The Force is in the Tissue

The tissue changes have the last word!

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Alberta Transplant Applied Genomics Centre (ATAGC)

http://atagc.med.ualberta.ca/Services/MolecularMicroscopeSystem
Relevant Financial Relationship Disclosure Statement

The Molecular Microscope® Diagnostic System  

Presenter: Phil Halloran

Our studies are supported in part by a licensing agreement with One Lambda/Thermo Fisher

- Phil Halloran has shares in Transcriptome Sciences Inc (TSI), a University of Alberta research company with an interest in molecular diagnostics
- Phil Halloran has been a symposium speaker for One Lambda/Thermo Fisher

https://www.molecular-microscope.com/
http://transcriptome.com/
http://atagc.med.ualberta.ca/Services/MolecularMicroscopeSystem/
Objectives

• To review the current standard of care for diagnosing rejection and tissue injury
• To understand the challenge of lacking a true ‘Gold Standard’ in diagnosing rejection
• To discuss the current gaps and needed steps in validating and calibrating non-tissue based diagnostics – cfDNA, DSA measurements
Follow Suttons Law
Go where the disease is – the tissue!

Willy Sutton
1901-1980
Medical Definition of Sutton's law: The principle of going straight to the most likely diagnosis.

Willy Sutton was asked why he robbed banks and replied: "because that is where the money is"
Two clinicians who pioneered disease classification

Robert Koch (1843-1910)
- A rigorous approach to studying etiology
- Koch’s postulates

Rudolph Virchow (1821-1902)
- Microscopic examination of diseased tissue

Understanding of diseases involves many dimensions, particularly examination of the diseased tissue and search for etiology and mechanisms
The changes in the tissue reflect rejection and injury.


Biopsies for cause ordered by 7 gene average

CXCL11
CXCL9
GZMB
CXCL9
GZMA
GNLY
PRF1
GAT1

All biopsies aligned by T cell burden (QCATs): Standardized with 8 control kidneys, PBTs from IQR filtered set

Kidney Biopsies For Cause (N = 234)

Comparing extreme phenotypes ‘sick versus well’

limited challenge bias
A common rejection module (CRM) for acute rejection across multiple organs identifies novel therapeutics for organ transplantation.

Discovery of a CRM consisting of 12 genes by leave-one-organ-out analysis.

CRM score correlates significantly with extent of graft injury.

The immunologic constant of rejection:
similar to autoimmunity, pathogen infection, and cancer

Activation of interferon-γ
stimulated transcripts

Recruitment of cytotoxic immune cells

Recruitment of immune effector functions

Activation of interferon-γ
stimulated transcripts

Recruitment of cytotoxic immune cells
Tens of thousands of genes “predict” outcome!
The single gene analysis must acknowledge this
Significant overlap in the molecular phenotype between disease entities: No transcript is specific

A molecular classifier for diagnosing AMR


Classifier score correlates with:

- Pathology (ptc, g, cg, l, cv, ah, ct, ci)
- Consensus amongst pathologists
- Presence of DSA
- outcome
Potential sources of variance with diagnostic classifiers

1) Sampling variance (random splits)
2) Label assignment (Gold Standard?!
3) Training set size (10-fold, 5-fold etc.)
4) Modelling strategy (which samples to exclude from the training sets)
5) Classifier type (LDA, SVM, etc.)

Classifier accuracies using a linear discriminant analysis (LDA) classifier. The histograms show the distribution of test set accuracies based on 1000 random 50:50 training:test set splits of the data. The N for each training and corresponding test set was either 20, 50, or 150 as indicated. The phenotypes being classified were either random (A-F) or rejecting vs non-rejecting (G-L). Each LDA classifier used the top 10 genes by Bayesian t-test. The left panel shows the results from properly conducted analyses where the gene selection was restricted to the training sets, while the right panel shows the resubstitution results, with genes selected using the combined training and test sets. The number on the left side of each graph is the average accuracy across all 1000 test sets.

Common Errors in the Implementation and Interpretation of Microarray Studies.
Reeve, Jeff; Halloran, Philip; Kaplan, Bruce
NK cells and macrophages in antibody mediated peritubular capillaritis

A.  

B.  

