

# **THROMBOTIC MICROANGIOPATHY (TMA)**

## **BANFF WORKING GROUP**

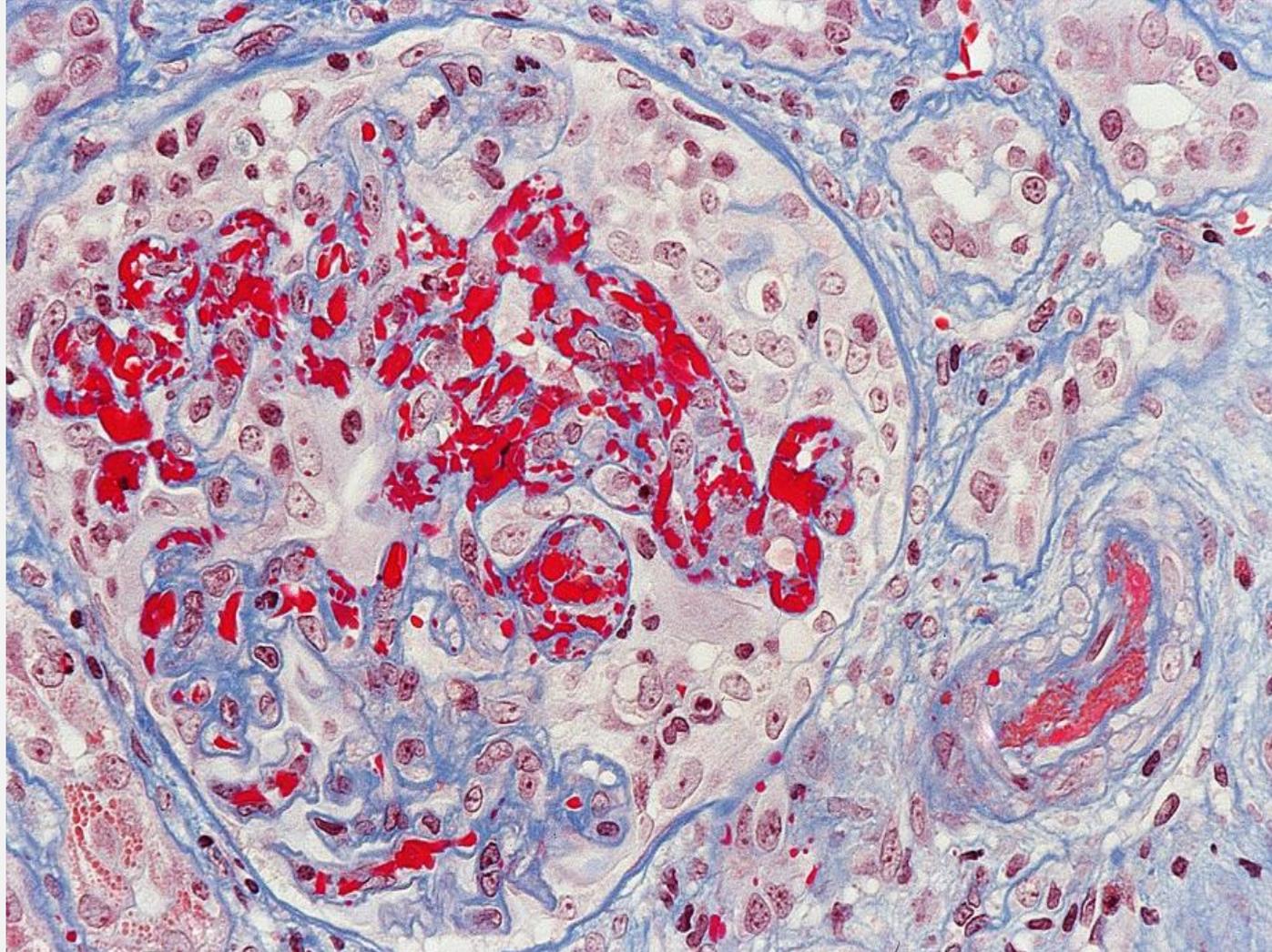
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**Assistant Professor Department of Pathology**

**University of Texas Medical Branch (UTMB)**

**USA**

# TMA in renal allograft can be a challenging diagnosis



# Causes of TMA in Native kidney

## 1. Shiga-like toxin-producing E Coli (typical HUS)

## 2. Other infections

- Meningococcus, *H. influenza*, *C. difficile*
- Viruses: Dengue, CMV, Influenza
- Parasites

## 3. Drugs

- Gemcitabine, mitomycin
- CNI, anti-vascular endoth. cell factor meds
- Clopidogrel, Quinine

## 4. Autoantibodies

- Auto-immune diseases : SLE, Scleroderma  
APLS, Anti-Factor H, I, disintegrin, ADAMTS13

## 5. Genetic mutations

- Factor H, I, Membrane cofactor protein, C3,  
ADAMTS13, coagulation factors (plasminogen,  
Thrombomodulin, VWF, Cobalamin C deficiency)

## 6. Pregnancy : Eclampsia/Pre-eclampsia

## 7. BM transplantation

# Causes of TMA in Tx kidney

## Recurrent TMA

### 1. Gene mutations

- Complement reg factor: Factor H, Factor I, MCP
- C3

### 2. Autoantibodies

- Anti-Factor H, Anti-ADAMTS13, APLS Antibodies,  
SLE and scleroderma

## De novo TMA

1. CNI
2. mTOR
3. AMR
4. Infections
  - PVB19
  - CMV
  - Hep C

# **TMA in renal allograft can be a challenging diagnosis**

- 1. Majority of transplant TMAs are de novo: No previous history**
- 2. Absence of systemic disease (thrombosis): Localized TMA**
- 3. Confounding lesions: g, ptc, C4d+, TG**
- 4. Lack of EM for transplant biopsies (TxBx)**
- 5. No established minimum diagnostic criteria**

# TMA WG Objectives

- **Survey the current practices for diagnosis of TMA in renal TxBx**
- **Define minimum diagnostic criteria for TMA in renal TxBx**
- **Develop recommendations for accurate diagnosis that would include morphological, clinical, laboratory and molecular findings**

# Part #1: The 2016 Survey on Current Practices

- 41 participants signed up
- Questionnaire of 20 questions was circulated
- 26/41 responded

