

The significance of preanalytical phase in immunohematology testing

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SUMMARY

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The preanalytical phase plays a very important role in immunohematology testing. Failure to identify and eliminate inaccuracies at that stage may lead to errors resulting in the adverse events and reactions. One common error is neglecting to verify the patient's identity or misidentification of the patient's identity. Such practice will potentially generate errors in immunohematology testing process and, what follows, also in writing orders for blood and its components; this, in turn, may lead to their administration to the wrong patient. Another consequence may be a need to postpone the transfusion due to incorrectly filled documents, containing inaccurate patient's data. Another problem is missing or incomplete information regarding serological and medical treatment history, which can complicate and significantly prolong the testing process conducted in a transfusion immunology laboratory or even result in erroneous requests for blood and blood components. A request containing inaccurate or incomplete information may consequently contribute to the transfusion being postponed. It is of note that a requisition form for blood and blood components may only be filled basing on a confirmed blood group report, i.e. the result yielded by two blood typing tests, the record in a military service book or in a blood group card. It is considered unacceptable to write blood requests relying on an incorrect, illegible or incomplete blood group record or the information from maternity record, a key ring, bracelet or a tattoo stating the blood type. Owing to a range of clinical situations, the security of selecting blood and blood components for transfusion requires, apart from strict adherence to the procedures, an individual approach to particular blood recipients and close cooperation with laboratory diagnosticians [2,4,11].

Key words: preanalytical phase, immunohematology, transfusion medicine, adverse events, adverse reactions

STRESZCZENIE

Znaczenie fazy przedanalizycznej w badaniach immunohematologicznych

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Faza przedanalizyczna pełni bardzo ważną rolę, jeśli chodzi o badania immunohematologiczne. Nieprawidłowości nie wychwycone na tym etapie mogą prowadzić do szeregu błędów a te z kolei do wystąpienia niepożądanych zdarzeń i reakcji. Jednym z najczęściej pojawiających się problemów prowadzących do pojawienia się niepożądanych zdarzeń jest zaniechanie identyfikacji pacjenta lub przeprowadzenie jej w nieprawidłowy sposób. Postępowanie to może spowodować błędy w oznaczeniach immunohematologicznych oraz nieprawidłowe wypisanie zamówień na krew i jej składniki, a nawet może prowadzić do podania krwi i jej składników niewłaściwemu pacjentowi lub odwołanie przetoczenia ze względu na nieprawidłowo uzupełnioną dokumentację niezgodną z danymi pacjenta. Brak informacji o przeszłości serologicznej pacjenta i stosowanym leczeniu może utrudnić i znacznie wydłużyć czas oznaczeń prowadzonych w pracowni immunologii transfuzjologicznej oraz czasem prowadzić do wypisania niewłaściwych zamówień na krew i jej składniki. Błędnie lub niekompletnie wypełnione zamówienie może skutkować opóźnieniem transfuzji. Zamówienie na krew i jej składniki może zostać wypełnione tylko na podstawie potwierdzonego wyniku grupy krwi czyli wyniku opartego na dwóch oznaczeniach grupy krwi, który może być w formie papierowej, lub karty grupy krwi albo wpisu zawartego w książeczce żołnierza zawodowego. Niedopuszczalne jest wpisywanie zamówień w oparciu o nieprawidłowy, nieczytelny, niekompletny wynik grupy krwi lub wynik zawarty w informacji z karty ciąży, breloczku, bransoletce lub tatuażu z grupą krwi. Ze względu na różne sytuacje kliniczne, bezpieczny dobór krwi i jej składników do przetoczenia, oprócz ścisłego stosowania procedur, wymaga też indywidualnego podejścia do poszczególnych biorców krwi i bardzo ścisłej współpracy lekarzy z diagnostami laboratoryjnymi [2,4,11].