Mean number of positive cells in five peritubular capillaries

C. CD56  

D. CD68  

E. CD3  

Hidalgo et al. AJT 2010; 10: 1812–1822
More DARC in ptc in areas of inflammation, only very focal in glomeruli in severe ABMR.
Kidney core 3-5 mm
1-2 EMB bites
1-2 TBB bites
Liver 3-5 mm

Collect Biopsy
Place immediately in RNALater

Shipping/Receiving
Room temperature

RNA Extraction, Clean-Up, and Quality Control

Molecular Microscope®
(MMDx-Kidney)

RNA Labeling

Scan Chips
Wash & Stain
Hybridization
Evidence supporting the claim for superiority of molecular to histologic diagnosis

1. Histology relies on relatively few (6) canonical lesions, semi-quantitatively scored with considerable variability\textsuperscript{1,2}.

MMDx uses hundreds of features (probe sets), measured on a continuous scale with high precision.

2. When predicting a phenotype with a well-defined gold standard (survival), molecular measurements outperform histology\textsuperscript{3-5}.

3. MMDx outputs are continuous rather than semi-quantitative or binary, and can indicate when a biopsy has values near boundaries, allowing the observer to calibrate their diagnosis accordingly.

4. The MMDx supervised classifiers were trained on histology labels using microarray data, thereby combining information.

5. Many/most of the genes used by MMDx make biological sense.

6. Historically, the molecular findings have been used to update the Banff classification e.g. recognition of C4d- ABMR\textsuperscript{6,7}.

7. MMDx can assess recent injury and correlates with function better than histology\textsuperscript{8-10}.


Reference List


Explaining the Molecular Microscope® report for core kidney transplant biopsies (MMDx-Kidney)

**Clinical interpretation**

**Summary of molecular changes** (Injury, rejection)

**Proportions** rejection-related molecular changes (Normal, TCMR, ABMR)

**Visualization** Relationship of biopsy to others in reference set PC2 vs. PC1

**Visualization** PC2 vs. PC3

**Survival of other kidneys like this one**

**% of biopsy that is cortex**

**Adherence index:** Low scores in biopsies 6m-5y post-transplant correlate with possible non-adherence or under-immunosuppression

**Histologic and molecular diagnoses in the molecular nearest neighbors of this biopsy**

**Additional detail** Rejection, injury-related binary classifiers and AKI transcript set

**Comparison to normal** Scores of this biopsy interpreted vs. relatively normal biopsies

### Proportions rejection-related molecular changes

<table>
<thead>
<tr>
<th>Proportion</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>-</td>
</tr>
<tr>
<td>TCMR</td>
<td>-</td>
</tr>
<tr>
<td>ABMR</td>
<td>-</td>
</tr>
</tbody>
</table>

### Visualization

PC2 vs. PC1

PC2 vs. PC3

### Clinical Characterization

- **Adherence index:** Low scores in biopsies 6m-5y post-transplant correlate with possible non-adherence or under-immunosuppression.
- **Histologic and molecular diagnoses in the molecular nearest neighbors of this biopsy:**
- **Additional detail:** Rejection, injury-related binary classifiers and AKI transcript set.
- **Comparison to normal:** Scores of this biopsy interpreted vs. relatively normal biopsies.

### Summary of molecular changes

- **Proportions rejection-related molecular changes**
  - Normal
  - TCMR
  - ABMR

### Visualization

- **Relationship of biopsy to others in reference set:** PC2 vs. PC1
- **PC2 vs. PC3**

### Survival of other kidneys like this one

- **% of biopsy that is cortex**

---

### Additional detail

**Rejection related**

- TCMR 1
  - 0.81
  - 0.02 - 0.1
  - 0.10 Normal
- TCMR 2
  - 0.81
  - 0.02 - 0.1
  - 0.10 Normal
- TCMR 3
  - 0.81
  - 0.02 - 0.1
  - 0.10 Normal

**Injury-related**

- AKI Score
  - 0.16
  - 0.01 - 0.2
  - Score 1
- Injury Score
  - 0.33
  - 0.01 - 0.2
  - Score 2
- AKI Score
  - 0.16
  - 0.01 - 0.2
  - Score 3
- Injury Score
  - 0.33
  - 0.01 - 0.2
  - Score 4

**Abnormality Index (AIA)**

- 0.82
  - 0.02 - 0.2
  - Score 5

**Classification based on histologic lesions**

- AKI Score
  - 0.16
  - 0.01 - 0.2
  - Score 5
- Injury Score
  - 0.33
  - 0.01 - 0.2
  - Score 4
- AIA
  - 0.82
  - 0.02 - 0.2
  - Score 5