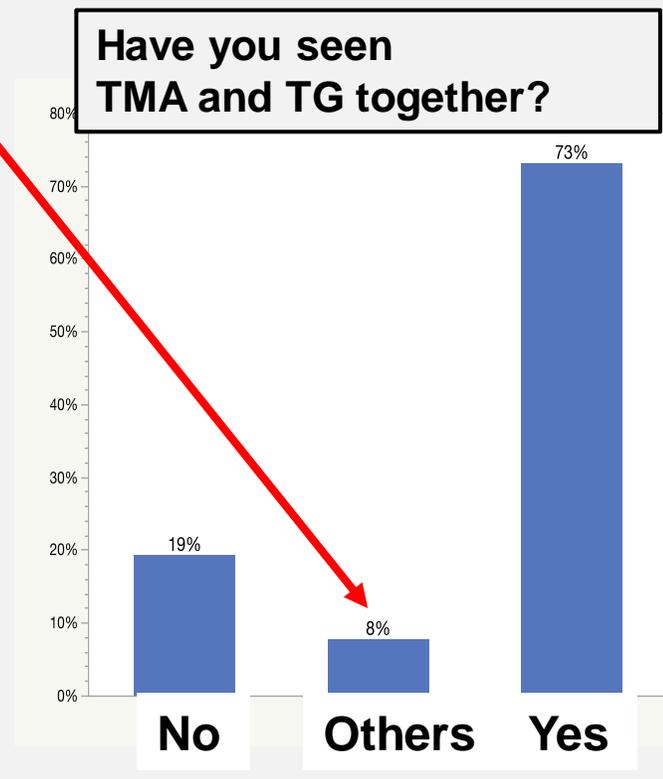
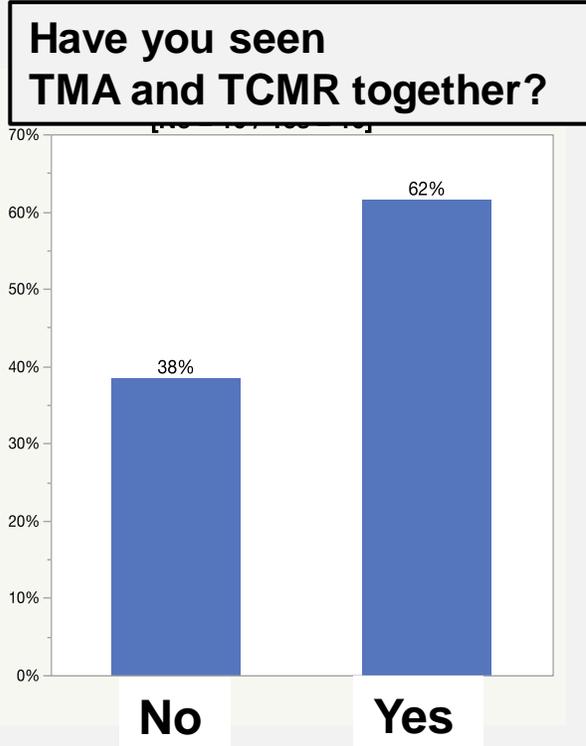
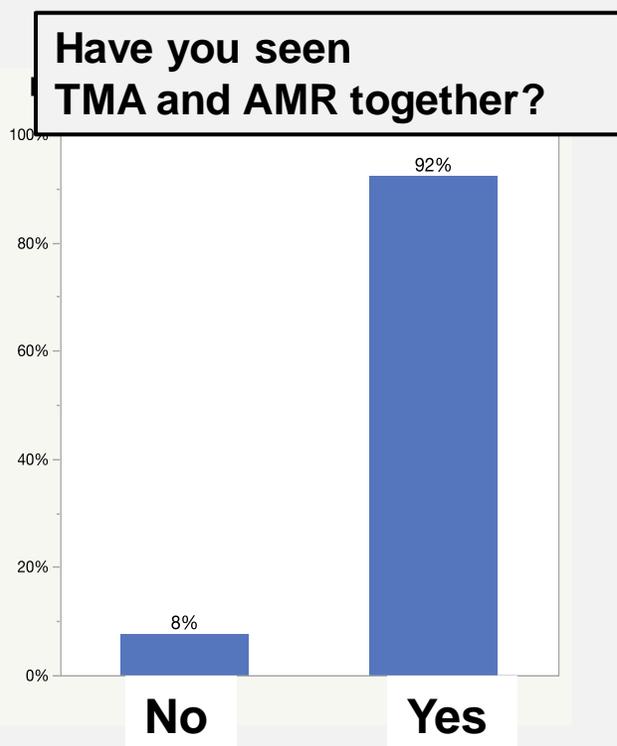
# 1 - Frequency and Spectrum

What is the estimated % of diagnosis of TMA in your services?

- 35% of participants → <5%
- 42% of participants → 5-10%
- 23% of participants → 10-20%

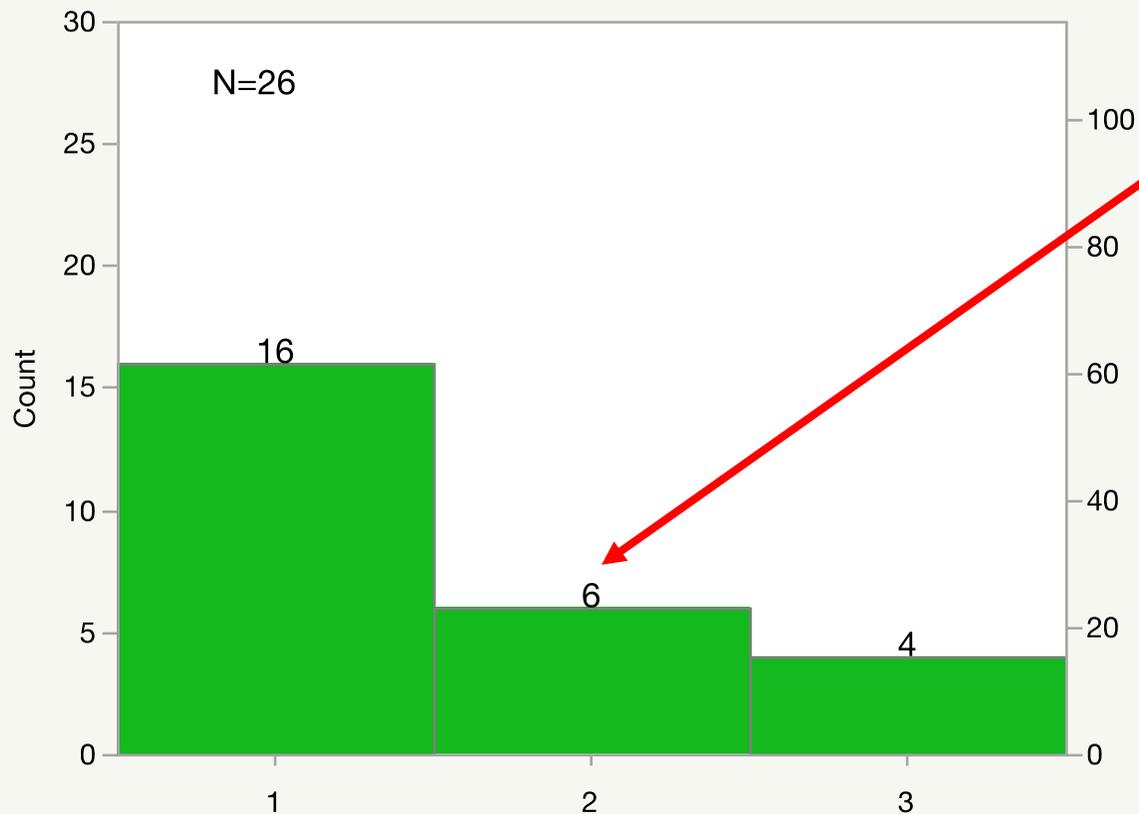
1-Do not know how to reliably separate late stages of TMA from TG.

2-Difficult to answer. To my opinion, TMA is one of the causes of TG.



## 2 – Stains for diagnosis of TMA

What stains do you use to make the diagnosis of TMA by LM?

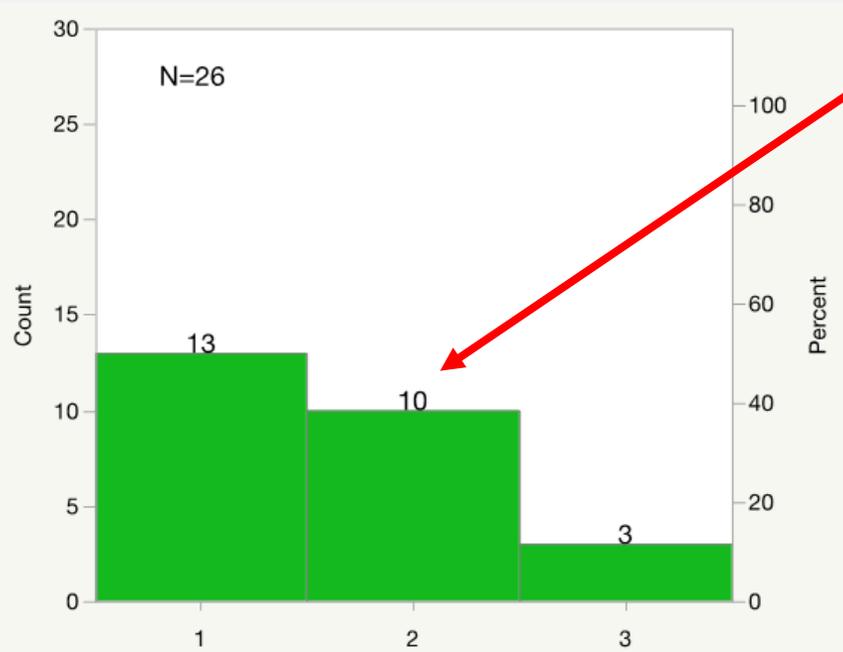


- 1- Jones + MT
- 2- Not a pathologist
- 3- H&E + MT+ PTAH
- 4- Trichrome AFOG (Acidic Fuchsin Orange G)
- 5- H&E + MT + PAMS (PAS to exclude hyaline)
- 6- H&E + PAS + MT + JMS

- 1. HE + MT
- 2. Others
- 3. HE + MT + MSB

## 2 – Stains and tests for diagnosis of TMA

Which of the following options is used in the diagnosis / confirmation of TMA?

