Institute of Transfusion Medicine
Transfusion Centre
University Medical Centre of the Johannes Gutenberg University Mainz

Changes in isoagglutinin titers and their clinical significance

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Changes in Isoagglutinin Titers and Clinical Significance

Key Points:

- Isoagglutinins are naturally occurring **alloantibodies**.
- The titer **fluctuate** due to genetic and environmental factors.
- Understanding isoagglutinin titer dynamics is **essential** in:
  - Transfusion medicine
  - Hematopoietic stem cell and Solid organ transplantation
  - Obstetrics and Neonatology
- **Accurate titration** is important because the titer ca influence clinical decisions.

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Case report
Hemolytic Transfusion Reaction after Platelet Transfusion

AB0-Blutgruppe	Rh-Faktor	Rh-Formel	Kell	Cw Antigen
A2B	**Rh(D)positiv**	**CCD.ee**	**K-**	**negativ**

- **Poster Abstract at virtual ISBT 2021**
- **Patient Details:**
  67-years-old patient, blood type AB, inpatient at an external clinic
- **Transfusion Event (01.04.2021):**
  Platelet unit, blood group O, transfused - manufactured by UMC-Mainz
  Symptoms 10 min after transfusion: malaise, chills, and tachycardia
  Initial suspicion: allergic or febrile transfusion reaction
- **Reporting Timeline:**
  03.04.2021 – External clinic informs us about the suspected **allergic** reaction
  29.04.2021 – German hemovigilance authority (PEI) confirms by letter a **severe transfusion reaction** which was unknown to us
  30.04.2021 – UMC-Mainz contacts external clinic to find out that:
  Patient's clinical condition deteriorated leading to acute renal failure
  External clinic informed PEI about the severe hemolytic reaction
  External clinic forgot to inform UMC-Mainz about the changes
- **Isoagglutinin Titer:**
  Anti-A IgM **1:1024**, suggesting a hemolytic transfusion reaction

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Case report

Hemolytic transfusion reaction after platelet transfusion

- Subsequent Events:
  - Same donor donated two additional platelet units in one double donation on 28.04.2021
  - The units were transfused to two group A hemato-oncological patients on 30.04.2021
  - We informed the hematology department of the possible hemolytic transfusion reactions
  - Hemolysis was confirmed for both patients

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Isoagglutinin Titration of Platelet Donors

Implementation and Clinical Integration in Mainz

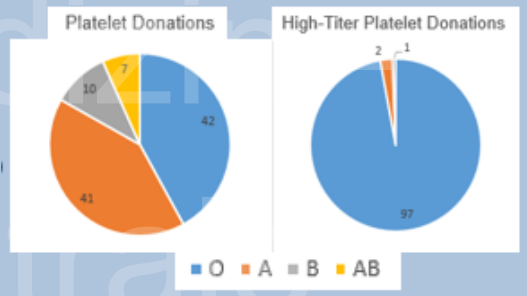
- Starting May 2021, isoagglutinin titration is part of the platelet units release process in Mainz
- All platelet donations are screened for isoagglutinins
- Titer results are transferred automatically to the laboratory information system
- These titers are taken into consideration when minor ABO incompatible platelet units are selected, to mitigate the risk of transfusion reactions.

IStatus	Art	Flag	lKonserven-Nr.	Spendedat.	Verfallsdatum	AB0	Rh	Formel	Cw	Kell	CMV	IsolgM-A	IsolgM-B	IsolgG-A	IsolgG-B
FREI	TK	x	27651302	10.11.2021	14.11.2021 23:59	0	+	CcD.ee	-	-	-	16	16	32	64
FREI	TK	x	27651302	10.11.2021	14.11.2021 23:59	0	+	CcD.ee	-	-	-	16	16	32	64
FREI	TK	x	27651302	11.11.2021	15.11.2021 23:59	0	+	CcD.Ee	-	-	-	32	16	32	>=128
FREI	TK	x	27651302	11.11.2021	15.11.2021 23:59	0	+	CcD.Ee	-	-	-	32	16	32	>=128
FREI	TK	x	27651302	10.11.2021	14.11.2021 23:59	0	-	ccddee	-	-	-	<16	<16	32	<16
FREI	TK	x	27651302	11.11.2021	15.11.2021 23:59	0	+	CcD.ee	-	-	-	16	16	16	<16
FREI	TK	x	27651302	11.11.2021	15.11.2021 23:59	0	+	CcD.ee	-	-	-	16	16	16	<16
FREI	TK	x	27651302	11.11.2021	15.11.2021 23:59	0	+	ccD.Ee	-	-	-	16	64	>=128	64