**Adherence index**

- Low scores in biopsies 6m-5y post-transplant correlate with possible non-adherence or under-immunosuppression.
MMDx-Heart
Endomyocardial biopsies
Results on the first 1000 biopsies

INTERHEART ClinicalTrials.gov NCT02670408
Figure 3. Molecular Microscope® Report for heart transplant biopsies (MMDx-Heart). The new biopsy is compared to the reference set of 889 endomyocardial biopsies and given a series of molecular scores culminating in the assignment of a molecular interpretation. This new biopsy was relatively normal with molecular features typical of well-differentiated parenchymal tissue with minimal injury or rejection. Patient information in the first table has been redacted. Archetype scores S1 (Normal), S2 (TCMR), S3 (ABMR), and S4 (Injury) from the 3-archetype model (3AA/model 1) or 4-archetype model (4AA/model 2) are given for the new biopsy in addition to corresponding binary classifier scores predicting the probability of molecular non-rejection, TCMR, and ABMR. The report provides a visualization of the new biopsy (yellow triangle) projected into the rejection-associated transcript-based principal component analysis of the 889 reference set biopsies. Biopsies in the reference set are colored according to their highest of four archetype scores in the 4AA model. Grey indicates that S1 (Normal) was the highest score, red corresponds to S2 (TCMR), blue to S3 (ABMR), and cyan to S4 (Injury). The right hand side of the report provides a table of additional molecular data including pathogenesis-based transcript (PBT) set scores and singular transcript expression scores relating to all rejections, TCMR, ABMR, and injury. Scores are represented as the log fold change in the new biopsy vs. normal biopsies (i.e. reference set biopsies with S1 (Normal) > 0.7). For each score a normal limit is given, defined as the 95th percentile score in the normal biopsies. Scores in the 95th-99th percentile are labeled “slightly abnormal” and scores in the 99th percentile are labeled “abnormal.” The report also has space for additional clinical information if provided.

MMDx-Lung
Transbronchial biopsies

Results on the first 250 biopsies

INTERLUNG ClinicalTrials.gov: NCT02812290
Explaining the Molecular Microscope® report for transbronchial lung biopsies (MMDx-Lung)

### Patient Information
- Date of transplant, date of biopsy, etc.

### Clinical Information
- Time post-transplant; indication, DSA (if provided)

### Additional Detail
- Rejection, injury-related transcript scores in this biopsy
- Comparison to normal scores interpreted vs. relatively normal biopsies

### Proportions
- Normal, TCMR, ABMR, injury

### Visualization
- Relationship of this biopsy to all others in the reference biopsies PC2 vs. PC1; PC2 vs. PC3

### Clinical Interpretation
- Abnormal biopsy with extensive parenchymal injury. Minimal ABMR-like changes. Minimal TCMR-like changes. Extensive injury (injury score 0.71, IN-4L, 11, and FISH Abnormal). The rejection states in this biopsy are unclear and may be the subject of the ongoing INTERLUCK study.

### Alveolar Content
- Too little makes interpretation difficult
Mucosal biopsies: much safer than TBBs

- Prospective collection of **mucosal biopsies** from indication or surveillance bronchoscopies in lung transplant recipients
  - **3rd airway bifurcation (3B-MB)**, typically between RLL and RML airway

- **1-2 pieces** for molecular analysis
  - Quantitative expression of 453 rejection-associated transcripts (RATs)
    - Originally identified by association with kidney transplant rejection histology

Image courtesy of Olympus
Lung Case #1
Report – Page 1

Relatively healthy lung transplant. Minimal ABMR-like changes. Minimal TCMR-like changes. Minimal parenchymal injury (Injury score 0.11, IRMATS and QCQMATs normal). (The relationship between the rejection archetype scores and actual rejection states is unclear and is the subject of the ongoing INTERLUNG study.)
# Lung Case #1