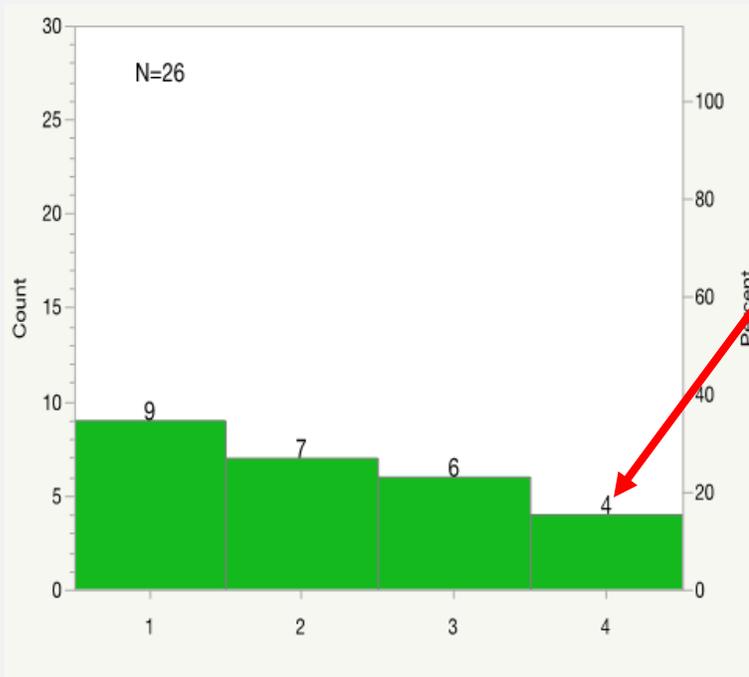


- 1- Fibrinogen (I.F.) + EM
- 2- C4d (I.F.)
- 3- C4d (IHC)
- 4- C4d, clinical data
- 5- C4d as per routine not confirmation
- 6- Diagnosis can be made on H&E / TCR
- 7- AFOG
- 8- C4d + C3
- 9- C4d is useful to distinguish AMR-assoc. TMA from other causes but not for the diagnosis of TMA
- 10- C4d + C3 + Fibrinogen

1. C4d + C3 + Fibrinogen + EM
2. Others
3. EM

### 3 – LM criteria of TMA in renal allografts

LM criteria for diagnosis of TMA (acute/organizing) in the Tx kidney should include presence of?

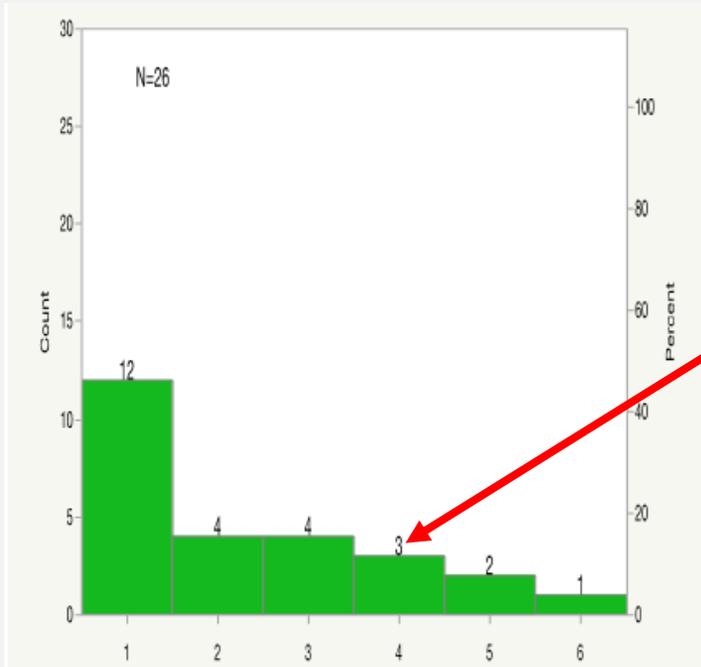


- 1- #1 + fragmented RBCs in arteries and arterioles
- 2- #1 for acute cases, no need for microthrombi for organizing cases
- 3- #2 and/or mesangiolyis
- 4- The spectrum of TMA is broad and not limited to thrombi and double contours: endothelial swelling, subendothelial edema, platelets thrombi (CD61 staining), mesangiolyis, "onion skin" changes, etc.

1. Glomerular microthrombi + extravasation of RBCs in arteries and arterioles
2. #1 ± double contours in glomerular capillaries
3. #2 ± microthrombi in peritubular capillaries
4. Others

### 3 – EM criteria of TMA in renal allografts

EM criteria for diagnosis of TMA should include which of the following?

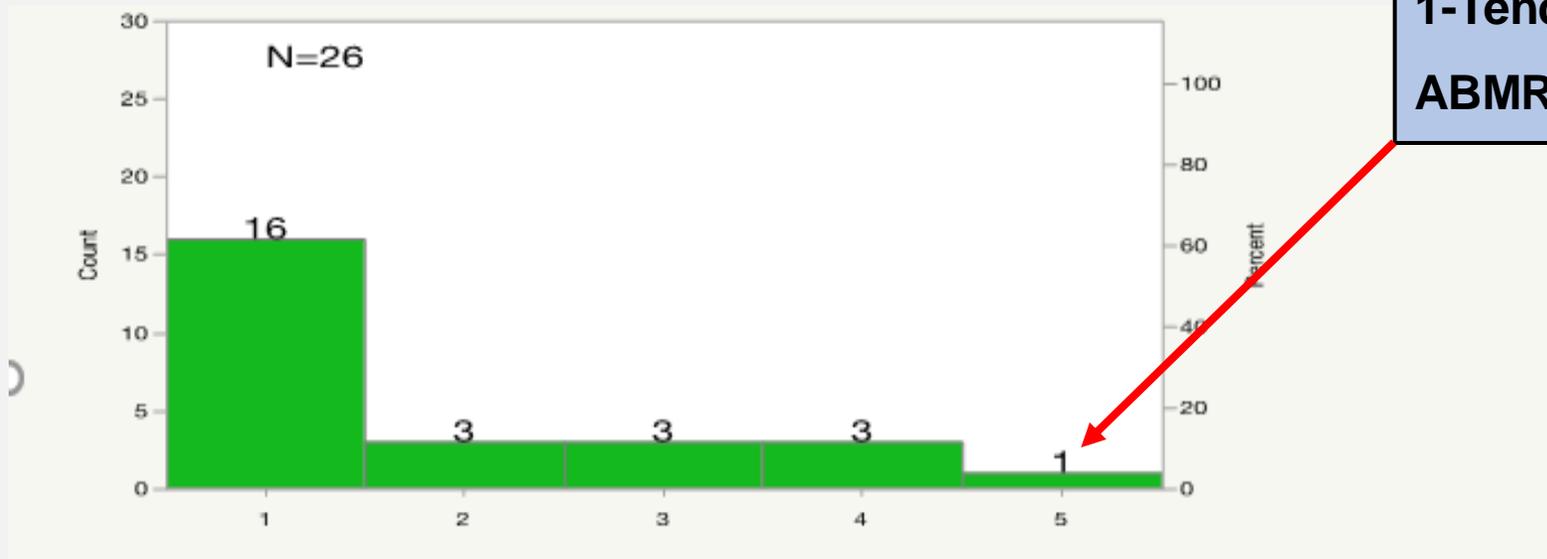


- 1- Mesangiolyis
- 2- EM changes can be extremely subtle or even absent
- 3- Fibrin deposition in acute; Sub-endothelial widening and accumulation of granular material ('Fluff') in chronic

1. Sub-endothelial widening and accumulation of “fluff” + Signs of endothelial cell injury
2. Signs of endothelial cell injury: Loss of fenestration, cytoplasmic fragmentation, platelet adhesion to endothelial cells
3. Sub-endothelial widening and accumulation of “fluff”
4. Others
5. #1 + mesangial interposition
6. #1 + #2 + mesangial interposition and/or GBM lamellation

## 4 – Criteria for recurrent and *de novo* TMA in allografts

Which of the following diagnostic steps are taken to establish the etiology of recurrent TMA?