Słowa kluczowe: faza przedanalizyczna, immunohematologia, transfuzje, zdarzenia niepożądane, reakcje niepożądane

Immunohematology aims to investigate the response of the immune system to cell antigens, analyse post-transfusion reactions, as well as to study disorders caused by antigen-induced immunisation. This field of study finds its practical application in transfusion medicine, transplantology (tissue and organ transplantation) and gynaecology and obstetrics in the study of serological conflicts and the resulting diseases of foetuses and newborns. However, the most common immunohematology tests include those conducted prior to blood transfusions. Blood and its components inter alia are used in emergency medicine and surgery in the case of acute blood loss and in the treatment of posthemorrhagic shock; in hematology they are used to treat anaemia, leukopenia, thrombocytopenia and clotting disorders and in neonatology for exchange or top-up transfusions in newborns.

It must be noted here that using blood and blood components is associated with the risk of adverse reactions and post-transfusion reactions. Immunohematology diagnostics addresses this issue by offering tests targeted at the selection of compatible, i.e. cross-matched blood or its components for the recipient. In order to achieve this aim, it is not only the testing process that must be adequately performed; other processes which include taking record of the reactions and interpretation of the obtained test results are likewise of great importance [2,7].

THE RANGE OF TESTS CONDUCTED IN TRANSFUSION IMMUNOLOGY LABORATORY

The Regulation of the Minister of Health of 8 July 2019 on the treatment with blood and its components in health care facilities offering on-site and 24/7 medical service specifies the minimal range of tests to be offered by an immunohematology laboratory operating at a health care entity. These include:

- Blood typing;
- Detection of antibodies targeted at red blood cells of the patient's serum as well as immune antibodies adsorbed on the red blood cells;
- Performing a cross-match test;
- Performing tests qualifying Rh D (-) women for anti-Rh D immunoglobulin administration during pregnancy or after delivery;
- Newborn's blood typing in the ABO and Rh systems.

These basic tests require great care and staff commitment in the assessment of the obtained reactions to ensure the right interpretation of the results, but also to detect any possible inaccuracies, which is a prerequisite to conduct further tests allowing secure transfusion.

Immunohematology laboratory Regional Blood Transfusion Centres (RBTCs) are, in turn, subject to substantive supervision by the Institute of Hematology and Transfusion Medicine. All the specimens posing analytical problems or requiring extended test panel which exceed the laboratory equipment and/or reagent capabilities are sent to supervisory units [1,5].

PREANALYTICAL PHASE: THE SIGNIFICANCE OF MEDICAL RECORDS AND SEROLOGICAL HISTORY

As has been mentioned above, immunohematology testing is a crucial stage in the process of selecting appropriate, compatible blood for transfusion and hence the importance of the preanalytical phase and the process of blood component selection. Prior to the delivery of a blood sample obtained solely for the purpose of immunohematology testing to the laboratory, the order forms for the tests and the request forms for blood and its components are completed, if ever done. Together with the required documents, all of them correctly completed, a patient's serological history should be provided. The information, which is of particular importance to the laboratory performing pre-transfusion tests, includes data on earlier blood transfusions, when and where they were performed, diagnoses, the results of previously conducted immunohematology tests, if ever done, and pregnancies which might be the cause of immunisation. It often allows to shorten test time, eliminate doubts connected with selection, and rapid blood transfusion. The examples illustrating the significance of serological history are presented below. When the patient has received a large quantity of no type-specific red blood cells concentrates within the period of 3 months before the test, it may lead to two populations of blood cells co-occurring in patient's blood and thus interfere with the selection of compatible blood. The phenomenon is manifested as follows: in the presence of diagnostic reagents some blood cells undergo agglutination, while others form a homogeneous suspension. The information on when and what type of blood was transfused eliminates doubts concerning the selection of the component, and facilitates and shortens the testing process. It is also important to know what antibodies the patient produced in the past, even if they are no longer detectable or are no longer found in the patient's plasma, as it the case with antibodies against Kidd antigens such as anti-Jh^a and anti-Jk^b. Such antibodies may lead to haemolytic transfusion reaction and tend to disappear in recipients' plasma quickly, so they may not be detected in the compatibility test. It is very important that information on the