## Report Page 2

### Molecular Phenotype

<table>
<thead>
<tr>
<th>Gene expression sets</th>
<th>Biopsy score</th>
<th>Normal Limit*</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABMR related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSA-dominant transcritps (DSAST)</td>
<td>-0.14</td>
<td>&lt;0.31</td>
<td>Normal</td>
</tr>
<tr>
<td>Endothelial DSA-dominant transcritps (eDSAST)</td>
<td>-0.16</td>
<td>&lt;0.46</td>
<td>Normal</td>
</tr>
<tr>
<td>NK cell bantran transcritps (NKB)</td>
<td>0.06</td>
<td>&lt;0.06</td>
<td>Normal</td>
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<tr>
<td>T cell bantran transcritps (TCP)</td>
<td>0.01</td>
<td>&lt;0.06</td>
<td>Normal</td>
</tr>
<tr>
<td>IFN-G (Interferon-gamma)</td>
<td>5.65</td>
<td>&lt;5.43</td>
<td>Normal</td>
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<tr>
<td>CTLA4 (Cell-penetrant)</td>
<td>4.55</td>
<td>&lt;4.92</td>
<td>Normal</td>
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<tr>
<td>Rejection and injury-related</td>
<td>4.10</td>
<td>&lt;4.14</td>
<td>Normal</td>
</tr>
<tr>
<td>Injury-related</td>
<td>0.23</td>
<td>&lt;0.44</td>
<td>Normal</td>
</tr>
<tr>
<td>Interleukin-1 (IL-1)</td>
<td>0.15</td>
<td>&lt;0.26</td>
<td>Normal</td>
</tr>
<tr>
<td>Interleukin-8 (IL-8)</td>
<td>0.93</td>
<td>&lt;2.67</td>
<td>Normal</td>
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<td>B-cell bantran transcritps (BCT)</td>
<td>0.81</td>
<td>&lt;0.44</td>
<td>Normal</td>
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<tr>
<td>IFN-G (Interferon-gamma)</td>
<td>0.65</td>
<td>&lt;0.46</td>
<td>Normal</td>
</tr>
<tr>
<td>CTLA4 (Cell-penetrant)</td>
<td>5.10</td>
<td>&lt;5.24</td>
<td>Normal</td>
</tr>
<tr>
<td>IFN-G (Interferon-gamma)</td>
<td>13.75</td>
<td>&lt;20</td>
<td>Normal</td>
</tr>
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</table>

*Normal range for all parameters is given in the table above. Interleukin-8 (IL-8) normal range: >0.44.

### Local Histology Phenotype

<table>
<thead>
<tr>
<th>ISHLT A Grade</th>
<th>ISHLT B Grade</th>
<th>ISHLT C Grade</th>
<th>C4d</th>
<th>Neutrophilic Capillaritis and/or Mavangurizya</th>
<th>Other</th>
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<tbody>
<tr>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tbody>
</table>

### Diagnosis

<table>
<thead>
<tr>
<th>ISHLT A Grade</th>
<th>ISHLT B Grade</th>
<th>ISHLT C Grade</th>
<th>C4d</th>
<th>Neutrophilic Capillaritis and/or Mavangurizya</th>
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<tbody>
<tr>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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</tbody>
</table>

### Clinical notes

*References*

MMDx-Liver

Molecular analysis of rejection and injury in human liver transplant biopsies:
First results of the INTERLIVER STUDY

INTERLIVER ClinicalTrials.gov NCT03193151
Archetype clusters

A1 = injury
A2 = relatively normal
A3 = TCMR

Early injury

“normal”

Fibrosis?
Liver Case #1 B1 (596 days post-Tx) Report

**General Information**

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Name</th>
<th>Sample Name</th>
<th>Date Reported (Y-M-D)</th>
<th>Date Received (Y-M-D)</th>
<th>Date of Transplant (Y-M-D)</th>
<th>Date of Biopsy (Y-M-D)</th>
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<tr>
<td>BED0001</td>
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<td>BED0001</td>
<td>2018-Dec-95</td>
<td>2016-May-02</td>
<td>2017-Dec-19</td>
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</table>

**Clinical Information**

- **Time of Biopsy Post-Tx**: 596 days
- **Biopsy Indication**: Primary Disease
- **For Cause**: Cirrhosis, Nonalcoholic Steatohepatitis

**Pure molecular interpretation**

Relatively healthy liver transplant. Minimal TCMR-like changes. Minimal parenchymal injury (Injury score, IRAT and QCAT scores normal). (The relationship between the rejection archetype scores and actual rejection states is unclear and is the subject of the ongoing INTERLIVER study.)

