THROMBOTIC MICROANGIOPATHY (TMA)

BANFF WORKING GROUP

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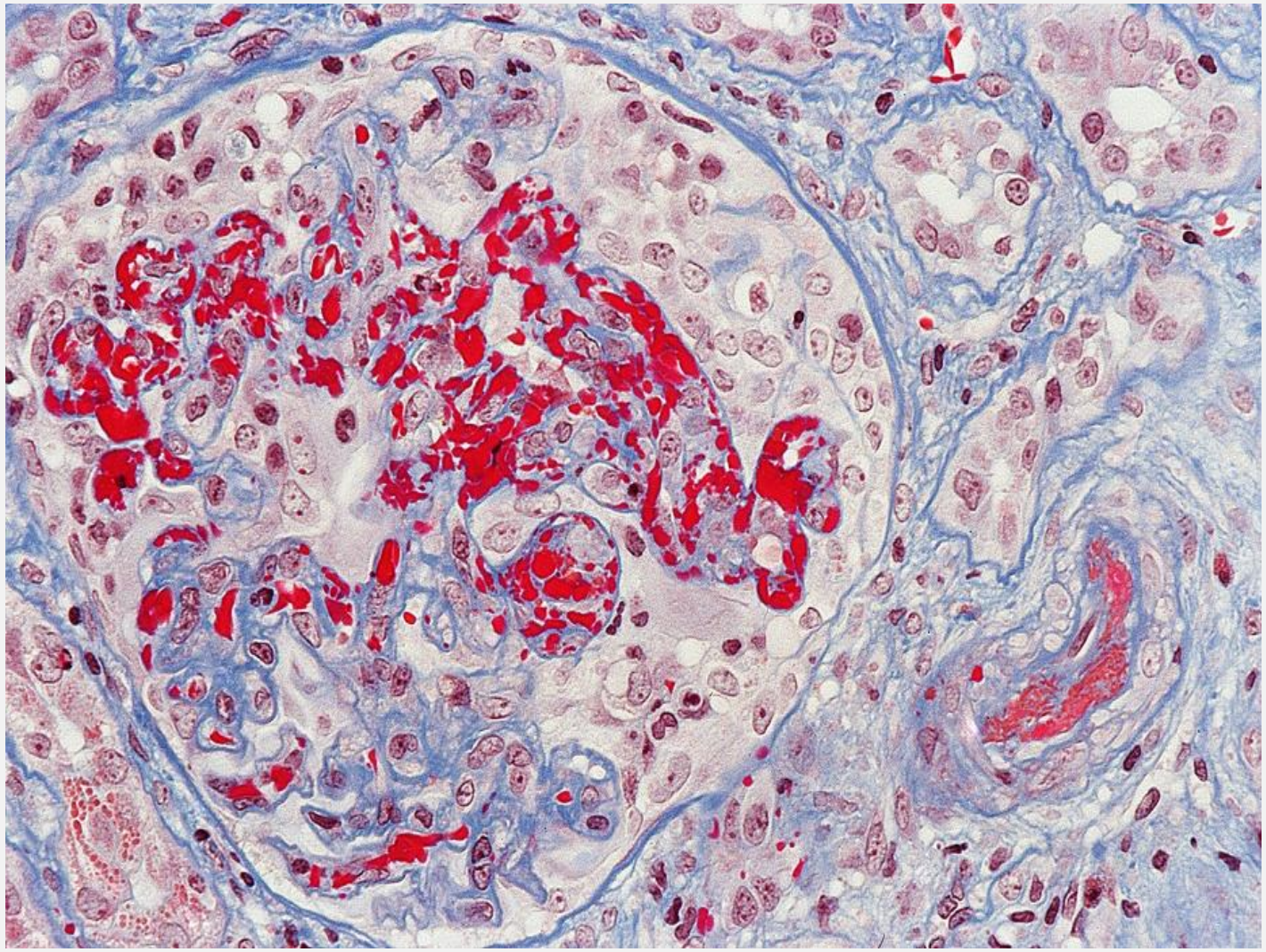
USA

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

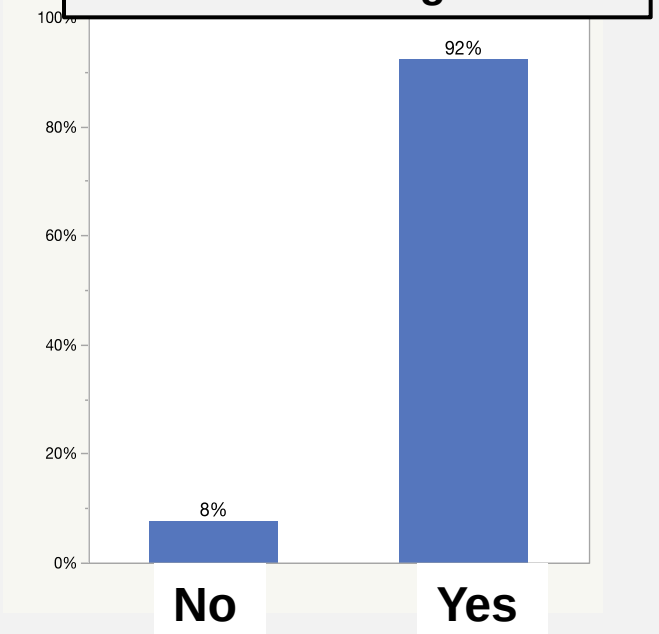
What is the estimated % of diagnosis of TMA in your services?

- **35% of participants ➔ <5%**
- **42% of participants ➔ 5-10%**
- **23% of participants ➔ 10-20%**

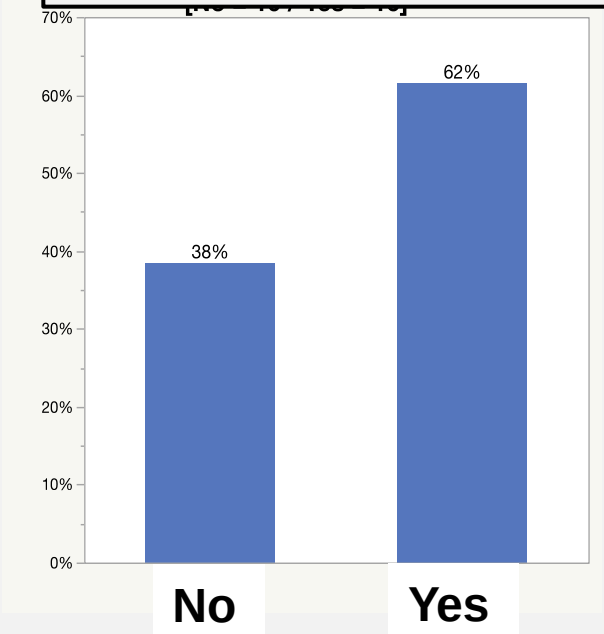
1-Do not know how to reliably separate late stages of TMA from TG.