patient's diagnosis is included by the doctor in the order for immunohematology tests. It allows the diagnostician to predict why the reactions are different from those expected. The examples of conditions which may complicate the interpretation of test results are multiple myeloma or Waldenström macroglobulinemia, as these are diseases in which, due to the presence of macromolecular proteins in the blood, pseudoagglutination of erythrocytes is observed; additionally, a positive Coomb's test and high cold agglutinin titer are sometimes found. Another example is autoimmune hemolytic anemia, where positive indirect and direct antiglobulin (IAT and BTA) test results are the effect of the presence of antibodies directed at red blood cells. Moreover, some viral infections, such as those caused by *Epstein-Barr* virus or cytomegalovirus, may be responsible for secondary production of cold (not showing activity in 37°C) antibodies, which may interfere with serological testing. Bacterial infections, in turn, are recognised as a cause of polyagglutination, being the effect of bacterial enzymes or other agents acting on the red blood cell membrane. The contact results in the exposure of the normally cryptic T, Tn and other determinants. Human sera are characterised by the presence of naturally occurring antibodies directed at these determinants, which causes agglutination of these blood cells. Polyagglutination is, thus, a reaction which takes place following the exposure of the cryptic determinants *in vivo*, often in the course of an infection [2,4].

MATERIAL FOR TESTING

Another very important process to take place before immunohematology tests can be conducted is obtaining material for these tests. This material is blood collected into tubes either with or without anticoagulant. Sampling blood from a vascular access device is allowed if the patient receives intravenous fluids. The fluid may react in immunohematology tests, so the cannula should be flushed with sodium chloride solution and the first few millimetres of the blood drawn should be discarded. The material collected for blood typing in newborns and infants up to 4 months of age should be taken to an EDTA tube with ethylenediamide tetraacetic acid, but in newborns with very low birth weight a sample of umbilical cord blood is also accepted. In order to obtain a confirmed blood typing result, the practice of taking one specimen of venous blood and another one of umbilical cord blood is accepted.

Transportation of the obtained specimens to a transfusion immunology laboratory must adhere to strictly defined standards. Blood samples should be stored at 18-25°C up to 8 hours following the collection, but

after this time elapses, and up to 5 days following blood collection, they should be stored and transported in the temperature of 2-8°C.

Blood samples for serological testing require special labelling and consistent patient's identification data. Thus, they should carry the following information:

- Patient's name and surname and personal identification (ID) number;
- Day and time of collection;
- Phlebotomist's signature;
- When data are missing: NN labelling is used, hospital register number and department register number are provided.

It is obligatory that every sample for immunohematology testing should be labelled in the patient's presence. After blood is taken and the tube is labelled, the data should be checked once again with the data on the order. Such practice prevents erroneous labelling. The prevailing number of errors are known to occur during the preanalytical phase and most adverse reactions can be attributed to misidentification of patients or samples. Requests for blood or its components are made on the basis of a confirmed blood typing test result. Such test result is based on two test results yielded by the specimens collected at two different times. Detailed recommendations regarding specimen identification and collection are found in the Regulation of 17 February 2021 of the Minister of Health for the treatment with blood and blood products in health care facilities offering on-site 24/7 medical service. The specimens accepted to the transfusion immunology laboratory should be checked with the submitted, valid test order which complies with a model order found in the currently binding regulation issued by the Minister of Health and must be always cross-checked regarding accuracy of the data placed on the order and the tube. Any discrepancies or doubts regarding patient's identification should be resolved and in the case of tubes which were erroneously labelled the specimens should again be collected, delivered and checked. A centrifuged specimen should not show haemolysis, as this is a positive reaction equivalent to agglutination [3,5,13].

EFFECTS OF MEDICAL DRUGS ON TEST RESULTS

Another attention-deserving issue is information about the administered medicines and colloids which may produce positive reactions in serological testing, such as indirect and direct antiglobulin test (IAT, DAT), and interfere with blood typing tests.

These drugs include:

- anti-D immunoglobulin,
- dextran,

- mannitol,
- methyl dopa, L-dopa
- penicillin and its derivatives,
- quinine, quinidine,
- nonsteroidal anti-inflammatory drugs,
- daratumumab.

