AIHA – The Laboratory Perspective on Testing

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Joint UK NEQAS (BTLP) & BBTS BBT SIG Annual Meeting

20th November 2018
Auto Immune Haemolytic Anaemia (AIHA)

• BSH guideline (Hill et al. 2017):

“AIHA is a decompensated acquired haemolysis caused by the host’s immune system acting against its own red cell antigens”

• Incidence of approximately 1:100,000 p.a.

• Idiopathic, or secondary to associated disorders / drugs (50/50 split)

• Manifests as a variety of types:
  – Warm type (IgG or sometimes IgA)
  – Cold type (IgM / C3d)
  – Mixed type (IgG / C3d)

Auto Immune Haemolytic Anaemia (AIHA)

- Warm Auto Immune Haemolytic Anaemia (WAIHA)
  - Warm autoantibodies are responsible for majority of AIHA cases.
  - 70% of sufferers are >40yrs old with peak incidence between 60-70yrs*
  - Associated with Lymphoproliferative disorders
    - Chronic Lymphocytic Leukaemia
    - Hodgkin's disease, non-Hodgkin's lymphoma
    - Waldenstrom's macroglobulinemia

Auto Immune Haemolytic Anaemia (AIHA)

- Cold AIHA as a result of two conditions:
  - Cold Haemagglutinin Disease (CHAD)
    - 16-32% of AIHA cases
    - Clinical symptoms more serious for patients whose cold agglutinin active at temps >30°C
    - Autoanti-I specificity antibody more commonly associated with CHAD
  - Paroxysmal Cold Haemoglobinuria (PCH)
    - Biphasic haemolysin
    - Anti-P, but can be other specificities.
    - Chronic / acute - Syphilis / mycoplasma pneumoniae
Auto Immune Haemolytic Anaemia (AIHA)

- Mixed type AIHA
- Combination of IgG / C3d reactivity
- Rare <5% of AIHA
- Associated conditions:
  - Systemic Lupus Erythematosus (SLE)
  - Lymphoma
Auto Immune Haemolytic Anaemia (AIHA)

Importance of a clinical history

- Age – AIHA more common in older people, but not exclusive
- Ethnic origin – Could there be an inherited abnormality of RBC
- Diagnosis – Is the AIHA secondary to underlying disease?
- Medications – is it drug mediated? e.g. pencillin
- Brisk haemolysis - How fast is it falling?
- Symptomatic?
- Urgency?

Get the full picture!
Laboratory testing – Haematology / Biochemistry

- Tests look for haematological and biochemical indicators of haemolysis
  - Reticulocyte count - increased
  - Lactate dehydrogenase (LDH) – may be normal or increased
  - Bilirubin – increased
  - Haptoglobin – reduced
  - Blood film – spherocytes, agglutination or polychromasia
  - Urinalysis/dipstick test positive for blood but urine microscopy negative for red cells - if haemolysis is intravascular haemoglobinuria rather than haematuria
Laboratory testing - Transfusion

• Testing aims to differentiate between what type of AIHA is present, to group the patient and identify any underlying clinically significant alloantibodies, in order to provide suitable units for transfusion.

• Results seen in ABO / D grouping / phenotyping and antibody screening can include:
  – Anomalous ABO reverse group due to cold-reacting (IgM) autoantibody
  – Unable to phenotype due to autoantibody (IgG) coating of cells – false positive reactions
  – Panagglutinating antibody, various strengths, temp ranges depending on type of AIHA
  – Positive autologous control (patient’s cells Vs patient’s plasma)

• Testing can be complex and time-consuming, therefore can delay supply of suitable units for the patient.
Serological Toolkit

Temperature

Techniques

DAT

Reagents

Genotyping

Pre-warming

Warm washing

Alloadsorption

Titration

Autoadsorption
Temperature

- Antibodies have different optimal thermal ranges.
- Cold-reacting antibodies are not generally considered to be clinically significant unless they react above 30°C.
- Testing following pre-warming and warm washing of a sample may help to remove and cold-reacting autoantibody coating the patient’s RBC and allow phenotyping.
- Pre-warming plasma may also remove the reactivity of any cold reacting autoantibody so that panagglutination is not seen in IAT tests.
Techniques

• Weak reacting autoantibodies by IAT can mask underlying, clinically significant alloantibodies.
• Use of different IAT techniques can aid in the investigation and resolution of ABID and XM issues.
• Dilution of plasma.
• Risk that weak reacting, underlying alloantibodies could be missed.

Titration

• Titration of directly agglutinating, cold reactive autoantibodies can give an indication of their likely clinical significance. Titres <64 generally not clinically significant.
• However consider PCH if clinical symptoms suggestive – Donath Landsteiner test
• Need experienced serologists, variability in practice between individuals.
**Donath Landsteiner test**

1. Screening cell 1 and 2 and patient’s serum

2. Incubate 0°C tubes on ice for 1hr

3. Incubate 37°C tubes for 1hr

4. Return 0°C tubes to 37°C waterbath for 1hr

5. Add 30 µl screening cells 1 and 2 to each tube

6. Incubate 0°C tubes on ice for 1hr

7. Add 150 µl pre-warmed fresh serum to all 0°C and 37°C tubes

8. Incubate @ 37°C for 30 mins, then spin and read. Look for signs of haemolysis.

9. SC1 plus pt. serum @ 0°C

10. SC2 plus pt. serum @ 0°C

11. SC1 plus pt. serum @ 37°C

12. SC2 plus pt. serum @ 37°C

13. SC1 0°C then 37°C

14. SC1 37°C only

15. SC2 0°C then 37°C

16. SC2 37°C only
Reagents

- For patients with WAIHA it may be possible to use directly agglutinating reagents to obtain a phenotype
  - Not all antisera available as directly agglutinating reagents
  - If patient has been transfused in past 3 months phenotyping result not reliable due to presence of transfused cells
  - Genotyping

