fluorescent In Situ Hybridisatie (FISH)

Experience from a population based lymphoma panel

• 6 hospitals (1 academic, 2 large regional, 3 small local)
• 3 pathology laboratories: specialised pathologists
• Bi-weekly meetings at multihead microscopy
• Registration of all lymphoma cases by comprehensive cancer centre IKO
  • Diagnosis
  • Stage
  • Treatment
  • Follow-up
Results: definition discordant

Clinically relevant
- Benign versus malignant
- Hodgkin lymphoma versus non-Hodgkin lymphoma
- Follicular lymphoma grade 1,2 versus grade 3
- Progressed lymphoma versus diffuse large B-cell l.
- Small lymphocytic lymphoma versus mantle cell l.
- Suspect lymphoma versus certain diagnosis

Clinically irrelevant
- Follicular lymphoma grade 1 or 2
- Nodal marginal zone lymphoma versus immunocytoma, or CLL

Concordant versus Discordant

- Total number of lymphomas
  - N = 302
    - Concordant: 232
    - Discordant: 50
    - No panel diagnosis: 20

- Total number of reactive lesions in panel
  - Concordant: 45
  - Discordant: 18

Changed diagnoses: B-NHL

- Mantle cell lymphoma to small lymphocytic lymphoma: 2
- Follicular lymphoma grade 1,2 to 3 or vice versa: 9
- Other type to follicular lymphoma: 6
- Follicular lymphoma to nodal marginal zone lymphoma: 2
- Follicular lymphoma to extranod. marg. zone lymphoma: 1
- Low grade B-cell lymphoma to splenic marg.z. lymph.: 1
- Various (Hodgkin, T-NHL, low grade B-cell) to DLBL: 8
- Diffuse large B-cell lymphoma to Burkitt lymphoma: 1
- DLBL to EBV-associated lymphoproliferation: 2

  total: 32

Changed diagnosis: T-NHL, others

- Hodgkin lymphoma to large cell anaplastic lymphoma: 3
- Unclassifiable to hepatosplenic T-NHL: 1
- B-cell lymphoma to Hodgkin’s lymphoma: 3
- Specific type to unclassifiable (too little tissue): 8
- Lymphoma to uncertain diagnosis: 2
- Lymphoma to reactive process: 1

  total: 18

- B-NHL and T-NHL and others: 50 cases

- Suspect lymphoma to certain lymphoma: 24
- Suspect lymphoma to reactive process: ??
Results: summary

- All patients diagnosed with lymphoma:
  - 82% concordancy
    - Including 8% uncertain diagnosis potentially solvable by translocation detection and/or clonality testing
  - 18% discordance (1% benign)

- 8% potentially solvable by translocation detection
- 8% potentially solvable by clonality testing

**Fluorescence in Situ Hybridization**

- BCL6 localizes to chromosomal band 3q27

**Fusion-Signal FISH**

- Disadvantages:
  - Translocations to alternative partner genes are missed
  - High false-positive rate (5-10%) caused by coincidental colocalisation

**Split-signal FISH**

- Advantages:
  - Detection of aberrations is independent of partner genes
  - Minimisation of false positivity
  - Identification of partner gene (or chromosome region), if metaphases are present
False positivity vs false negativity ....

Microscope:  
Sample/nucleus:  
Slide:  
Split-signal FISH: Translocation-positive  False-negative  
Fusion-signal FISH: Translocation-negative  False-positive

Case 1
Morphology:
Diffuse large cell lymphoma.

FISH analysis of paraffin embedded tissue sections

Case 1

Myc
split-apart probe:

Large cell lymphoma

Case 1

Viewing with one filter set allows the first of the two probes to be visualised
Viewing with the second filter set reveals the sites of hybridisation of the other probe.

When the two signals are visualised together it is clear that all signals are fused.

FISH analysis of paraffin embedded tissue sections

**Case 2**

**Morphology:**
Diffuse large cell lymphoma. Some features suggestive of Burkitt’s lymphoma.
Large cell lymphoma

Case 2

Myc split-apart probe:

FISH analysis of paraffin embedded tissue

Interpretation of results

Case 1

Case 2

Signals (even in truncated cells) are fused, excluding a translocation.

Some nuclei contain split signals, indicating a translocation.
Results

FCL stained with TCRG probe

Multiple signals/nucleus

This case was karyotyped in the dept. of Human Genetics (89-94 chromosomes) with multiple aberrations

When to use clonality testing?