**Pathology**

- **Acute Rejection**
  - Portal Venule inflammation
  - Bile Duct inflammation
  - Various Endothelial Inflammation

- **Chronic Rejection**
  - Bile Duct Degeneration
  - Focal Cholestasis
  - Cholestasis
  - Chronic Fibrosis
  - Arterial IIR

**Other Disease**

- Autoimmune Hepatitis
- Steatosis/steatohepatitis
- Recurrent CMV/Herpesvirus

**Clinical notes**

References

Liver biopsy case 2 (751 days post-Tx)

General Information

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>DE000065</th>
<th>Name:</th>
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<tbody>
<tr>
<td>Sample Name</td>
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<tr>
<td>Biopsy Number</td>
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<tr>
<td>Date Reported (Y-M-D)</td>
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<td>Date Received (Y-M-D)</td>
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<td>Date of Transplant (Y-M-D)</td>
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<td>Date of Biopsy (Y-M-D)</td>
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Patient Age at Biopsy: NA

Clinical Information

<table>
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<tr>
<th>Time of Biopsy Post-Tx</th>
<th>751 Days</th>
<th>Biopsy indication</th>
<th>For Cause</th>
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<tbody>
<tr>
<td>DSA Status</td>
<td>NA</td>
<td>Primary Disease</td>
<td>Primary Biliary Cholangitis</td>
</tr>
<tr>
<td>PRA Status</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Molecular Phenotype

<table>
<thead>
<tr>
<th>Gene Expression</th>
<th>Biopsy score</th>
<th>Normal Limit</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABM-related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DSAXA</td>
<td>0.09</td>
<td>&lt;0.18</td>
<td>Normal</td>
</tr>
<tr>
<td>Endothelial DSAXA</td>
<td>-0.05</td>
<td>&lt;0.2</td>
<td>Normal</td>
</tr>
<tr>
<td>NK cell burden transcripts (NKB)</td>
<td>0.18</td>
<td>&lt;0.27</td>
<td>Normal</td>
</tr>
<tr>
<td>TCMM-related</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytoxotic T cell transcripts (COT)</td>
<td>1.63</td>
<td>&lt;0.25</td>
<td>Abnormal</td>
</tr>
<tr>
<td>T cell burden transcripts (TGB)</td>
<td>2.19</td>
<td>&lt;0.4</td>
<td>Abnormal</td>
</tr>
<tr>
<td>IFN-γ (Interferon gamma)</td>
<td>5.97</td>
<td>&lt;4.55</td>
<td>Abnormal</td>
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<tr>
<td>CTLA4 (T cell checkpoint)</td>
<td>4.62</td>
<td>&lt;3.39</td>
<td>Abnormal</td>
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<tr>
<td>Rejection and injury-related</td>
<td></td>
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<tr>
<td>IFNγ-inducible transcripts (GRIT)</td>
<td>0.67</td>
<td>&lt;0.13</td>
<td>Abnormal</td>
</tr>
<tr>
<td>Injury transcripts (RINAT)</td>
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</tr>
<tr>
<td>Microglia transcripts (QCMAT)</td>
<td>0.48</td>
<td>&lt;0.15</td>
<td>Abnormal</td>
</tr>
</tbody>
</table>

Pathology

| Acute Rejection |
|-----------------|----------------|
| Portal Vein Inflammation | 0 |
| Bile Duct Inflammation | 0 |
| Venous Endothelial Inflammation | 0 |

<table>
<thead>
<tr>
<th>Chronic Rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile Duct Degeneration</td>
</tr>
<tr>
<td>Focal Obstruction</td>
</tr>
<tr>
<td>Cholestasis</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune Hepatitis</td>
</tr>
<tr>
<td>Steatohepatitis</td>
</tr>
<tr>
<td>Fibrosis</td>
</tr>
<tr>
<td>HCC</td>
</tr>
<tr>
<td>Recurrent HCV Hepatitis</td>
</tr>
<tr>
<td>Recurrent CMV Hepatitis</td>
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</tbody>
</table>

Pure molecular interpretation

Abnormal biopsy with extensive TCMM-like changes. Some parenchymal injury (IRRAT and QCMAT scores abnormal). (The relationship between the rejection archetype scores and actual disease states is unclear and is the subject of the ongoing INTERLIVER study.)

Proportion Rejection and Injury:

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Relatively Normal</th>
<th>TCMM-Like</th>
<th>Injury-Like</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td></td>
<td>1.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Clinical notes

References:


Complementary diagnostic tools in transplant pathology

- Final diagnosis
- HLA
- Light microscopy
- IHC
- IF
- EM
- Chemistry
- Clinical history
- MMDX