Implementation of Isoagglutinin Screening for all Platelet Donations

- Isoagglutinin Screening Tests for IgG and IgM using Neo® Immucor®/Werfen
  Automated technology ensures consistent and standardized results
- Titration steps: 1:16, 1:32, 1:64 and 1:128 - titer results: <16, 16, 32, 64 and ≥128
- Units with titers ≥128 are restricted to patients of the same ABO blood group

- 2024: 10,157 units from 5,209 platelet donation
  21% were high-titer ≥128
  majority (97%) of all high-titer donation were blood group O
  half of blood group O donations were high-titer



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Changes in Isoagglutinin Titers

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Changes in Isoagglutinin Titers

Isoagglutinins

Titer Dynamics

Clinical Significance

Isoagglutinins Origin

- They were first discovered in 1900 by the Austrian pathologist Karl Landsteiner.
- The isoagglutinins are naturally occurring alloantibodies produced by individuals lacking the corresponding antigen on the surface of their erythrocytes.
- Anti-A and anti-B are the two common isoagglutinins.
- Produced in the first six months of life, prior to any exposure to human antigens, they reach adult concentrations between 5 and 10 years of age.
- Their development is linked to environmental exposure to structures mimicking A and B antigens, particularly from intestinal flora.

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Isoagglutinins Characteristics

- The antibody response to non-protein antigens, such as ABO blood group antigens, is primarily mediated by IgM, produced by B1 B lymphocytes, independently of T-cell help.
- Early studies found only IgM isoagglutinins in non-immunized individuals (no prior transfusion or pregnancy).
- Later reports identified both IgM and IgG isoagglutinins, and after immunization with human antigens, IgA was also detected.

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Isoagglutinin Titration

- The titration involves serial dilutions followed by agglutination testing. The titer is reported as the highest dilution with a macroscopic agglutination.
- **IgM** titration, **when** performed with precision, correlates well across both manual and automated methods.
- **IgG** titration is challenging due to interference from IgM antibodies.
  Techniques like DTT treatment or heat inactivation are employed to selectively neutralize IgM.
- The **solid-phase red cell adherence assay** is effective in quantifying IgG antibodies without IgM interference and correlates with results after DTT-treatment.

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Reproducibility of Titration Methods

- **Standardization of antibody titration** remains a significant challenge in transfusion medicine.
- **Manual titration methods** – are labor-intensive, time-consuming, and subject to inter-operator, but also technique and inter-laboratory variability, have low reproducibility.
- Different factors - test cell phenotype, diluent, pipetting technique, incubation conditions, and centrifugation can contribute to inconsistent results.
- A study involving 35 international laboratories using a standardized titration protocol found that variability was mainly due to **subjective grading of agglutination strength**.
  Clearer grading definitions helped reduce variability. (AuBuchon, 2008, Arch Pathol Lab Med).
- However, a follow–up study showed no significant improvement in inter-laboratory consistency despite the protocol's implementation (Bachegowda, 2017, Arch Pathol Lab Med).
- **Automated titration methods** – automated column agglutination (IgM/IgG), solid-phase red cell adherence (IgG), and microplate agglutination (IgM)
  - offer higher **reproducibility** and **efficiency**
  - but remain **cost-prohibitive** for smaller laboratories

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Changes in Isoagglutinin Titers

Isoagglutinins

Titer Dynamics

Clinical Significance

Titer Dynamics

- Isoagglutinin titers can vary significantly between individuals.
- These variations are influenced by both genetic and environmental factors.
- On the **genetic** side, factors such as ABO blood group and sex have measurable impact.
- However, **environmental** or **environmentally modulated factors** play a major role as well. They include:
  - Age
  - Gender-related lifestyle factors
  - Nutritional status
  - Exposure to microorganisms
  - Vaccination
- A study examining isoagglutinin titers among relatives from 74 families provided valuable insights:
  - Only 20-30% of the variability could be attributed to **genetic factors**

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Titer Dynamics
Age

- **Aging** is associated with a progressive immune system dysregulation: a decline in cell-mediated immunity with preserved aspects of humoral immunity. For example:
  - B-lymphocyte function is largely retained
  - Levels of IgA and IgG may increase with age
  - However, antibody affinity tends to decline
- The reduced immune response observed in older adults is influenced by several modifiable factors, including:
  - Poor nutrition
  - The presence of subclinical diseases
- Early studies suggested a gradual age-related decline in isoagglutinin titers.
- However, later research did not confirm these findings.
  - Lower titers have been observed mainly in the very elderly, over 90 years old individuals.
  - Still, some individuals in this age group have shown relatively high isoagglutinin titers.