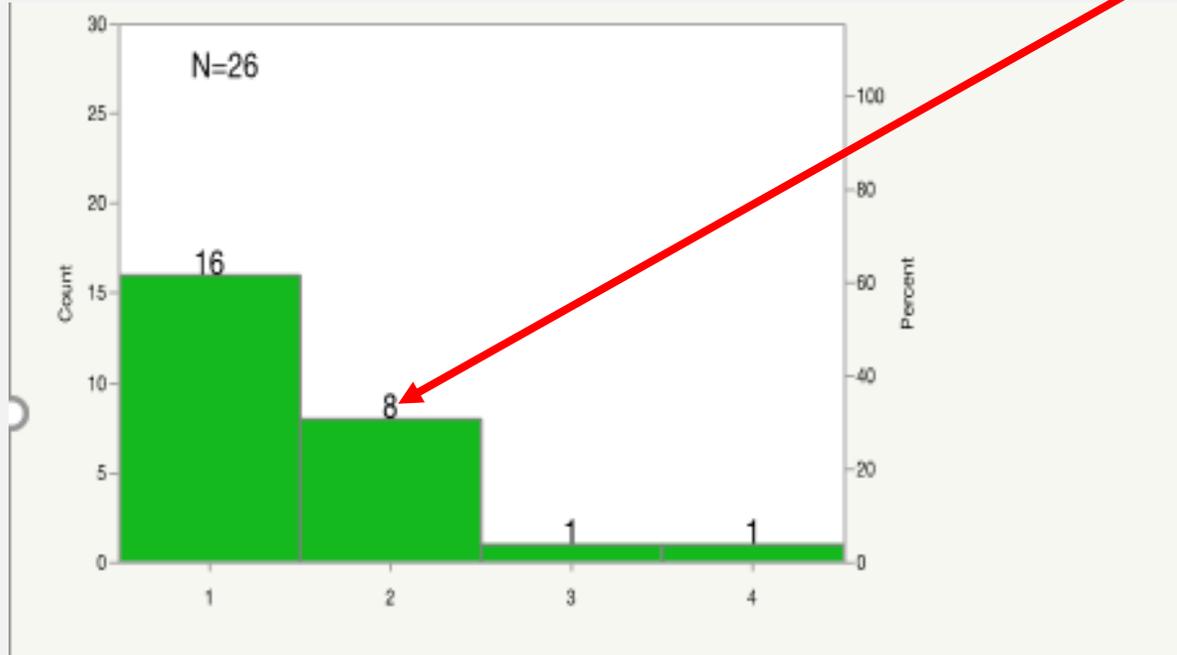


1-Tendency to blame CNIs if no TCMR or ABMR found and original disease is not HUS

1. Pre-Tx clinical diagnosis of aHUS based on serological  $\pm$  genetic testing
2. Post- Tx clinical diagnosis of aHUS based on serological  $\pm$  genetic testing
3. Pre- and Post clinical diagnosis of aHUS based on serological  $\pm$  genetic testing
4. Pre-Tx clinical suspicion of aHUS in the native kidney without laboratory proof of aHUS
5. Others

## 4 – Criteria for recurrent and *de novo* TMA in allografts

Which of the following clinical, laboratory and histologic findings may help establish the diagnosis of recurrent TMA in your institution?

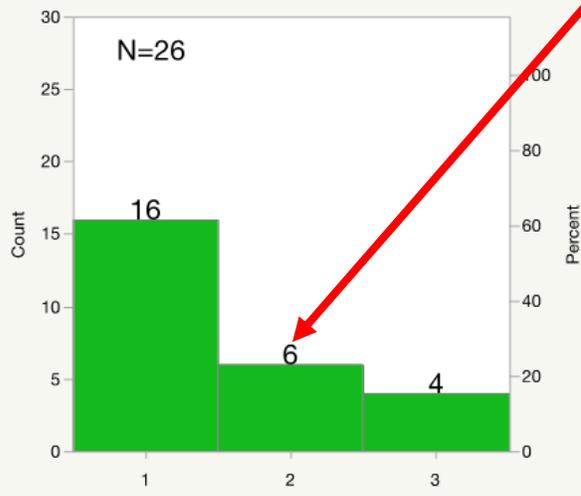


1. Histologic signs of AMR+ correlation with DSA & C4d or CNI Toxicity with correlation with drug level or presence of malignant HTN
2. Others
3. Histologic signs of CNI Tox + correlation with drug level
4. Presence of malignant HTN

- 1- Original disease must be HUS
- 2- Presence of DSA excludes recurrence from primary TMA
- 3- TMA in native kidney + TMA in allograft biopsy
- 4- #1 + patient's history such as preceding diarrhea
- 5- Not sure any of the above options establish a diagnosis of recurrent TMA as they aren't variables present in the native kidney before transplant (with exception of malignant HTN)
- 6- Histological diagnosis of TMA + Exclusion of other causes such as AMR, CNI toxicity + Genetic/serological findings consistent with aHUS
- 7- Not sure if any fit
- 8- Clinical findings (renal dysfunction, hematologic findings) + microthrombi with or without other associated conditions such as AMR

## 5 – Role of complement

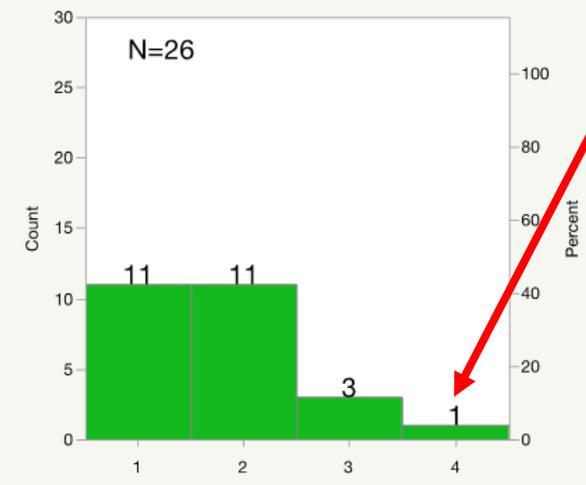
To assess the role of complement in Tx TMA you prefer to use:



1- IF or IHC seem OK  
 2- IHC for C4d and IF for C3 and C1q  
 3&4- IF plus IHC (C4d)  
 5- In our experience, C5b-9 antibody is difficult to use on both FFPE and frozen tissue  
 6- IF is routine, IHC is available if needed

1. IF
2. Others
3. IHC

To assess the role of complement in Tx TMA you prefer to use:

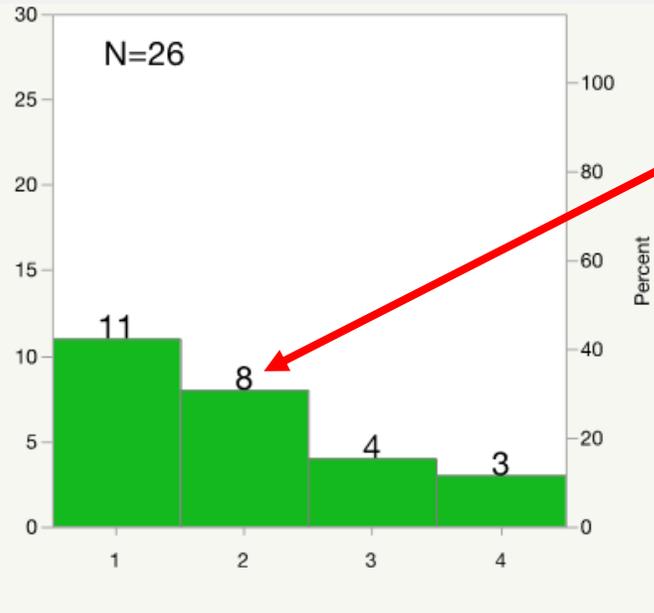


1-C5b-9 is theoretically a good marker but the antibody is difficult to use on both FFPE and frozen tissue