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

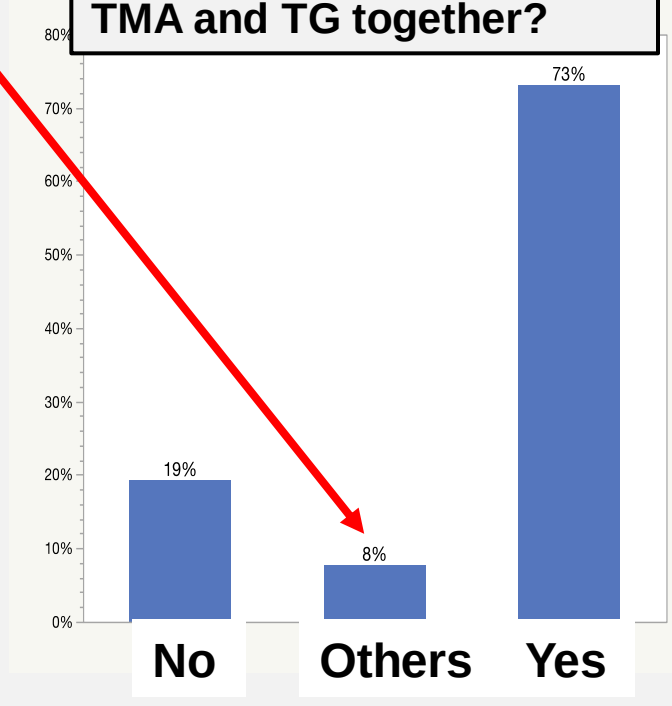
Have you seen TMA and AMR together?



Have you seen TMA and TCMR together?

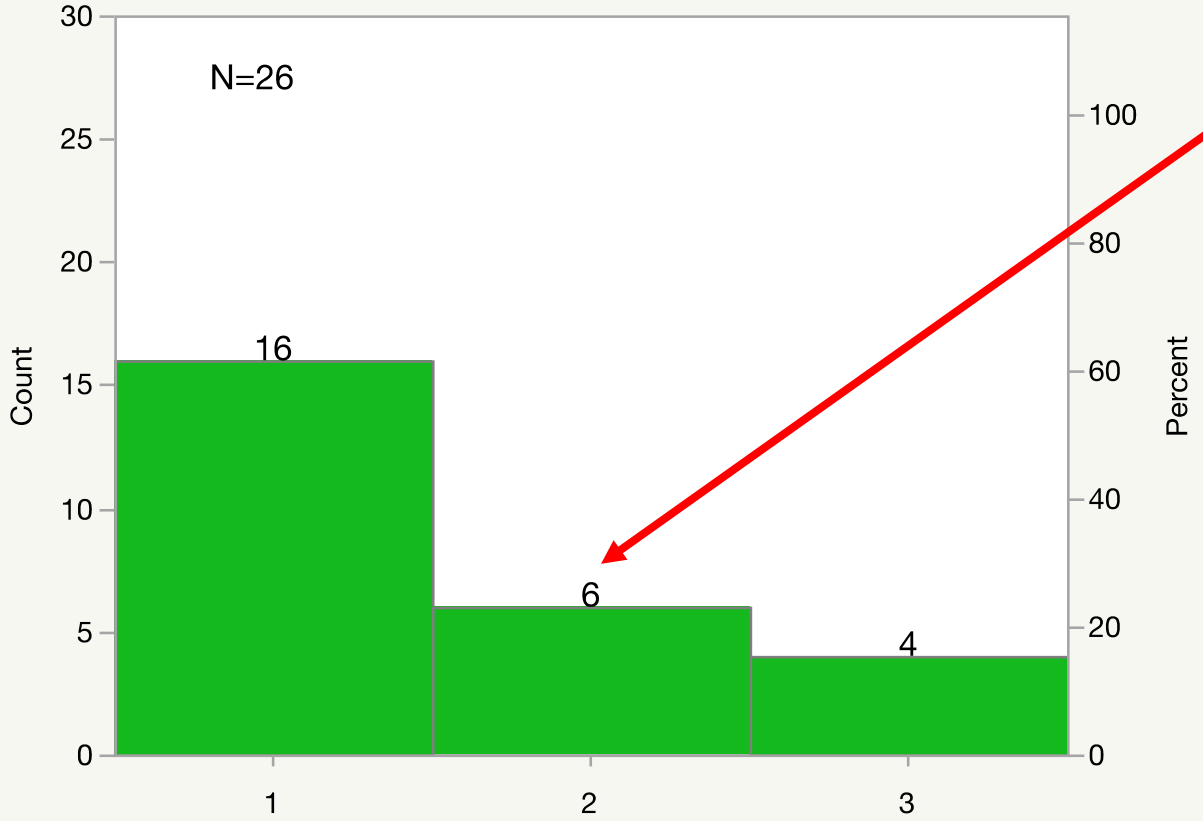


Have you seen TMA and TG together?



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)

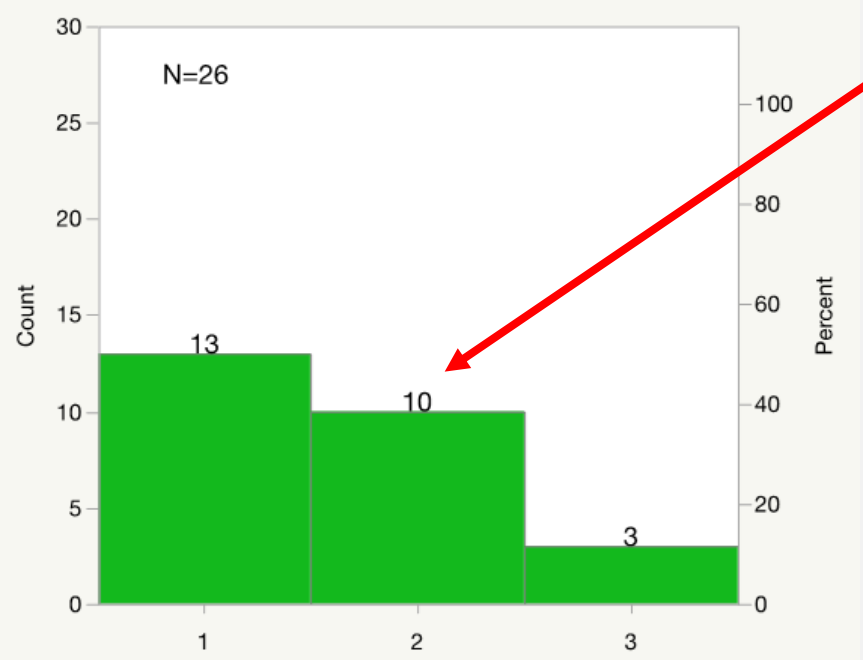
5- H&E + MT + PAMS (PAS to exclude hyaline)