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Titer Dynamics
Sex and Gender

- Both **sex-specific biological factors** and **gender-related social factors** significantly influence immune responses.
- **Sex hormones** play key roles in immunoglobulin production:
  - Estrogen enhances the production of IgM and IgG immunoglobulins
  - In contrast, testosterone tends to inhibit their production
- Furthermore, **gender influences lifestyle factors** that affect immune function, including:
  - Antigen exposure through environmental and occupational activities
  - Health-seeking behaviors
  - Patterns of domestic or physical activity
- Usually, isoagglutinin titers are higher in females compared to males.

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Titer Dynamics
Nutrition

- **Nutritional status** plays a key role in regulating immune function.
- **Diet** is important not only in supplying nutrients, but also in shaping the **gut microbiota**, which then affects **isoagglutinin production**.
- Diets high in processed foods, common in developed countries, are associated with altered gut microbiota and reduced isoagglutinin levels.
- A striking example comes from **longitudinal** data in Japan, where:
  - Isoagglutinin levels have **declined** over a period of 15 years
  - The decline was linked to dietary westernization, compared to populations in Thailand and Laos, where more traditional diets are still common.
- A comparative study involving healthy individuals from:
  - Amazonian Amerindians
  - Rural Malawians
  - Urban Americans
- found that:
  - Gut microbiota varied significantly between rural and urban populations
  - Cohabitating individuals shared microbial similarities, regardless of genetics or culture
  - Microbial variation was also influenced by age and geography

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Titer Dynamics
Probiotics

- **Probiotics** are dietary supplements containing live microorganisms that may express **ABO-like antigens** and can stimulate the production of isoagglutinins.
- A notable case report (Daniel-Johnson, 2009, Transfusion) investigating a hemolytic transfusion reaction found elevated isoagglutinin titers in a platelet donor, that used a high-potency probiotic supplement.
  - The high-potency probiotic contained **11 microbial strains** (lactobacilli - 7 strains, bifidobacteria - 3 strains, and *Bacillus subtilis*)
  - The platelet donor had elevated **anti-B IgM and IgG titers of 16,384**
  - During a period of three years the donor took low-potency probiotics and had consistently elevated titers, ranging from 4,096 to 8,192
  - The donor was blood group A; elevated titers are commonly found in group O donors

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Titer Dynamics
Microorganisms and Vaccines

- Several **microorganisms** (gram-negative bacteria, viruses, parasites) express ABO-like antigens. **Infection** with these microorganisms are often associated with increased isoagglutinin titers.
- Historically, some **biologically derived vaccines** (for influenza, pneumococcal disease, tetanus) contained ABO-like antigens that originated from **bacterial or viral extracts, manufacturing residues** and stimulated **IgG** isoagglutinin production.
  - When administered to blood donors, during whole blood transfusion era, they could cause hemolytic transfusion reactions.
  - When administered to pregnant women could lead to hemolytic disease of the newborn in ABOi pregnancies.
- **Modern biosynthetic** or **highly purified vaccines** have no significant effect on isoagglutinin titers.
- A 2016 study that evaluated the immune response after pneumococcal vaccination found a subtle increase in IgG only with a highly sensitive method (surface plasmon resonance). This change was **not detectable with standard agglutination**, which indicate an insignificant effect with no clinical relevance.

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Titer Dynamics
Temporal Factors

- It was found that some immune parameters show **seasonal and circadian variations.**
- In a 2021 study (Wyse, iScience) was shown that:
  - lymphocytes and neutrophils show seasonal and circadian variations
  - while monocytes remain stable
- Interestingly, these fluctuations showed no correlation with lifestyle, population demographics, or environmental influences.
- Further insight comes from a **retrospective longitudinal study** conducted over 18 months, involving active military blood donors.
- The study revealed seasonal changes in IgM isoagglutinin titers (Bailey, 2019, Transfusion):
  - Anti-A IgM was highest in spring and lowest in autumn.
  - Anti-B IgM was lowest in spring and autumn, with peaks in summer, when compared to winter months.

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Changes in Isoagglutinin Titers

Isoagglutinins

Titer Dynamics

Clinical Significance

Clinical Significance

- In cases of **ABO incompatibility**, isoagglutinins bind to corresponding antigens, forming immune complexes that can induce **hemolysis**. The severity of hemolysis depends on both the quantity and affinity of the isoagglutinins involved.
- The isoagglutinins, along with changes in titer over time, play a critical role in:
  - **Transfusion medicine**
    - Platelet transfusion, as ABO minor incompatible are sometimes necessary
    - Whole blood transfusion: as Low-Titer Group O Whole Blood (LTOWB), in military and civilian traumatology
  - ABOi kidney or stem cell **transplantation**
  - ABOi **pregnancies**