1. C4d + C3
2. C4d + C3 + MAC
3. C4d alone
4. Others

## 6 – TMA in the donor biopsy

**Do you think the outcome is affected when TMA is seen in the donor biopsy?**

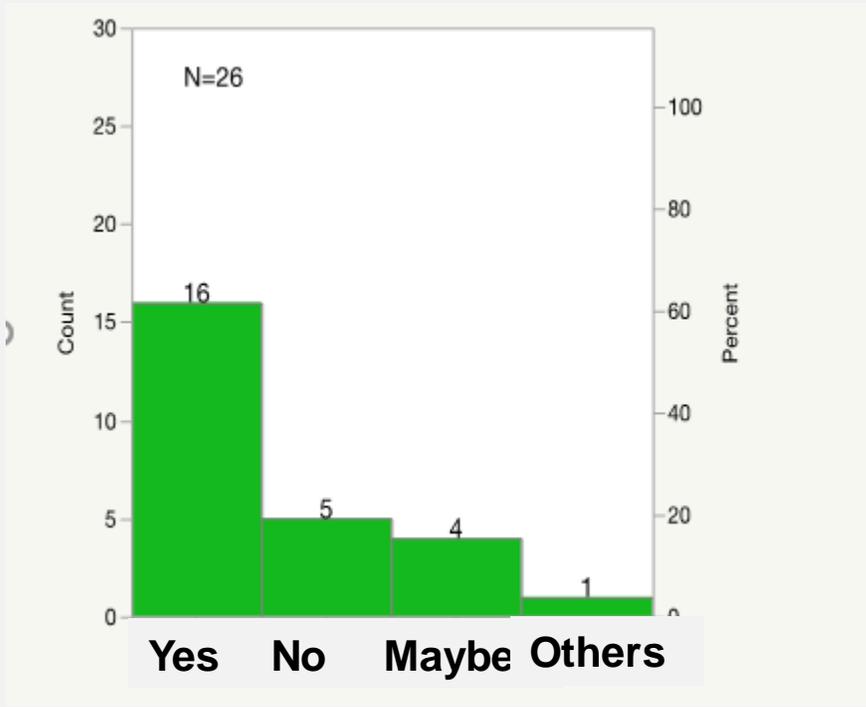


- 1- Don't know
- 2- Others
- 3- No - Graft outcome is unaffected
- 4- Yes - Graft outcome will be poor

- 1- Depends on severity
- 2- Based on my mentor opinion
- 3- Depends on the etiology
- 4- Depends on the pathogenesis of the TMA
- 5- Acute TMA doesn't affect outcome, have no experience with chronic TMA
- 6- There will be delayed function and baseline creatinine may be higher after functioning
- 7- Recent paper of Batra, et al. (Am J Transplant 2015) seems to indicate that glomerular fibrin thrombi do not impact negatively graft outcome
- 8- If you are referring to deceased donors, these kidneys would not be accepted in our center if there is diffuse TMA. Kidneys with a few microthrombi in glomerular capillaries would be accepted, and the outcome is probably not affected.

# 7 – Pathology before and after Eculizumab therapy

If you have Tx TMA cases treated with Eculizumab, do you have Bx before and after treatment?



# 8 – Endothelial cell genes

Would you be able to measure a set of endothelial gene transcripts in paraffin blocks of renal allograft biopsies at your institution?



# Part #1- Conclusions

- **Considerable heterogeneity of practices among pathologists:  
Stains; LM criteria; EM and EM criteria; Laboratory criteria**
- **Usage of complement for diagnosis is not standardized**
- **Diagnosis of recurrent TMA is made using different tools: Pre-Tx history of HUS versus post Tx serologic  $\pm$  genetic testing**
- **Majority of participants do not know about the meaning of donor TMA, its incidence and its effect on graft outcome**
- **Pathology after treatment with Eculizumab is not known**
- **Questions to answer: Endothelial cell injury studies: miRNAs? Other?  
Exploration of the role of endothelial cell damage in peritubular capillaries in TMA?**

Lake Moraine  
Banff National Park, Canada



# Part #2: Consensus generation

- **Consensus generation and the Banff Classification On Allograft**

## **Pathology:**

- **Main tool used to define all Banff lesions**
  - **Since the first Banff group was formed in 1991**
- 
- **The term **consensus** is defined as**
    - **General agreement**
    - **Not necessarily unanimity**
    - **Resolution of objections**
    - **Fair consideration of all comments**

# Consensus methods

- **The Nominal Group Techniques (NGT) – Structured meeting**
- **The NIH’s Consensus Conference – Consensus panel**
- **The Glaser state-of-the-Art Approach**

**Rennie on NIH consensus statements about coronary artery bypass surgery:**

**“As I read such statements, I have the sensation that I am being provided the bland generalities that represent the lowest common denominator of a debate-the only points on which the experts can wholeheartedly agree- and that these points must be so mild, so far from the cutting edge of progress, and so well-established that surely everybody must already know them.... “.**

# The Delphi methodology\*

- **Structured process**
- **Panel of experts: The panelists**
- **Iterative fashion:**
  - **Repeat rounds**
  - **Controlled feedbacks given by the facilitator**

# The Delphi methodology

- **Difference with other techniques**
  - **Anonymous**
  - **Participants are polled individually**
  - **Does not require the physical presence of the participants in an actual meeting**
  
- **Steps**
  - **Definitions**
  - **Rules**

## A. Definition of an Expert Panel (The panelists)

- **Inclusion criteria:**
  - **Nephropathologists who have reported TMA in the past 3 years (2014-2017):**
    - **TMA WG participants**
  
- **Exclusion criteria:**
  - **The leaders of the Banff-TMA-WG (the facilitators) are excluded to ensure elimination of any bias.**

- **The role of the facilitators:**
  - **Carry out programming of the survey rounds**
  - **Keep track of the responses**
  - **Recode the items**
  - **Host digital slides**
  - **Collect, analyze and present the data**
  
- **A biostatistician and an expert in Delphi methodology**
- **Total number of potential panelists is 26**

## B. Commitment to participation

- **Panelists will be contacted at the beginning of the study by e-mail**
- **Requirement for participation: to sign a document, committing to respond to ALL surveys and not discuss the project with any other individual**
- **Acknowledgement of the panelists in any publication derived from the project**