- Reactive versus Malignant
- Recurrence versus new lymphoma
- Lineage verification
- Lymphoproliferations in immune deficiency
- NOT: Hodgkin versus non-Hodgkin
- Know the pitfalls
- Interaction pathologist-molecular biologist

WHO classification of malignant lymphoproliferations

<table>
<thead>
<tr>
<th>B-cell malignancies</th>
<th>T-cell malignancies</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Precursor-B-lymphoblastic leukemia/lymphoma</td>
<td>1. Precursor-T-lymphoblastic leukemia/lymphoma</td>
</tr>
<tr>
<td>2. B-cell chronic lymphocytic leukemia</td>
<td>2. T-cell prolymphocytic leukemia</td>
</tr>
<tr>
<td>5. Lymphoplasmacytic lymphoma</td>
<td>5. Adult T-cell lymphoma/leukemia (HTLV1+)</td>
</tr>
<tr>
<td>10. Follicular lymphoma (characterized)</td>
<td>10. Mycosis fungoides/Sézary syndrome</td>
</tr>
<tr>
<td>11. Mantle cell lymphoma</td>
<td>11. Anaplastic large cell lymphoma (cutaneous type)</td>
</tr>
<tr>
<td>14. EBV-related B-NHL in immunodeficiency</td>
<td>14. Anaplastic large-cell lymphoma (systemic type)</td>
</tr>
</tbody>
</table>

Complementarity of Ig targets for clonality detection in B-cell malignancies

<table>
<thead>
<tr>
<th>IGK</th>
<th>IGH</th>
<th>all IGH</th>
<th>Drs-JH</th>
</tr>
</thead>
<tbody>
<tr>
<td>VH-JH</td>
<td>Dλ-JH</td>
<td>VH-JH + Dλ-JH</td>
<td>Ve-Jk</td>
</tr>
<tr>
<td>MCL (n=54)</td>
<td>100%</td>
<td>11%</td>
<td>100%</td>
</tr>
<tr>
<td>B-CLL/SLL (n=56)</td>
<td>100%</td>
<td>43%</td>
<td>100%</td>
</tr>
<tr>
<td>FL (n=109)</td>
<td>84%</td>
<td>19%</td>
<td>86%</td>
</tr>
<tr>
<td>MZL (n=41)</td>
<td>86%</td>
<td>91%</td>
<td>95%</td>
</tr>
<tr>
<td>DLBCL (n=109)</td>
<td>79%</td>
<td>30%</td>
<td>85%</td>
</tr>
<tr>
<td>TOTAL (n=369)</td>
<td>88%</td>
<td>28%</td>
<td>91%</td>
</tr>
</tbody>
</table>

* Combination of two Ig gene rearrangements without somatic mutations
Complementarity of TCR targets for clonality detection in T-cell malignancies

<table>
<thead>
<tr>
<th></th>
<th>TCRB</th>
<th>TCRB</th>
<th>TCRB</th>
<th>+ TCRG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vβ-Jβ</td>
<td>Dβ-Jβ</td>
<td>Vγ-Jγ</td>
<td>+ Dγ-Jγ</td>
</tr>
<tr>
<td>T-PLL (n=33)</td>
<td>94%</td>
<td>47%</td>
<td>100%</td>
<td>94%</td>
</tr>
<tr>
<td>T-LGL (n=28)</td>
<td>86%</td>
<td>62%</td>
<td>96%</td>
<td>96%</td>
</tr>
<tr>
<td>PTCL-U (n=47)</td>
<td>85%</td>
<td>67%</td>
<td>98%</td>
<td>94%</td>
</tr>
<tr>
<td>AILT (n=37)</td>
<td>70%</td>
<td>61%</td>
<td>89%</td>
<td>92%</td>
</tr>
<tr>
<td>ALC (n=43)</td>
<td>70%</td>
<td>48%</td>
<td>74%</td>
<td>74%</td>
</tr>
<tr>
<td>TOTAL (n=188)</td>
<td>80%</td>
<td>58%</td>
<td>91%</td>
<td>89%</td>
</tr>
</tbody>
</table>

20% to 25% of anaplastic large cell lymphomas do not have TCR gene rearrangements (null-ALCL).