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

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

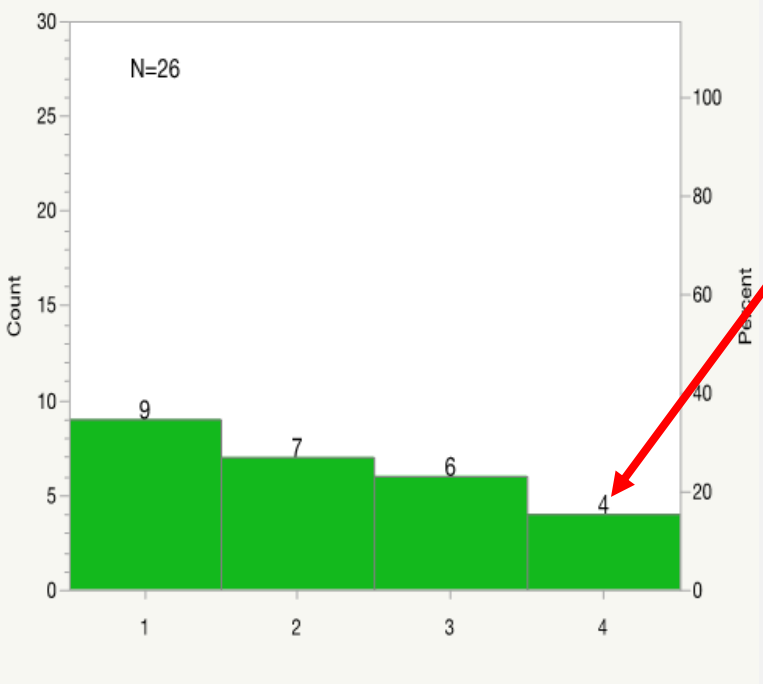
8- C4d + C3

9- C4d is useful to distinguish AMR-assoc. TMA from other causes but not for the diagnosis of TMA

10- C4d + C3 + Fibrinogen

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

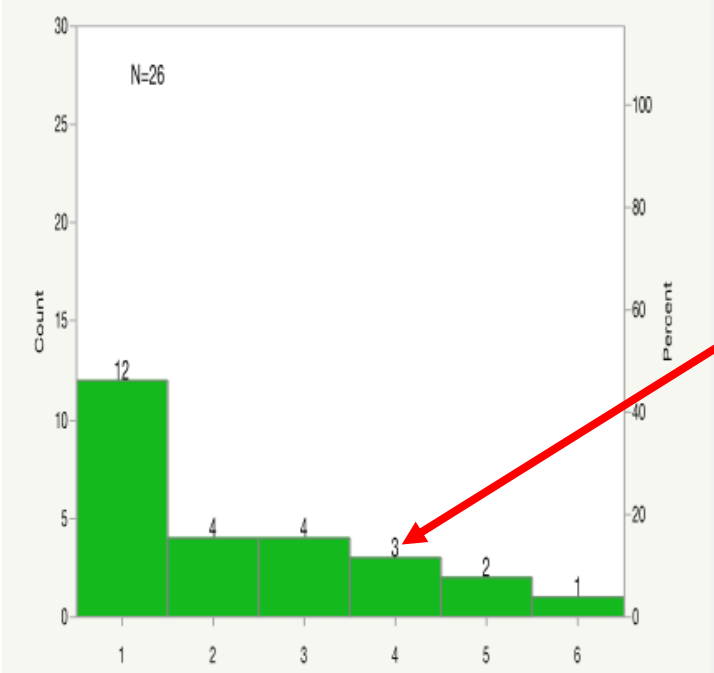
3- #2 and/or mesangiolysis

4-The spectrum of TMA is broad and not limited to thrombi and double contours: endothelial swelling, subendothelial edema, platelets thrombi (CD61 staining), mesangiolysis, "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- Mesangiolysis

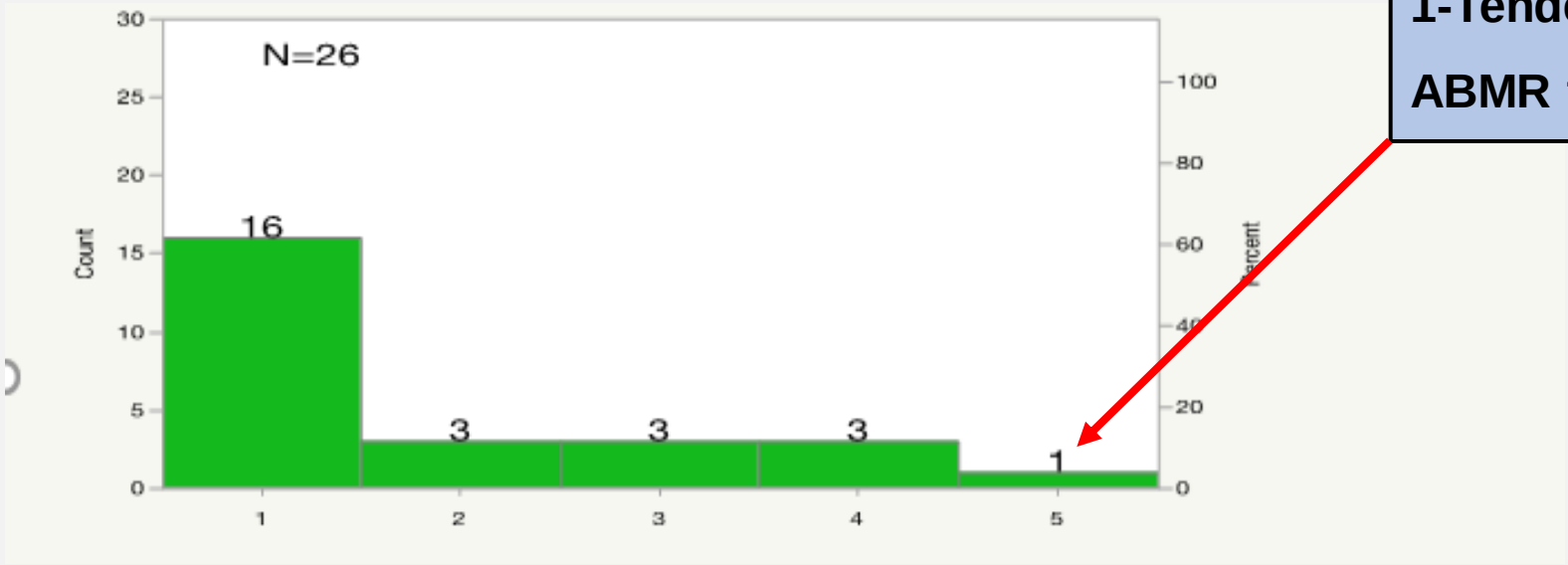
2- EM changes can be extremely subtle or even absent

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?

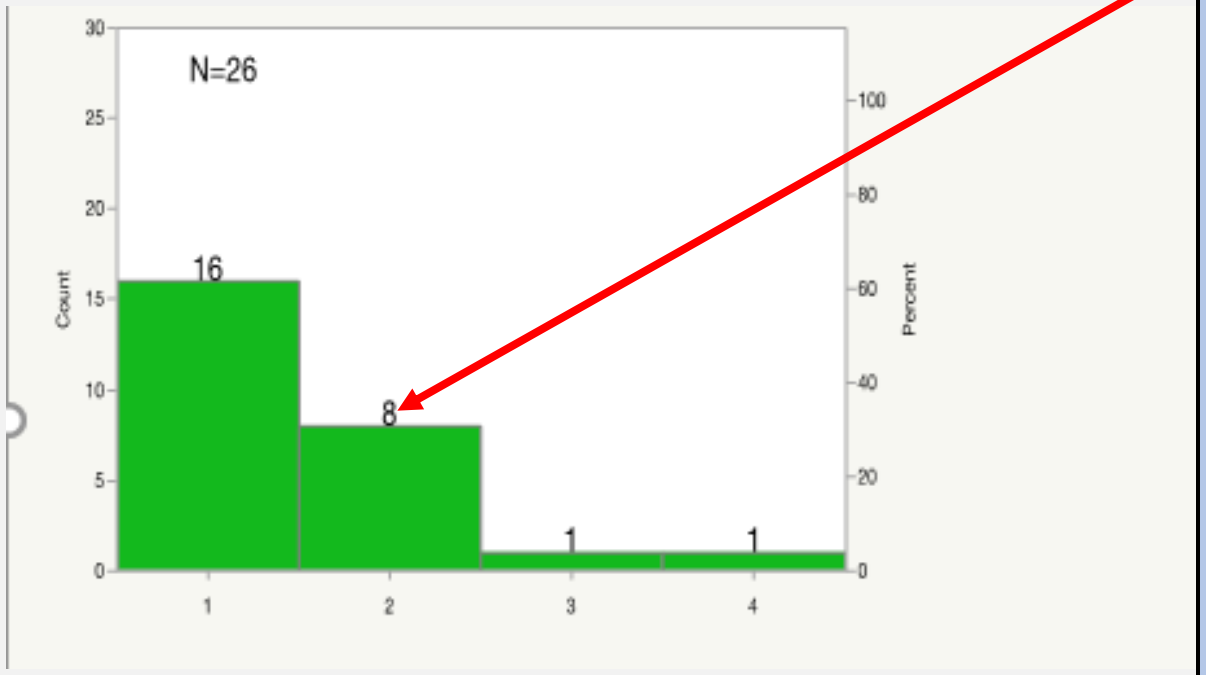


1-Tendency to blame CNIs if no TCMR or ABMR found and original disease is not HUS

1. Pre-Tx clinical diagnosis of aHUS based on serological ± genetic testing
2. Post- Tx clinical diagnosis of aHUS based on serological ± genetic testing
3. Pre- and Post clinical diagnosis of aHUS based on serological ± 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)

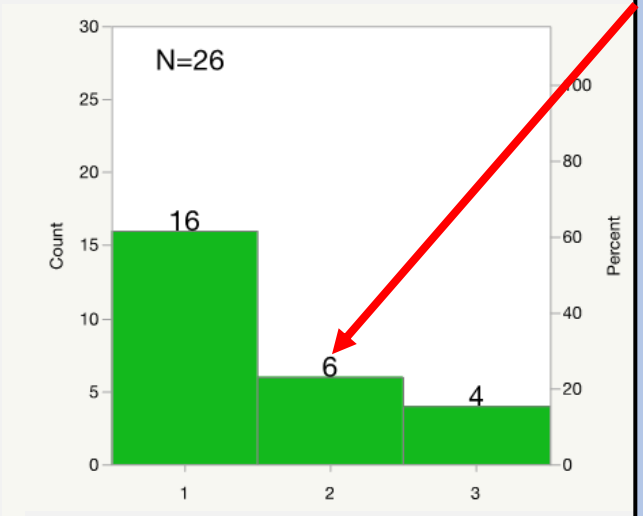
6- Histological diagnosis of TMA + Exclusion of other causes such as AMR, CNI toxicity + Genetic/serological findings consistent with aHUS

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
2. Others
3. IHC

1- IF or IHC seem OK

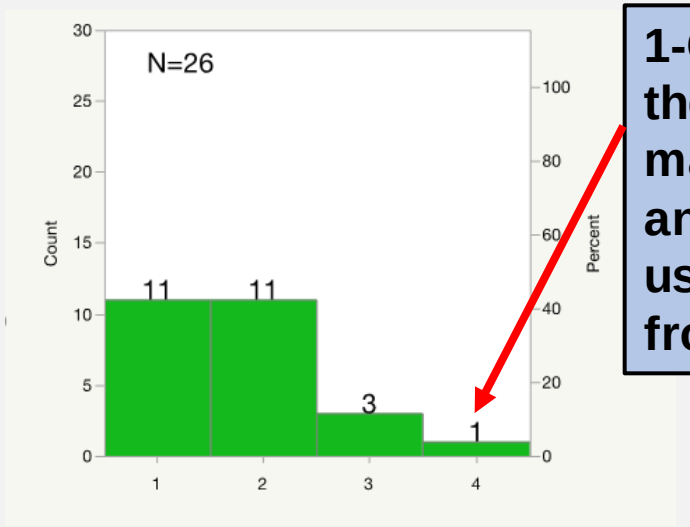
2- IHC for C4d and IF for C3 and C1q

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

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

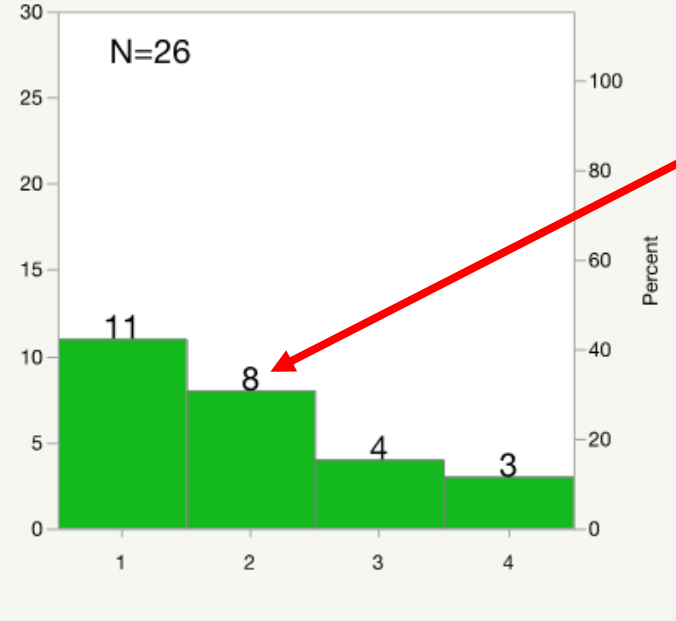


1. C4d + C3
2. C4d + C3 + MAC
3. C4d alone
4. Others

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

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

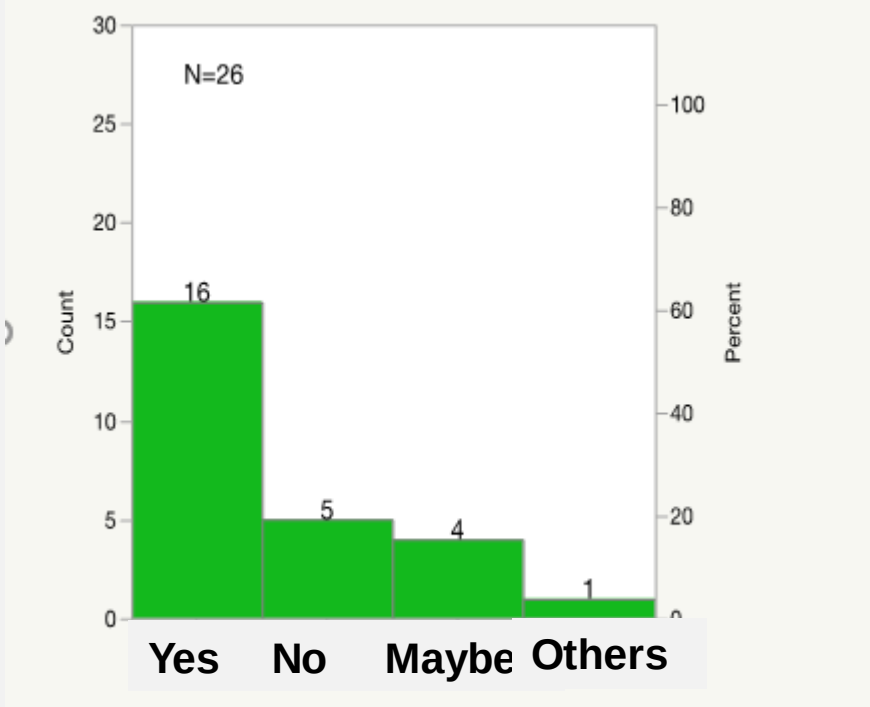
6- There will be delayed function and baseline creatinine may be higher after functioning

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 ± 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

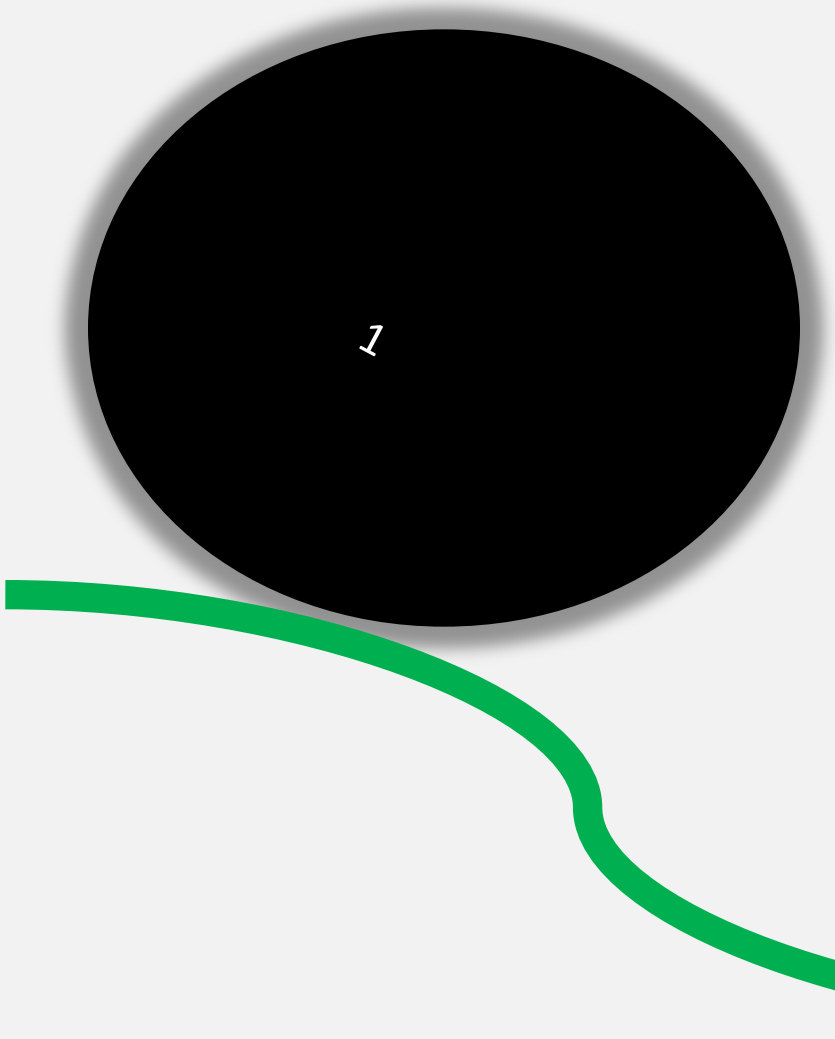
- Renal TxBx collection from both the panelists and the facilitators of the WG

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

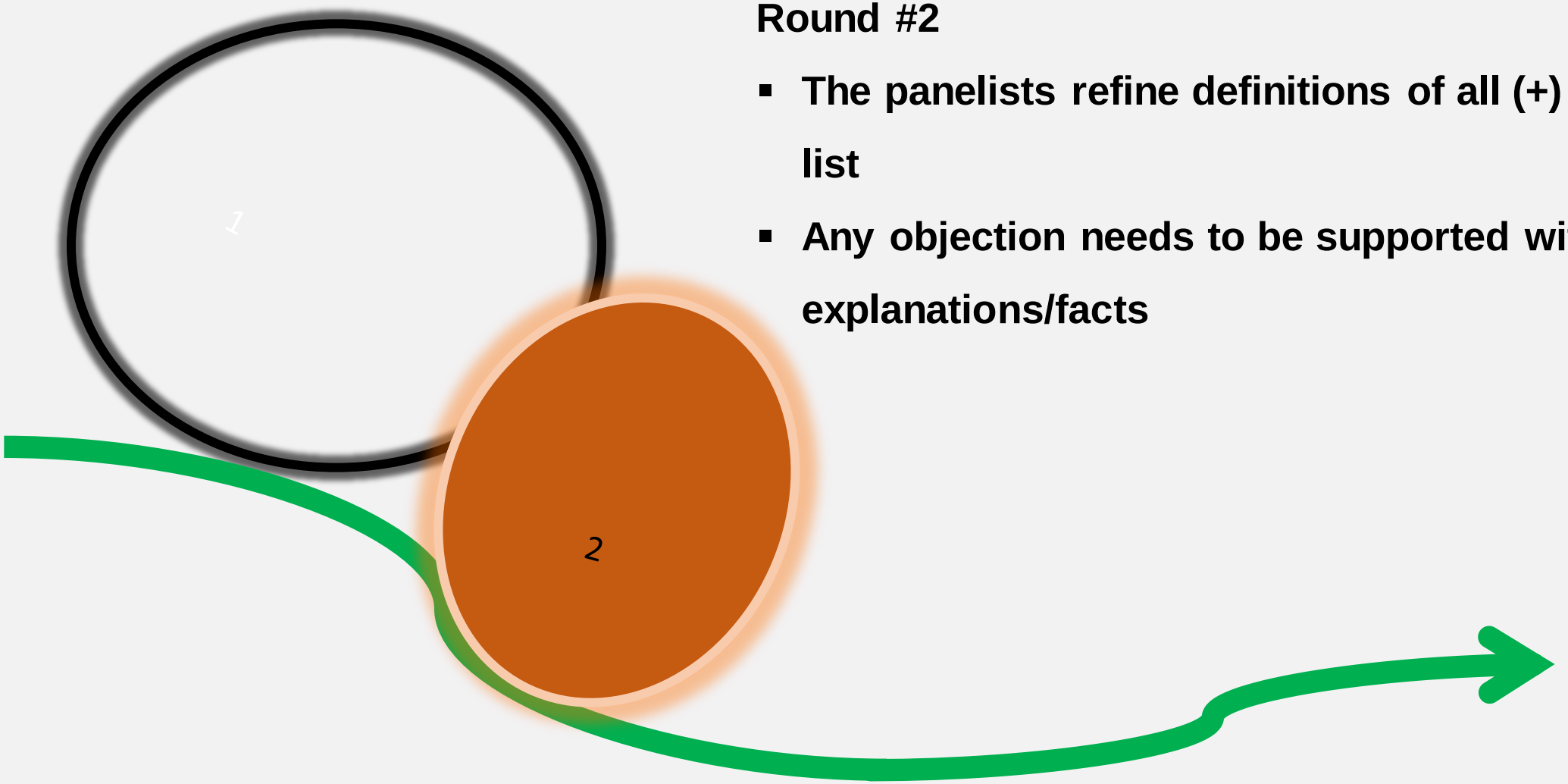
To develop a core set of diagnostic histopathological criteria, 6 rounds are designed:



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

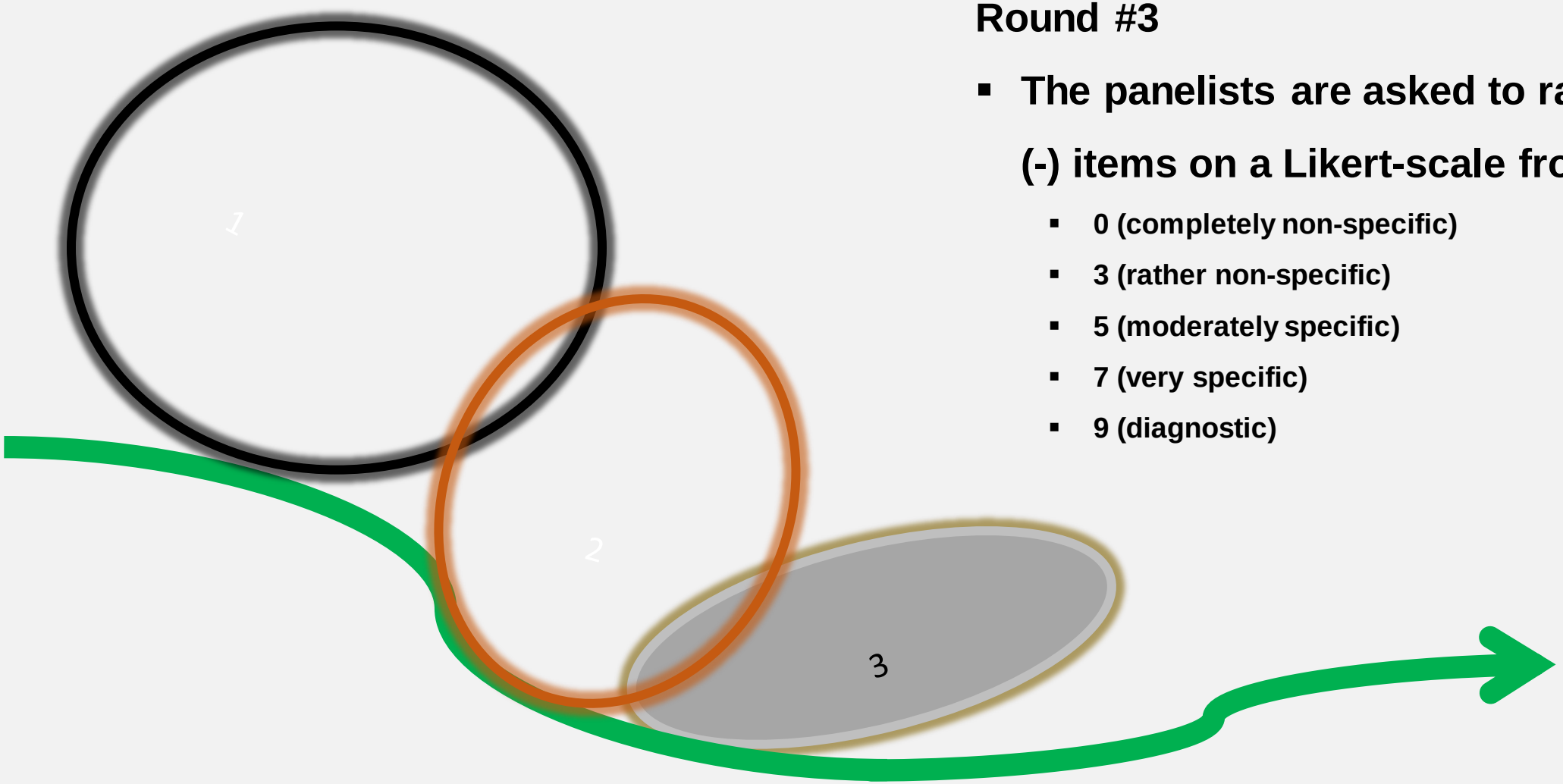
The facilitator will create a curated list of all (+) and (-) items (with %) and sent back to the panelists