## C. Validation of the histopathological criteria

- Renal TxBx collection from both the panelists and the facilitators of the WG

### **Inclusion criteria:**

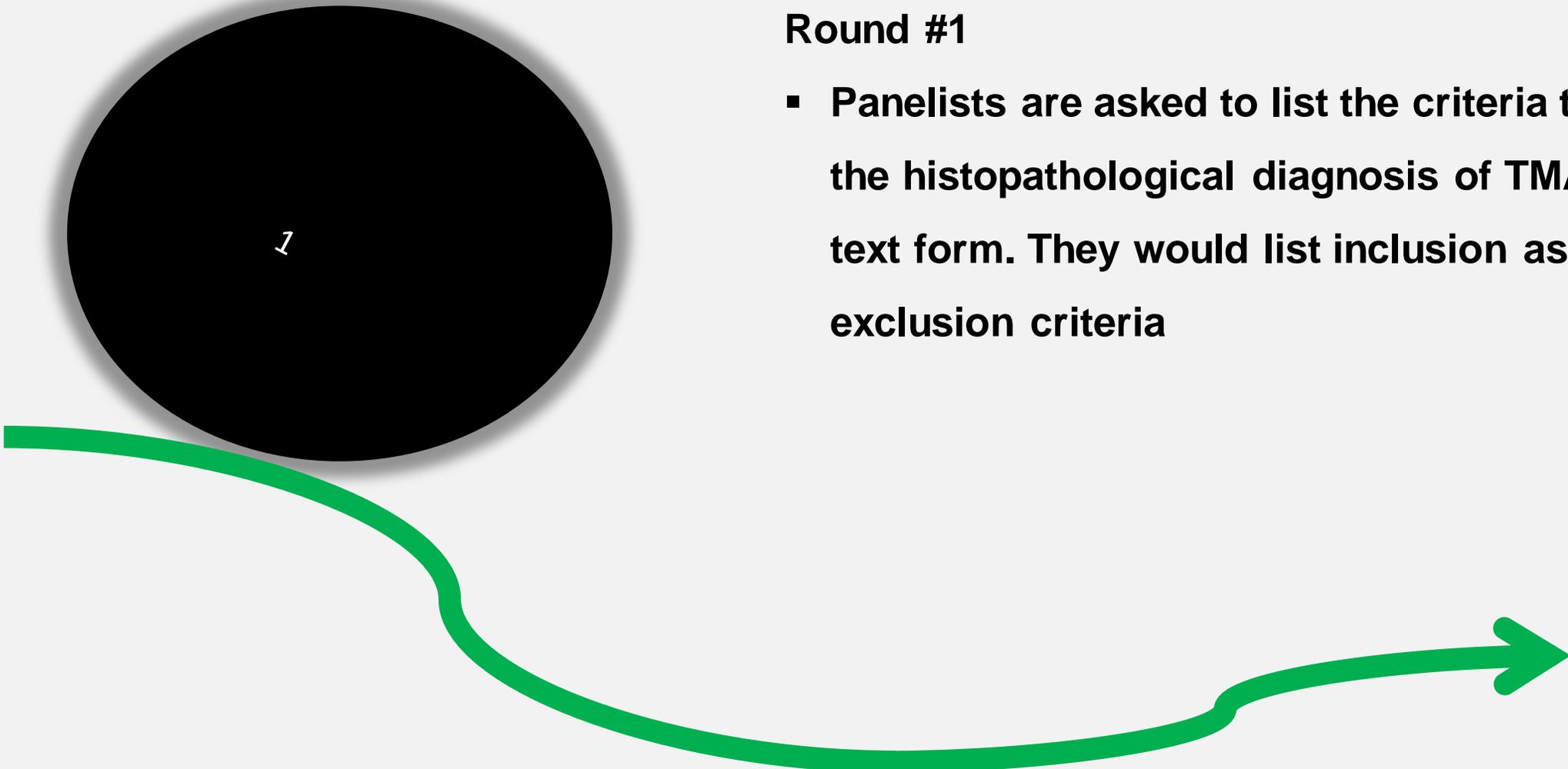
- Cases will include TxBx (procurement or 0-hr Bx excluded)
  - Straight forward cases of TMA
  - “Look-alike” cases
  - Positive controls: Native biopsies (Lupus nephritis associated with Anti-phospholipid syndrome)

## **D. Development of a core set of histopathological criteria for the diagnosis of TMA: Multiple rounds**

**To develop a core set of diagnostic histopathological criteria, 6 rounds are designed:**

## Round #1

- Panelists are asked to list the criteria they use for the histopathological diagnosis of TMA in free text form. They would list inclusion as well as exclusion criteria

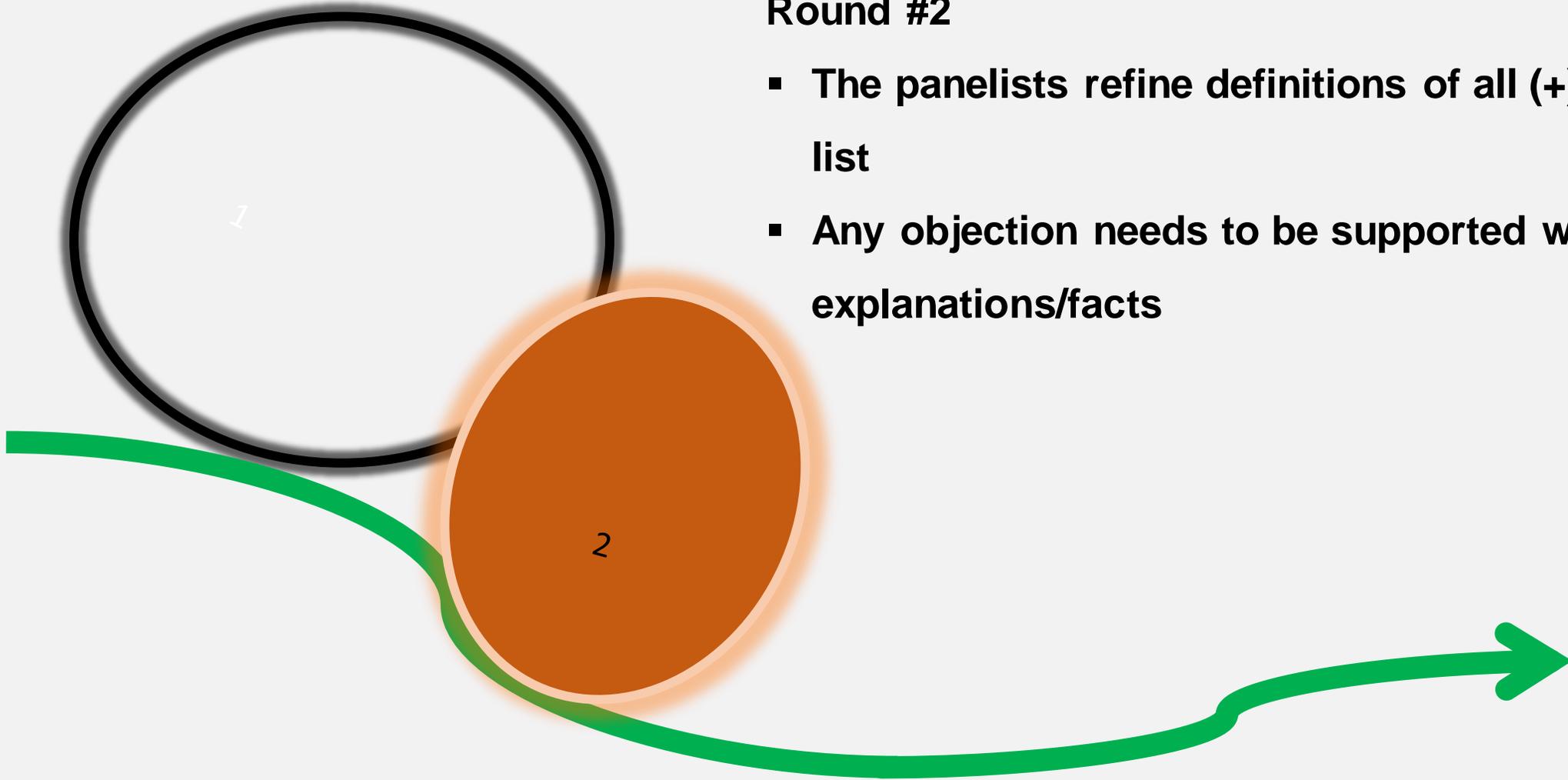


1

The facilitator will create a curated list of all (+) and (-) items (with %) and sent back to the panelists

## Round #2

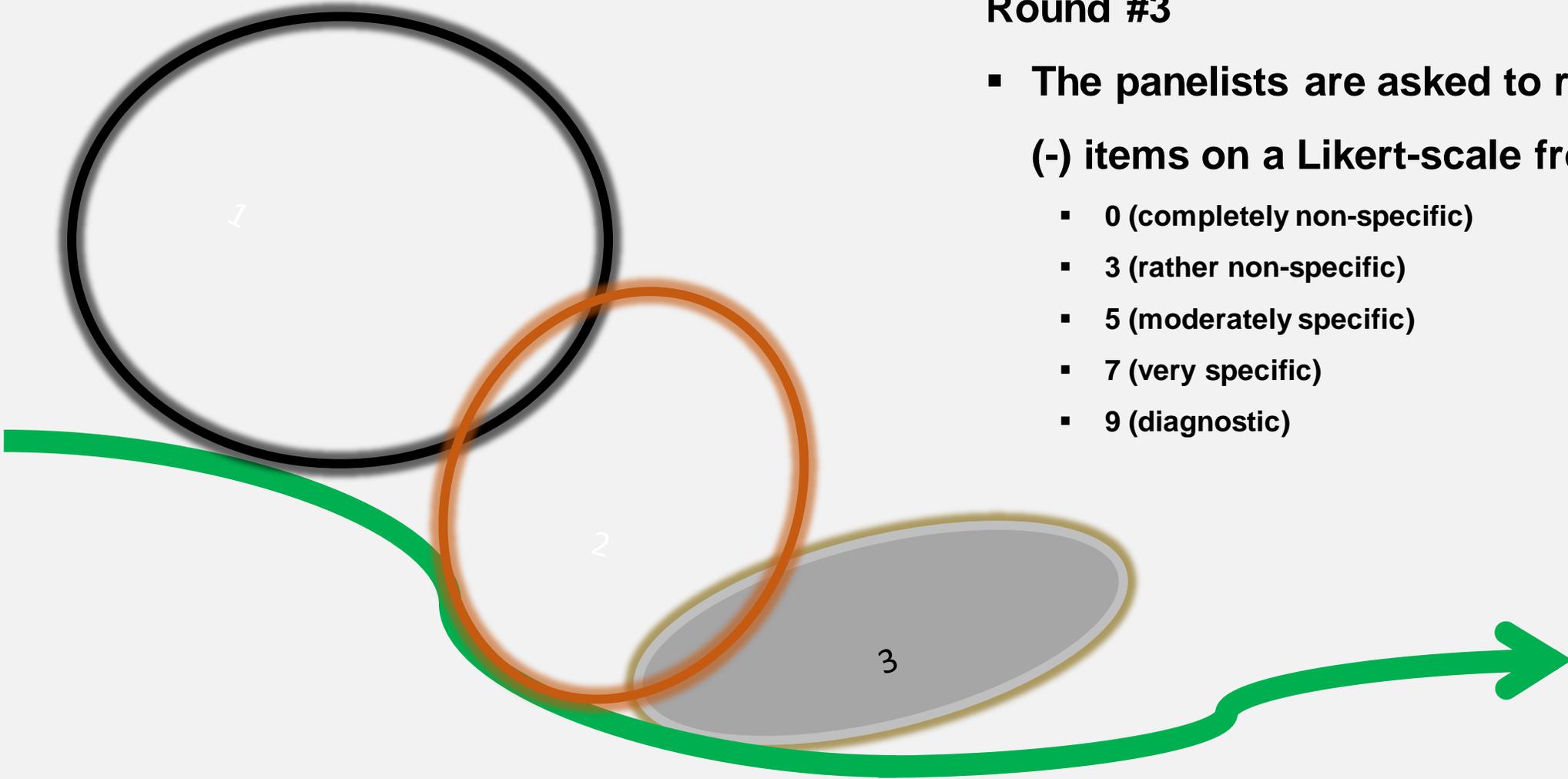
- The panelists refine definitions of all (+) and (-) on the list
- Any objection needs to be supported with explanations/facts



**The facilitator rewrites definitions according to the feedback from round #2 and re-sends the new list to the panelists**

## Round #3

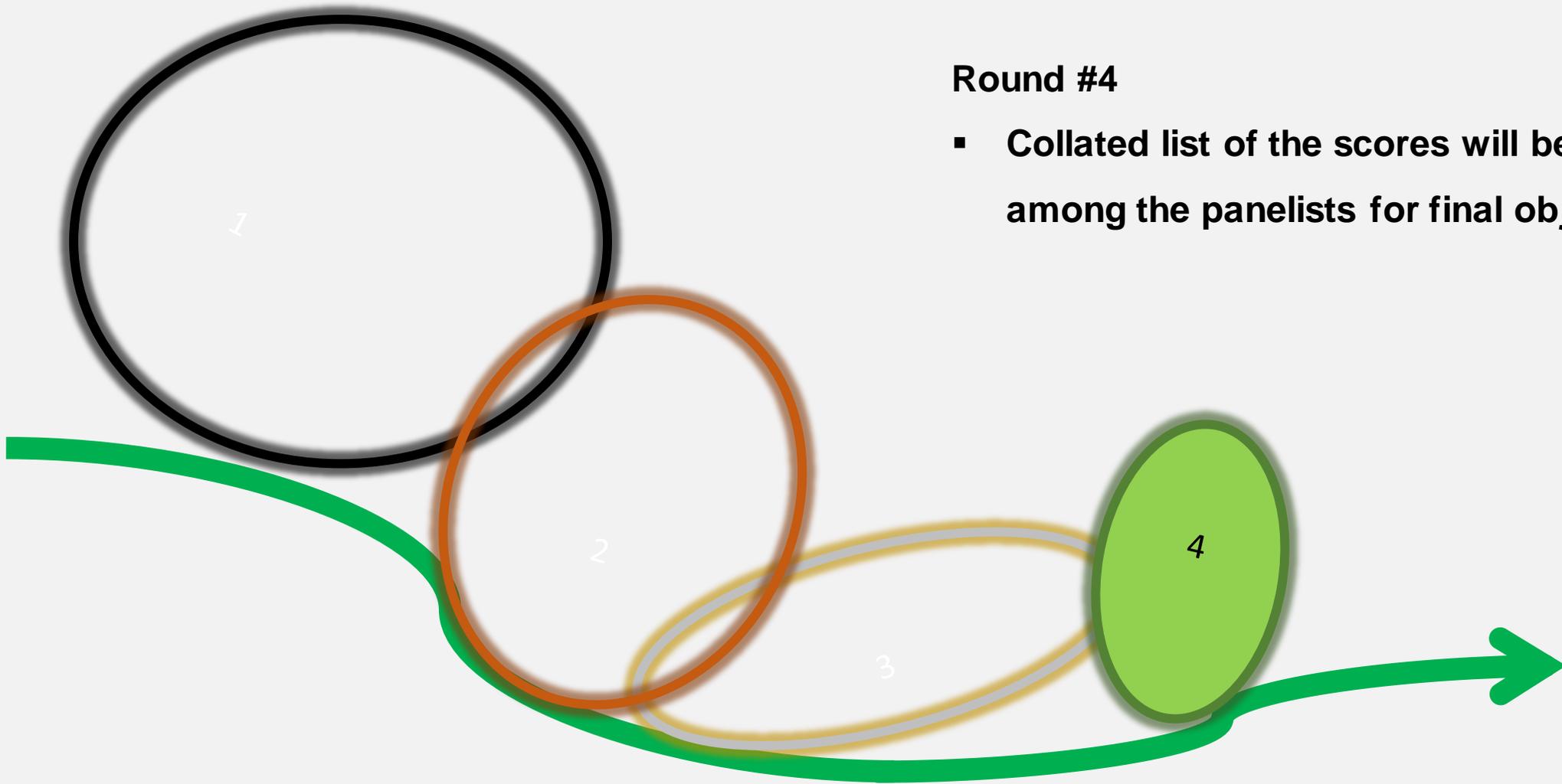
- The panelists are asked to rank all (+) and (-) items on a Likert-scale from 1 to 9:
  - 0 (completely non-specific)
  - 3 (rather non-specific)
  - 5 (moderately specific)
  - 7 (very specific)
  - 9 (diagnostic)



The facilitator will collate the specificity scores and create the list for round #4.

## Round #4

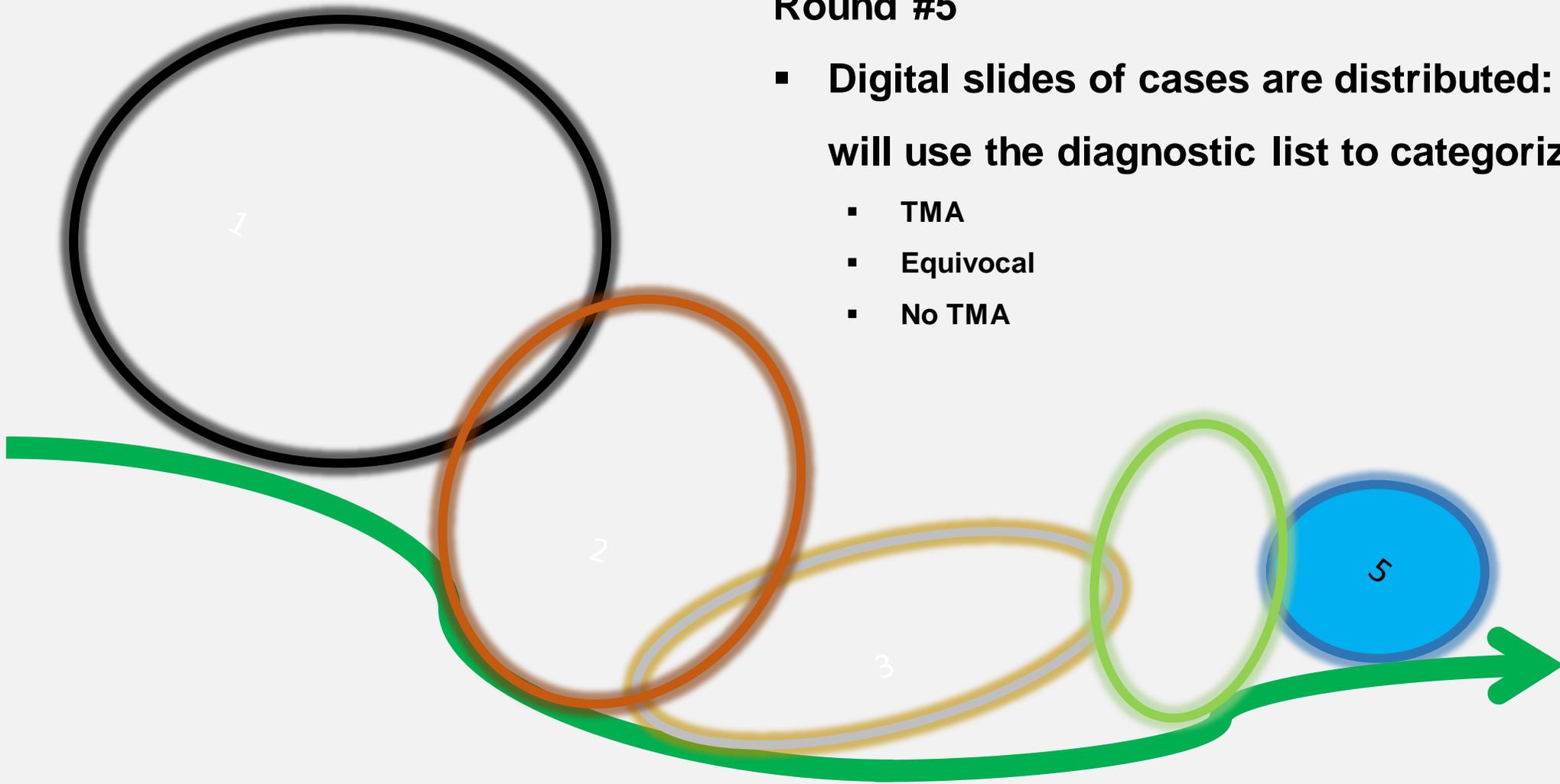
- Collated list of the scores will be circulated among the panelists for final objection



**Final list will be created by the facilitator**

## Round #5

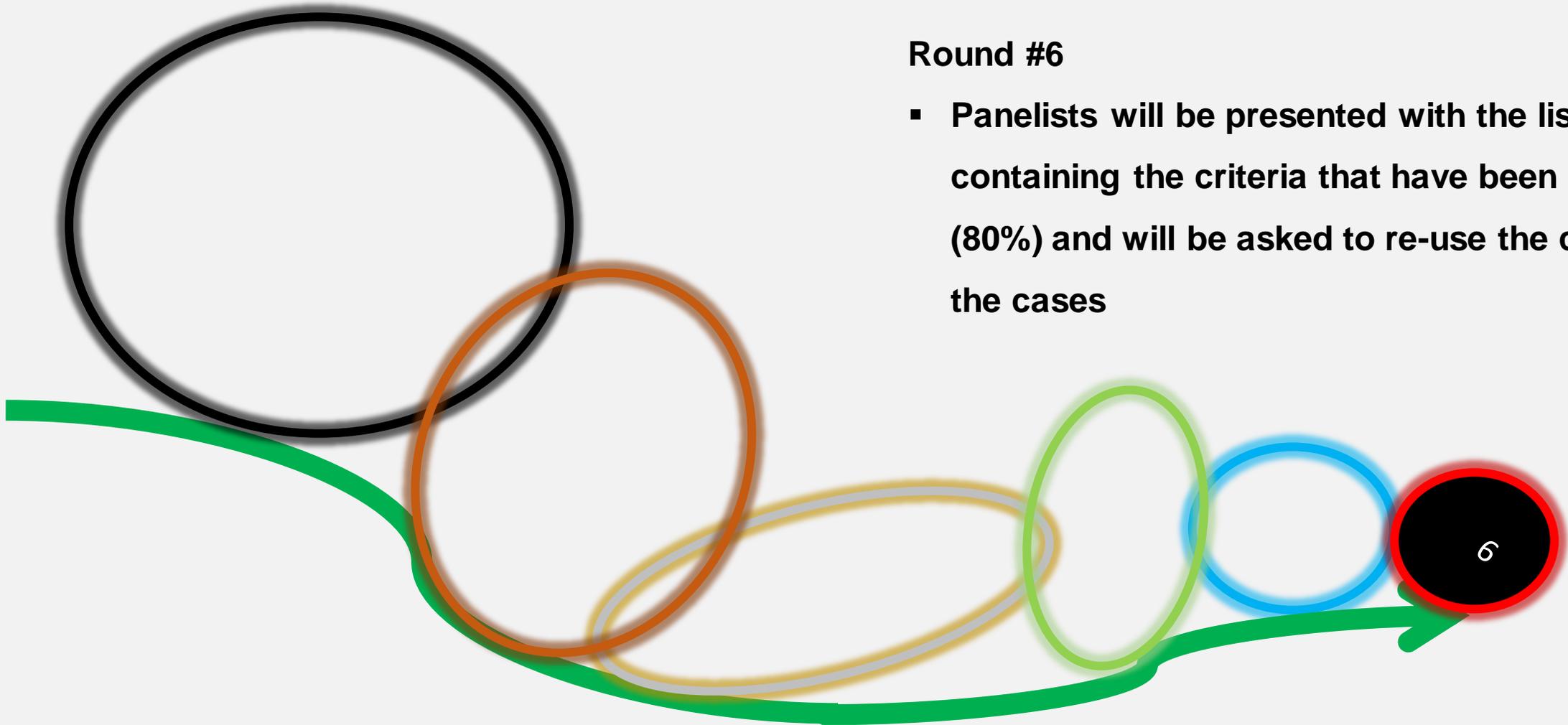
- Digital slides of cases are distributed: The panelists will use the diagnostic list to categorize the cases as
  - TMA
  - Equivocal
  - No TMA



The panelists will also check on the item on the +/- lists to explain what criteria they have used for their diagnosis. The facilitator will collate the answers. All cases with a consensus  $\geq 80\%$  are retained in the collection

## Round #6

- Panelists will be presented with the list containing the criteria that have been used (80%) and will be asked to re-use the criteria on the cases



The panelists will also check on the item on the +/- lists to explain what criteria they have used for their diagnosis. The facilitator will collate the answers. All criteria with a consensus  $\geq 80\%$  are retained. Final results are communicated to the panelists

- **The items will be tested for reproducibility among participants**
- **The performance of all candidate algorithms will be determined**
- **Results will be communicated to the panelists**
- **A statistician will perform the statistical analysis using the appropriate methods**
- **The WG chair assigns tasks for manuscript preparation**



# TMA Working Group Members involved in Project #1

**Alachkar, Nada**

**\*Afrouzian, Marjan**

**Alpers, Charles E.**

**Ambruzs, Josephine**

**Baran, Dana**

**Baydar, Dilek**

**†Becker, Jan (Delphi method)**

**Broecker, Verena**

**Buob, David**

**Chander, Praveen**

**Dadhania, Darshana M**

**De Almeida Araujo, Stanley**

**Farris, A. Brad**

**†Fischer, Wayne (Statistics)**

**Kan, Amanda**

**\*Liapis, Helen**

**Muthukumar, Thangamani**

**Ozluk, Yasemin**

**Rabant, Marion**

**Regele, Heinz**

**Rhandawa, Parmjeet**

**Royal, Virginie**

**\*Seshan, Surya**

**Sis, Banu**

**Stevenson-Lerner, Heather**

**Taheri, Diana**

**\* Co-chair**

St. Johns/ Newfoundland/ Canada/ May 2009



**TMA-WG meeting**

**Date: Thursday March 31th**

**Time: 1:15 PM**

**Location: Room Aula Magna**