Anti-D immunoglobulin is prescribed to women as a prevention of serological conflict as well as to Rh D (-) recipients (particularly for young women) of platelet concentrates obtained from Rh D (+) donors. This drug produces positive reactions in the antiglobulin test, so it must be ascertained if testing positive in IAT screening results from the presence of immune antibodies against red blood cells or if it is caused by the drug (passive anti-D antibodies). The information on when the drug was administered and on the result obtained directly before administration of the drug is essential for immunohematology testing, which is to yield reliable results. Other commonly used drugs which may interfere with serological testing are: dextran, used in the early treatment of hemorrhagic shock, burns or surgical procedures and an osmotic diuretic, mannitol. These drugs can affect the serological testing through erythrocyte rolling (rouleaux formation) and its misinterpretation as agglutination. There is a range of drugs which may lead to the development of immune hemolytic anemias. A possible underlying mechanism here is the production of autoantibodies, as it may be the case with the patient receiving methyl dopa, levodopa or interferons. In immunohematology testing there are warm autoantibodies adsorbed on red blood cells (positive DAT). Penicillin and its derivatives, cephalosporins, can modify erythrocyte cell membrane, where plasma proteins are non-specifically adsorbed, with positive DAT observed. Another mechanism is the formation of immunological complexes, which results in the formation of drug-specific antibodies. This mechanism and positive DAT result can be induced by quinine, quinidine and some nonsteroidal anti-inflammatory drugs. In many cases drug discontinuation proves sufficient to inhibit the process of haemolysis, but in situations when the drug cannot be discontinued, and blood transfusion is necessary, the information about the drug that the patient received is vital [6]. An important drug group showing the potential to significantly affect pre-transfusion test results are anti CD monoclonal antibodies. One of these drugs is daratumumab used in the therapy of recurrent and refractory multiple myeloma. This is a human IgG1 κ monoclonal antibody which binds to CD38 molecule, abundant on neoplastic myeloma cells. This marker is also found on red blood cells, which may lead to the reaction of these monoclonal antibodies with the antigen found on the blood cells, generating positive IAT and compatibility test results.

This effect may persist up to 6 months following the completion of therapy. While the reaction of this type can mask antibodies found in the serum, it has no effect on ABO and Rh antigen test results. According to the recommendation included in the amending Decree of the Minister of Health of 5 November 2020, prior to the commencement of anti-CD38 monoclonal antibodies therapy, the specimen obtained from the patient should undergo the following tests: screening for the presence of immune antibodies, which, if present, should be identified, DAT, and phenotype determination (Rh, Kell, Kidd, Duffy and MNS systems). If the patient underwent blood transfusions within 3 months before the test or is BTA positive, genotype identification is indicated. If the tests were not performed before the commencement of therapy, using Dithiotreitol (DTT) or another reagent able to destroy or block CD38 molecule may be necessary for testing [8]. A novel drug, an anti-CD47 monoclonal antibody, is currently in a trial, which holds a promise as an agent to be used in the treatment of hematological and solid malignancies. Its role is to block CD47 antigen, and thus stimulate phagocytosis and anticancer response. An anti-CD47 monoclonal antibody binds to CD47 antigen, highly expressed on erythrocytes. Its interference is seen with all pretransfusion tests, also in screening for ABO and Rh antigens. Developing the procedures allowing appropriate selection of blood for transfusion dedicated to patients on anti-CD47 therapy is the subject of study and publications. There has been evidence that anti-CD47 interfering quality may be eliminated by using anti-IgG immunoglobulin in an antiglobulin test or using the method of antibody adsorption [8,10,12].

HEMOVIGILANCE IN TRANSFUSION

The transfusion of blood and its components must be efficient and safe; hence the significance of all the stages of the preanalytical phase leading to the issue of a correctly selected component for a particular patient. In order to ensure the transfusion is performed according to the protocol, it is critical to select the proper component for transfusion and to complete the order form for immunohematology tests correctly, to adhere to the standards during blood collection and to label the specimens as required; accurate identification of the recipient and double-checking of the documentation before the transfusion, followed by patient's monitoring during and after the transfusion are likewise crucial. Proper blood collection and patient's identification represents a nurse's responsibility. The collected blood specimens together with the request forms for blood or its components and test orders are sent to a blood bank and a trans-

fusion immunology laboratory. There specimens, orders and request forms for blood or its components are checked and if they are incompatible they are returned for correction [4,11]. Such mistakes are called non-life-threatening adverse events. A serious adverse event is defined as an event connected with testing, preparation, storage, issue and transportation of blood or its components or their transfusion, which could be fatal or life-threatening, may result in a disability, deterioration of health, a disease, or may lead to hospitalization or prolonged illness/hospital stay. An adverse event is an unfavourable, unintended event connected with testing, preparation, storage, issue and transportation of blood or its components occurring before, during or after transfusion of blood or its components which may lead to an adverse reaction. Any adverse reaction taking place during the transfusion should be classified as transfusion-induced reaction until determined otherwise, even before its causative factors are confirmed, indicating high probability of the serious adverse reaction in the recipient being caused by the transfused blood or its component or, in the donor, the probability of the serious adverse reaction occurring during blood donation. The responsibility to report serious adverse events and reactions is on the hospital where such a reaction was observed. The case is reported to the Institute of Hematology and Transfusion Medicine through the competent Regional Blood Donation and Transfusion Centre within 24 hours following the reaction. Reporting and analysing the events and reactions in blood recipients requires a close cooperation of the hospital and the Blood Donation and Transfusion Centre. The centre notifies the Institute by sending collective reports on severe and mild transfusion reactions on an annual basis. The role of the Institute of Hematology and Transfusion Medicine is to assess and examine the adverse events reported by all the Regional Blood Donation and Transfusion Centres and to submit the prepared analyses to the competent institutions of the European Union.

Hemovigilance is a set of established surveillance procedures implemented in the case of serious adverse events, serious adverse reactions in donors and recipients and to ensure the epidemiological surveillance of donors. Hemovigilance in Poland is regulated by the Act of 22 August 1997 on Public Blood Service with further changes (Journal of Laws, No. 106, item 681, as amended), Minister of Health Regulation of 16 October, 2017 on the treatment with blood and its components in health care facilities offering on-site 24/7 medical service, the Minister of Health Directive of 6 March 2019 on the standards of good practice for the storage, issue and transport of blood for or-

ganizational units of public blood service. The analysis of every adverse event and reaction is conducted on many levels, including institutional (hospital), regional (the Blood Donation and Transfusion Centre), national (the Institute of Hematology and Transfusion) and international (the European Council, the European Commission) levels [11].

CONCLUSION

Realizing the importance of preanalytical phase and its role in immunohematology testing is the first step towards ensuring reliable test results, issuing correct requests for blood and its components and performing safe transfusion. Reducing the number of errors committed in this phase would translate to improved patient care and lower laboratory costs. In fact, any inaccuracies missed at that stage may lead to a range of errors, and those may, in turn, result in adverse events and reactions which could be life-threatening or fatal. It is therefore vital that, for the purpose of immunohematology testing, patient's identity is verified according to the procedures before blood collection and their serological history is known. Immediate access to information on the treatment the patient received, previous test results and transfusions saves the time needed for testing and facilitates the process of selecting the right component for transfusion. The stages of the preanalytical phase include also the validation of tests and processes, qualification of equipment and reagents and inspection of reagents on an everyday basis. Each method or process involved in immunohematology testing must undergo validation before it is introduced into routine practice, and later regular re-validation. All the reagents and equipment, before being approved for use in immunohematology testing, are subject to qualification, calibration and maintenance in accordance with their specifications. Qualification means confirmation that the devices, equipment and reagents meet operating standards and yield expected results. In conclusion, adherence to high standards in preanalytical phase may, while being nearly cost-free, effectively shorten testing time and contribute to the reduction of adverse events and post transfusion reactions [1,2].

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