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



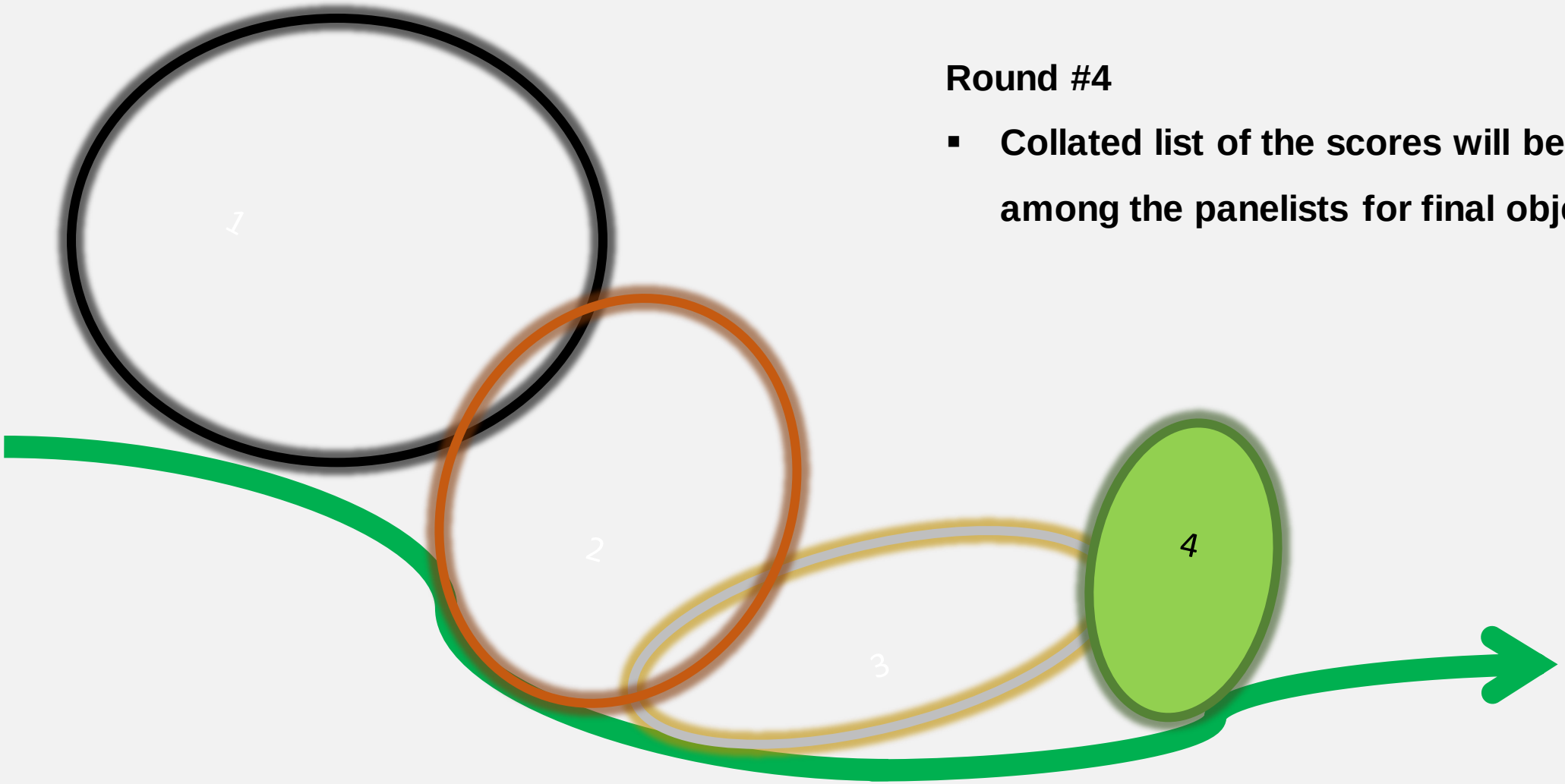
Round #3

- 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

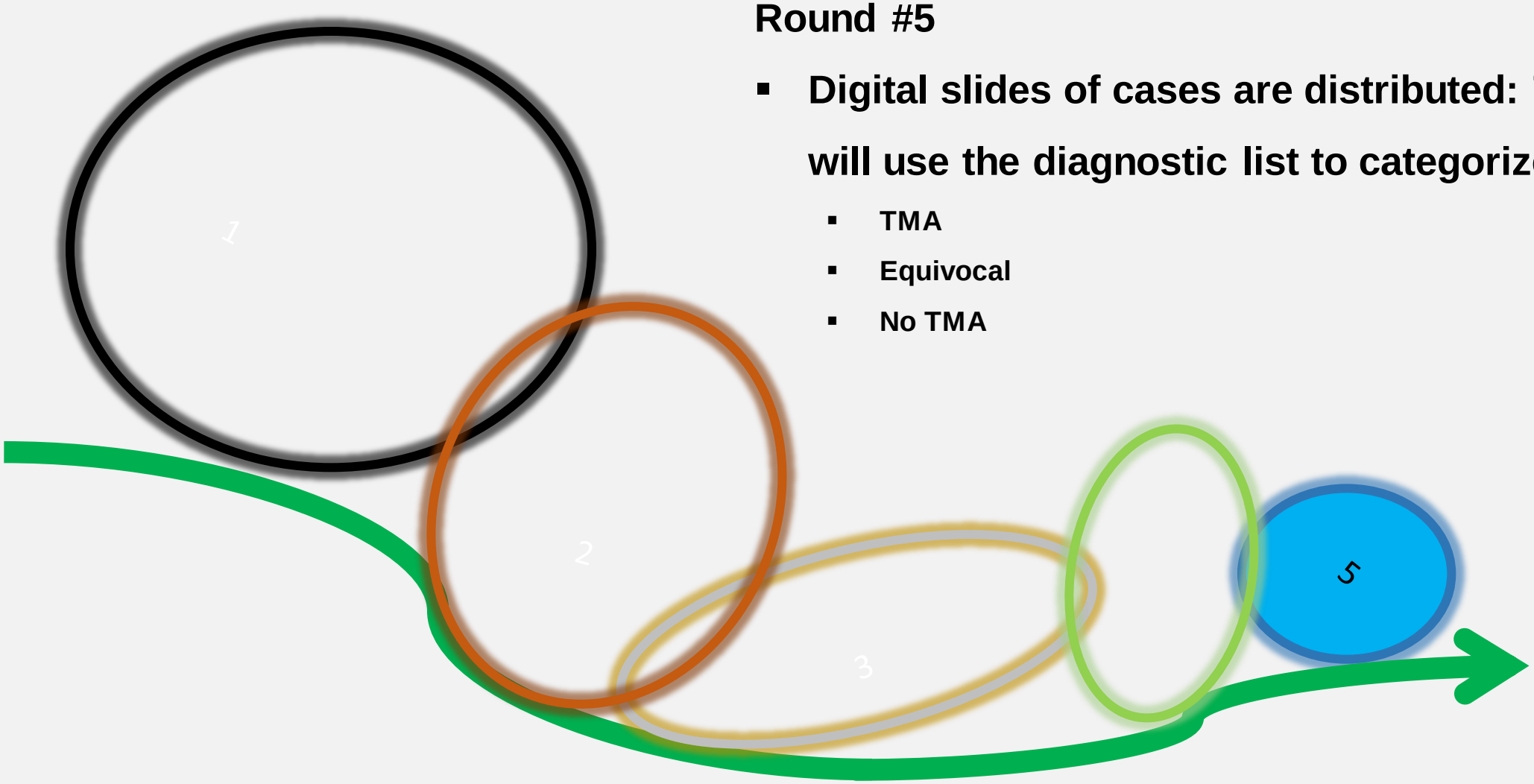
- Collated list of the scores will be circulated among the panelists for final objection



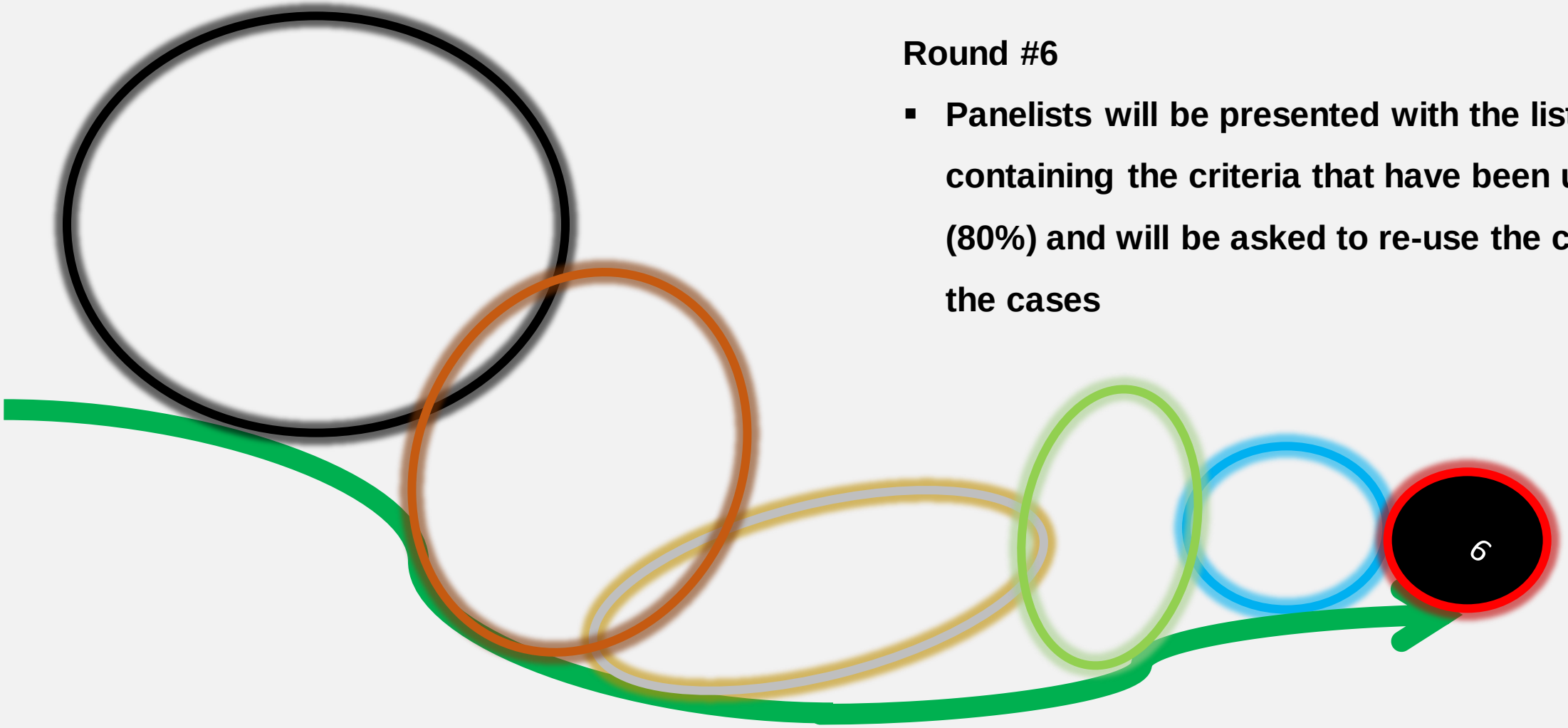
Final list will be created by the facilitator

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 ≥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 ≥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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* Co-chair

TMA-WG meeting

Date: Thursday March 31th

Time: 1:15 PM

Location: Room Aula Magna