- Chloroquine Diphosphate treatment can be used to remove bound antibody and phenotype
  - Antigens destroyed by CDP treatment include
  - Availability of genotyping has led to the decline of CDP treatment in many reference labs

- 0.01M DTT treatment used to abolish activity of cold-reacting IgM antibodies to permit phenotyping, typically only for ABO and Rh.
Direct Antiglobulin Test (DAT)

- Direct Antiglobulin Test (DAT) looks for what is coating the cells in vivo.
- Must include monospecific testing for IgG and C3d as a minimum.
  - Warm type (IgG or sometimes IgA)
  - Cold type (IgM / C3d)
  - Mixed type (IgG / C3d)
- Take clinical history into account when interpreting results
  - Recent upper respiratory tract / viral infection - PCH
  - Malignant disorders – CLL, Lymphoma
  - Post transplant
  - Transfusion history
- Positive DAT does not necessarily indicate AIHA
WAIHA – DAT

- Typically IgG but may be IgA
- Cells coated with antibody, may include donor cells if previously transfused <3 months.
## AIHA – Panels

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- **Ctrl**: 0

**Conclusion**

- **Autoantibody Panagglutinin by IAT/ENZ**

**Group**

- **A Dpos**

**Pheno**

- **R⁺r⁻ K⁻**

**Reporting BMS**: ………… Checking BMS…...
Alloadsorption

- Differential adsorption using papain treated R₁R₁ and rr cells, used to remove pan-agglutinating autoantibody and detect underlying, clinically significant alloantibody/ies
Alloadsorption

- Alloadsorption performed at 37°C – optimum temp for WAIHA
- If cold autoantibodies present (CHAD) then can perform alloadsorption at 4°C to remove cold reacting agglutinins.
- If mixed type AIHA then can perform one adsorption step at 4°C and then additional steps at 37°C
- Beware antibodies to HFAs take note of the autologous control and DAT result. (Note: DAT neg AIHA exists!)
- Small risk of dilution of weak reacting alloantibodies
- Alloadsorbed plasma used to XM against ABO compatible Rh / K matched units, which are issued as SUITABLE.
- If there are any underlying alloantibodies then antigen negative units, in addition to the specification above should be selected.
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**Group**

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**Pheno**

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**Conclusion**

Autoantibody Panagglutinin by IAT/ENZ

Reporting BMS: ✔ Checking BMS: ✗
### Alloadsorption Panels

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<th>Group</th>
<th>Pheno</th>
</tr>
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<tr>
<td>Anti⁺</td>
<td></td>
<td>A⁺ B⁺</td>
<td>D⁺ O⁺</td>
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<tr>
<td>Anti⁻</td>
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<tr>
<td>DAT Batch No</td>
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</table>

### Conclusion

Auto antibody
Panagglutinin by IAT/ENZ

Reporting BMS: Checking BMS.
Allohaosorption Panels
negative

YOU'VE GOT THIS!
Autoadsorption

- It is possible to perform autoadsorption, but in WAIHA cases this is not always the best option because:

  - Autoantibody coating the RBC may have a blocking effect

- Use a mixture of 1% Papain / 0.2M DTT (ZZAP treatment) to dissociate bound autoantibody prior to autoadsorption.

  - ZZAP treatment removes Kell system, MNSs, Fy\(^a\) and Fy\(^b\) antigens

- Not possible to perform autoadsorption if the patient has been transfused in the past 3 months – risk of adsorbing alloabs

- In addition, quite often these patients have a low Hb due to their clinical condition and therefore a low HCT, which does not allow for sufficient cells to undertake autoadsorption.
Genotyping

• Genotyping can be useful in the following circumstances:
  – DAT+ cases when extended typing is required to inform blood selection or alloantibody exclusion, especially where alloadsorption has been unsuccessful
  – Previously transfused patients when extended typing is required to inform exclusion of additional specificities

NOTE: Fully genotyped matched blood is rarely required, does not significantly reduce alloimmunisation beyond full Rh / K matching / matching for antigens where there is an associated alloantibody and is not sustainable for large cohorts of the UK population.
Easy to match?

CCDee, K-, M-, S-, Jk(a-b+), Fy(a-b+)

CCDee: 20%
K-: 18%
S-: 9%
Jk(a-): 2.2%
Fy(a-): 0.7%

1 in 143!
Easy to match?

ccDEE, K-, s-, Jk(a+b-), Fy(a+b-)

ccDEE
K-
s-
Jk(b-)
Fy(b-)

1 in 7,143!
Crossmatching and issue(s)

• Issue of units as suitable – RCI issue as suitable using alloadsorbed plasma vs the hospital time taken to get to this point

• Time taken to perform testing can add delay to patient care if not communicated / expectations not managed / can be complex!
  – URGENT! Match O pos / O neg / Gp specific
  – No underlying alloabs / trf dependent / urgent and full ABO / Rh / K known – Match ABO / Full Rh / K
  – Planned / known pt / additional alloantibodies - Match ABO Full Rh / K / Additional antigens

• Issue of units as suitable – RCI issue as suitable vs the hospital time taken to get to this point

• Who would feel confident doing this?
Communication and collaboration

- Ensure best quality care and treatment – collaboration and communication between scientists and clinical staff
- Let clinical staff know where blood provision may be delayed because of a complex serological picture
- Advise on blood provision in the interim, should blood be required sooner
- Advise on blood provision in an emergency
- Ensure staff know what to do in each scenario when a panagglutinin is present
Thank you