	AB0 Blood Group Compatibility	
Patient	Red Cells / Major Compatibility	Plasma / Minor Compatibility
0	0	0, A, B and AB
A	A and 0	A and AB
B	B and 0	B and AB
AB	AB, A, B and 0	AB

Clinical Significance
Platelet Transfusion

- Platelet units can be transfused out-of-group when ABO-identical products are unavailable. This is common practice and often necessary due to inventory constrains.
- Because platelets must be stored at room temperature, they have a **short shelf life**. This limits the availability of ABO-identical units and often necessitates out-of-group transfusions.
- Minor ABO-incompatible platelet transfusions account for **10-40%** of all transfusions.
- Generally well tolerated, the risk of hemolytic transfusion reactions is estimated at **1 in 9,000** cases. The reactions are often **underdiagnosed**, particularly in critically ill patients where symptoms can be masked or attributed to other causes.
- To mitigate risk, some institutions **titrate** platelet donations and have a **titer threshold** for ABO incompatible transfusion.

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Clinical Significance
Whole Blood Transfusion

- Whole blood transfusion has a long-standing history, especially in military settings.
- Compared to component therapy, WB simplifies logistics and provides balanced resuscitation in a single unit, and also reduces donor exposure.
- WB can be **ABO-identical** or Low-Titer Group O Whole Blood (**LTOWB**)
- Group O WB can be used in emergency transfusions but requires **low isoagglutinin titers** to ensure safe transfusion of non-O patients.
- A **standardized** isoagglutinin titration is important for reliable identification of low-titer donors.
- WB is also used in civilian trauma care in some countries.
- In Germany, the use is limited to autologous transfusion, which is gradually declining.

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Clinical Significance
Hemolytic disease of the Newborn (HDN)

- HDN is caused by **maternal IgG** antibodies that cross the placenta and target fetal antigens.
- **15-25%** of pregnancies are ABO incompatibility
- Less than **1%** develop **clinically significant HDN**
- **ABO-HDN** mainly found in **non-group O newborns** born to **group O mothers** that have elevated **antenatal IgG** isoagglutinins.

  Anti-A are frequently implicated than anti-B isoagglutinins..
- Isoagglutinin titers may
  increase during ABO-incompatible pregnancies,
  influencing the risk of HDN.

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Clinical Significance

Allogeneic Hematopoietic Stem Cell Transplantation (HSCT)

- HSCT involves the **transfer of multipotent stem cells** from a healthy donor to a patient with a high-risk hematological malignancy.
- While donor selection is based primarily on HLA compatibility, ABO compatibility is not required.
  - **50%** are ABO-incompatible
- In **ABO minor-incompatible** HSCT, hemolysis is due to:
  - passive transfer of isoagglutinins from the donor
  - or de novo production of isoagglutinins by the transferred B-lymphocytes – (Passenger Lymphocyte Syndrome)
- In **ABO major-incompatible** HSCT:
  - host isoagglutinins can survive the conditioning regimen
  - inhibit red cell engraftment (Pure Red Cell Aplasia)
- Isoagglutinin titers between 1 and 8 are usually associated with successful engraftment.

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Clinical Significance
Solid Organ Transplantation (SOT)

- SOT is the treatment of choice for patients with terminal organ failure.
- **ABO compatibility** is crucial in SOT, unlike stem cell transplantation.
- Due to the growing gap between **demand** and **organ availability**, desensitization protocols have been developed to enable transplantations from ABO-incompatible living donors.
- ABO-incompatible SOT complications are caused by:
  - **recipient isoagglutinins** targeting **donor blood group antigens** found on **graft endothelium**
    - Followed by complement activation, thrombosis, and tissue infarction
- Managing and monitoring **isoagglutinin titers** before and after transplantation is critical to:
  - prevent antibody-mediated rejection
  - maintain graft function
- **Desensitization techniques** include plasma exchange and immunoadsorption, aiming to **reduce isoagglutinin titers.**
- However, **threshold titers** and **testing methods** vary across institutions.
- **Accurate** isoagglutinin titration is essential in this setting because isoagglutinin titer can **impact** clinical decisions.
- Kidney transplantations have the highest success rates in ABOi settings.

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Clinical Impact of Isoagglutinin Titer Changes

Take-Home Messages:

- Isoagglutinins titers are **influenced** by multiple factors: genetic and environmental.
- Elevated titers can lead to **hemolytic transfusion reactions**, **transplant rejection**, and **hemolytic disease of the newborn**.
- **Standardization** of isoagglutinin titration remains challenging due to variability in manual methods; automated techniques offer promise but are costly.
- **Continuous monitoring** and **careful management** of isoagglutinin levels are vital in transfusion and transplantation settings to optimize patient safety.

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Thank you for your attention