Initial histopathological diagnosis
• Non-specific lymphadenitis, often follicular hyperplasia (n=74)
• EBV / CMV / HIV infection (n=5/1/1)
• Dermatopathic lymphadenopathy (n=4)
• Cat scratch disease (n=3)
• Toxoplasmosis (n=3)
• Sarcoidosis (n=2)
• Tuberculosis (n=2)
• Progressively transformed GC (n=2)
• Spleen in spherocytosis / spleen in ITP / trauma spleen (n=1/1/1)
• Myoepithelial sialo-adenitis (n=1)
• Kikuchi’s lymphadenitis (n=1)
• Skin pseudo lymphoma (n=1)
• Hashimoto (n=1)
• Reactive, suspect B / T (n=1/1)

Results
• clear clonal results (categories I + II): 11% (12 / 107)
  - missed lymphomas (n=2)
  - unexplained clonal results (n=10)
• ambiguous molecular results (category III): 14% (15 / 107)
  - mainly oligoclonality (restricted repertoire) in polyclonal background
• clear polyclonal results (category IV): 75% (80 / 107)

I : clear clonal products in multiple loci and multiple tubes
II : clear clonal products, but in single locus
III : ambiguous, mostly clonality with single technique or single tube
IV : polyclonal in every locus and tube
Molecular results

1. Clear clonal results  \( n = 15 \)
   1. Clerical errors  \( n = 3 \)
   2. Missed lymphomas  \( n = 2 \)

2. Unconfirmed clonal result  \( n = 32 \)

**BIOMED-2 WP2a category: reactive tissue lesions**

**Examples**

**Missed lymphomas (only recognised after molecular results!)**
- involved by MF / Sezary syndrome  (DE 069; cat. I)
- involved by MZL  (DE 105; cat. I)

**Unexplained clonal results (n = 10)**
- skin pseudolymphoma  (NL 108; cat. I)
- EBV infection  (ES 189; cat. I)
- ruptured spleen (remark. plasma cells!)  (PT 021; cat. I)
- early AILD in M. Sjögren?  (PT 024; cat. I)
- large GC in frozen tissue  (GBN017;cat.I)
- reactive lymph node (treated for DLBCL)  (DE 107; cat. I)
- reactive, but difficult (skin; B-NHL?)  (GBN037;cat.II)
- traumatic spleen (unusual T-cells)  (NL 111; cat. II)
- spleen ITP  (NL 156; cat. II)
- reactive (progressively transformed GC)  (NL 172; cat. II)
Specific Ig/TCR gene patterns in B-cell malignancies

- (Virtually) all B-cell malignancies have IGH rearrangements;
- Incomplete DH-JH rearrangements occur in 30-40% of cases, except of MCL (~10%) and FCL (~20%), most of which contain BCL1-IGH or BCL2-IGH rearrangements on the second allele;
- All Igκλ B-cell malignancies have IGK deletions on at least one allele (80% have biallelic deletions);
- Igκλ B-cell malignancies rarely contain IGL rearrangements (<5%), but 30% have IGK deletions on one allele;
- TCR gene rearrangements occur in 5 to 15% of mature B-cell malignancies and in 80 to 90% of immature B-cell malignancies (precursor-B-ALL).

Specific TCR/Ig gene rearrangements in T-cell malignancies

- Virtually all TCRαβ T-cell malignancies have TCRG gene rearrangements and ~20% have TCRD gene rearrangements on one allele.
- A large part of TCRγδ T-cell malignancies (30 to 40%) contain TCRB gene rearrangements, particularly incomplete Dβ-Jβ rearrangements.
- Approximately 5 to 20% of T-cell malignancies contain IGH gene rearrangements, including incomplete DH-JH rearrangements.
- IGK or IGL gene rearrangements are generally rare (<5%) in T-cell malignancies.

Conclusion: TCR/Ig gene rearrangements are not lineage specific, even not for TCRαβ versus TCRγδ lineage.

Conclusions concerning BIOMED-2 multiplex tubes

Unprecedentedly high level of clonality detection based on:
- primers recognise majority of functional gene segments (multiplexing of multiple primers)
- introduction of incomplete rearrangements: DH-JH and Dβ-Jβ
- usage of unmutated targets: DH-JH and IGK-Kde
- complementarity of Ig/TCR targets (e.g. IGH and IGK; TCRB and TCRG)

Clonal results in more than one tube!

Minimal sets of BIOMED-2 multiplex tubes:
- suspected B-cell proliferation: VH-JH and IGK (5 tubes)
- suspected T-cell proliferation: TCRB and TCRG (5 tubes)
- TCRγδ T-cell proliferation: TCRG and TCRD (3 tubes)

BIOMED-2 clonality strategy

Clonal results in more than one tube! Minimun sets of BIOMED-2 multiplex tubes: