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Transfusion medicine

[1] Dennis Arinze Nwachukwu, [2] Anih Chinaza Doris, [3] Olokodana Babatunde Kazeem, [4] Oyayinka Omotolani Anthonia, [5] Abarigu Priye Gift, [6] Okpara Onyedikachi Martins, [7] Njoku Perfect Izuchukwu, [8] Aishatu Aminu Ribadu, [9] Felix Ogbuta Kalu, [10] Adibe-Ikpo Victor Ugochukwu, [11] Egeonu Kelvin Kelechi, [12] Chibuzor Prince Okwor, [13] Okpara Friday, [14] Uchenna Favour Ogechi, [15] Eze Peter Chinweuba, [16] Faith Chinasa Abel, [17] Ogungbenro Precious Adebola, [18] Onwuatuelo Oscar Damian, [19] Obatu Ifeanyi Daniel, [20] Danjuma Seth Wadalo, [21] Danjuma Atnadu Joseph, [22] Ugwu Kingsley Monday, [23] Maduagwu Chukwuebuka Rufus, [24] Udeagu Ugochukwu Franklin, [25] Eyamah Sylvester, [26] Popoola Kolapo Oluwatosin

[1] Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Nigeria
[2] Department of Nursing, People's Friendship University of Russia (RUDN), Moscow, Russia
[3] Department of Anatomy, Crescent University, Ogun State, Nigeria
[4] Department of Anatomy, Olabisi Onabanjo University, Ogun State, Nigeria
[5] Department of Human Anatomy, University of Port Harcourt, Rivers State, Nigeria
[6] Department of Medicine and Surgery, Niger Delta University Wilberforce Island Amasoma Bayelsa State, Nigeria
[7, 25] Department of Public Health, Epidermiology and Evidence-Based Medicine Division, Sechenov University, Moscow, Russia
[8] Nigerian Defense Academy, PMB 2109 Kaduna, Kaduna State, Nigeria
[9, 11] Department of Medical Laboratory Science, Abia State University, Uturu, Abia State, Nigeria
[10] Department of Medical Laboratory Science and Technology, Nnamdi Azikiwe University, College of Health Science and Technology, Anambra State, Nigeria
[12, 15, 16, 22] Department of Medical Laboratory Sciences, University of Nigeria, Enugu Campus, Enugu State, Nigeria
[13] Department of Medical Laboratory Science, Ebonyi State University Abakaliki, Ebonyi State, Nigeria
[14] Osisatech Girls Secondary School Enugu, Enugu State, Nigeria
[17] Sciences, Muslim Grammer School Ipapo Oyo State, Nigeria
[18] Department of Human Physiology Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria
[19] Department of Medical Radiography, University of Nigeria, Enugu Campus. Enugu State Nigeria
[20] Faculty of Medicine, Shenyang Medical College, China
[21] Rostov State Medical University, Rostov, Russia
[23] Department of Microbiology, Imo State University Owerri, Imo State, Nigeria
[24] Department of Medical Laboratory Science, Enugu State University of Science and Technology, Enugu State Nigeria
[26] National Defence College, Abuja Clinic, FCT, Nigeria

Corresponding Author: **Dennis Arinze Nwachukwu**

Abstract

Blood transfusion medicine started with xenotransfusion. A French physician, Jean-Baptiste Denis initiated xenotransfusion in 1667. He performed the first documented blood transfusion on June 15, 1667 to a 15-year-old boy using blood of a lamb. His treatment choice led to the patient's recovery from violet fever. This paved way for xenotransfusion until 1900 when Karl Landsteiner discovered the human ABO blood group system; then xenotransfusion was abandoned. Recently, due to advances in rDNA technology, xenotransplantation, biomedical researches and the pressing needs for blood transfusion, xenotransfusion is being considered again in humans and pigs are the best choice species. Studies have shown a very close relationship between the haematological parameters and physiology of pigs and humans. Also, there is increasing ability to genetically-modify pigs to reduce immunogenicity. Three antigens present in pig RBCs (Gal, Neu5Gc and Sd[a]) are the recognised issues with immunogenicity of xenotransfusion using pigs. However, development of gene-knockout technologies has proven effective in overcoming these issues. In animals, xenotransfusion is practiced but only used in emergency cases. Xenotransfusion in humans may resurface as researches and technologies improve.

Keywords: Xenotransfusion, Immunogenicity, Blood Transfusion, Transfusion Medicine, Haematological Parameters

Introduction

The word "Xeno" came from the Greek word "Xenos", which means "strange". "Transfusion" on the other hand is the transfer of blood or blood products from one person to another. Xenotransfusion therefore refers to a strange transfer of blood involving

organisms from different species. In other words, xenotransfusion is the transfer of blood from one species into the veins of another species. Major cases of xenotransfusion involves the transfer of blood from a non-human species of animal into a human. Xenotransfusion was the starting point for both xenotransplantation and human blood transfusion [1]. Xenotransplantation is an important scientific research area aiming at providing solutions to serious diseases such as tissue and organ damages.

Blood transfusion as a practice is a success when there is no significant alteration in the blood physiology of the recipient after transfusion. Transfusion failures usually occur due to post-transfusion haemolytic reactions (the destruction of the red blood cells that were given during transfusion). This results from the incompatibility of the physiological parameters between the donor and the recipient. Antigens present in cells are the major determinants of transfusion compatibility. These antigens have created many blood group systems in humans and animals. The international society of blood transfusion has recently recognized 33 blood group systems [2]. However, only few of the blood group systems are relevant to transfusion. In humans, the ABO and Rhesus antigens are present in the red blood cells and other blood cells. According to Klein and Anstee [3], the ABO antigens are also present on platelets and other tissues of the body. These antigens are the major key role in compatibility factor of blood transfusion. Many non-human mammals have similar blood group systems that can match with the ABO blood group system in humans. The ABO blood group system can be found in apes and monkeys. Cats have AB blood group system. Unlike humans that can have A, B, AB or O type bloods, cats only have A, B and AB types. Pigs have AO blood types. The B type blood has not been described in pig [4]. Similar blood group systems in different species shows that blood from one species may function properly in another species. However, for proper function of blond from different species in another species, there should be close similarities in the haematological parameters between the species involved. Porcine Reb Blood Cells (pRBCs) share the most similarity to human Red Blood Cells than any other animals. For instance, the pRBC diameter is about 6µm while that of humans is about 7.2µm. The RBC count in pigs is about 5.7 – 6.9 million per microlitre while that of humans is about 4.7 – 6.1 million per microlitre. The average life space of RBC of pigs is 86 days while that of humans is 120 days. This close relation between pRBCs and that of humans has made pigs the species of interest in Xenotransfusion.

Definition of Key Words

- **Xenotransfusion:** This is defined as the blood transfer from an organism to another organism of different species, usually from an animal to a human.
- **Xenotransplantation:** This is defined as the transfer of cells (stem cells), tissues and organs from animals to humans for therapeutic purposes.
- **Blood Group:** This refers to the entire antigens present in the erythrocytes of an organism.

Important Abbreviations

DEA: Dog erythrocyte antigen
MHCII: Major histocompatibility complex II
Gal: Galactose-α (1,3)-galactose
Neu5Gc: N-glycolylneuraminic acid
SIRP-α: Signal-regulatory protein-α
GTKO: α-1,3-galactosyltransferase gene-knockout
TKO: tipple-knockout

History of Xenotransfusion

The early blood transfusions in humans were xenotransfusions, carried out by Jean-Baptiste Denis beginning in 1667 [1]. The first xenotransfusion was performed by Jean-Baptiste Denis, a French physician and Paul Emmerez, a surgeon on June 15, 1667. Denis and Emmerez transfused blood from a lamb into the vein of a 15-year-old boy and this led to a successful recovery from violet fever. The transfusion was done by direct transfer of blood from the lamb to the boy due to lack of knowledge on how to preserve blood from coagulation. The patient complained of feeling a strong heat moving through his arm. He however, worked and ate normally and was calm and jovial [1]. Denis performed the second transfusion in the same June, 1667. The transfusion involved a healthy 45-year-old man who was paid for his participation. About 300ml (10 ounces) of blood was drawn out from his vein and the same quantity of blood was taken from a lamb and injected into his vein [1]. Like the first transfusion, the recipient also complained of heat sensation running through his vein. On June 24, Denis performed the third transfusion on a young Swedish nobleman. Before the transfusion, the patient was practically unconscious. After the first transfusion, the man felt better until his condition grew worse. A second transfusion was performed but was unsuccessful, the patient died.

On November 23, 1667, an English physician, Richard Lower, successfully transfused blood from a lamb into a 22-year-old man. Another xenotransfusion was performed on December 19, 1667 by Denis and Emmerez to a 34-year-old deranged man, Antoine Mauroy. About 10 ounces of blood was drawn out of the patient's vein and about 6 ounces were taken from calf and injected into him. He only got a slight relieve and so, Denis performed the second transfusion two days later with about one pound of calf's blood. The patient as well complained of strong heat running through his vein and pains in his kidneys. Few days after, his urine was black as if it had been mixed with soot of chimneys; this was the first reported post-transfusional acute haemolytic reaction [1]. Denis performed another transfusion on February 10, 1668 to a paralytic woman using 12 ounces of lamb's blood. Not only did she get well, but her paralysis disappeared almost immediately.

Many other xenotransfusions were practiced; some successful and some unsuccessful. Blood transfusion as a practice was banned by the French and English parliaments on January 10, 1670 following many controversies. However, the practice was resurrected about a century later on December 14, 1799 by William Thornton. In 1816, John Henry Leacock, a Scottish physician showed, in his eight transfusion trials, that the best results of transfusions were obtained if the donor and the recipient were of the same species [1]. He was the first to recommend inter-human transfusion. Xenotransfusion was abandoned after the discovery of ABO blood group system by Karl Landsteiner in 1900 as many people are willing to donate blood for transfusion. Currently, due to new discoveries in xenotransplantation and the demand level of blood, xenotransfusion in humans is been considered again and pig is recognized as the best potential donor as there are close

relations between the porcine red blood cells and that of human. Also, pigs can easily be genetically modified to produce only O-type bloods.

Fig 1: A Physician Transfusing Blood from a Lamb to a Patient.[1]

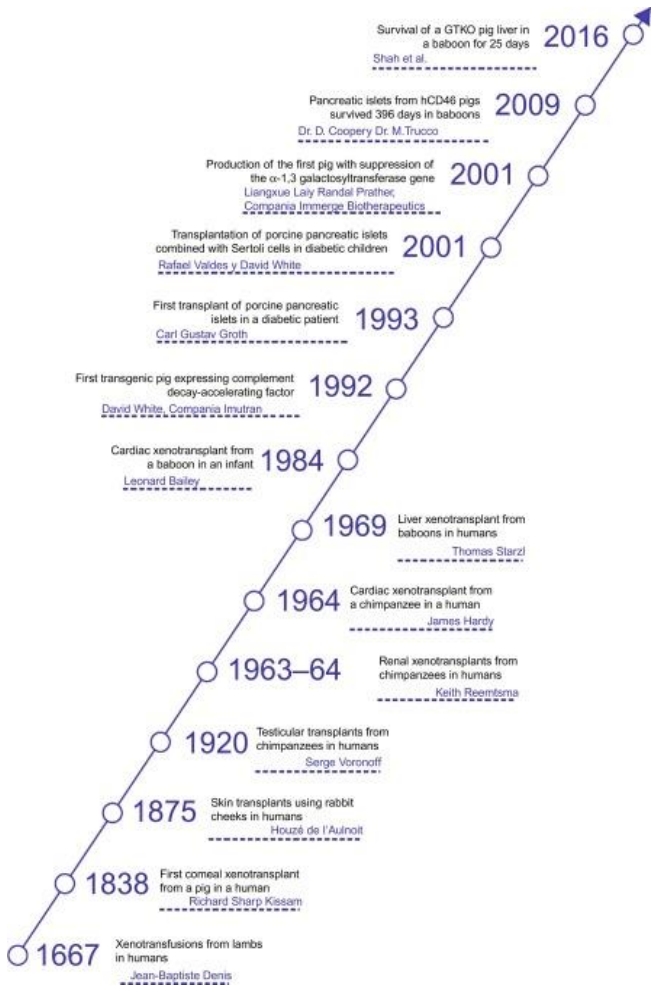

Fig 2: Progress of xenotransplantation Initiated by Xenotransfusion.[5]

Blood Transfusion Medicine

Blood transfusion is the transfer of blood or blood products from an individual to another. Blood and blood products provide unique and life-saving therapeutic benefits to patients (World Health Organisation). According to the American Red cross, there are four common types of blood transfusion [6]:

- **Red Blood Cell transfusion:** This involves transfusing packed red blood cells into the recipient (Human or animal). This is required in cases of blood loss by accident or surgery, iron deficiency anaemia or blood disorder such as sickle cell anaemia.
- **Platelet transfusion:** In cases of low platelet count, platelet transfusion can be given. Platelet given can be Random Donor Platelet (RDP) or Single Donor Platelet (SDP). One unit of RDP increases platelet count by approximately 5 X 10^9/L [7]. SDP is very expensive and have limited applications.
- **Plasma Transfusion:** Plasma is blood in which the blood cells have been removed. A patient May receive a plasma transfusion if he/she has experienced a severe burns, infections, or liver failure [6].
- **Whole blood transfusion:** This is the most practiced transfusion. Here, the whole blood involving the blood cells and the plasma is given to patience.

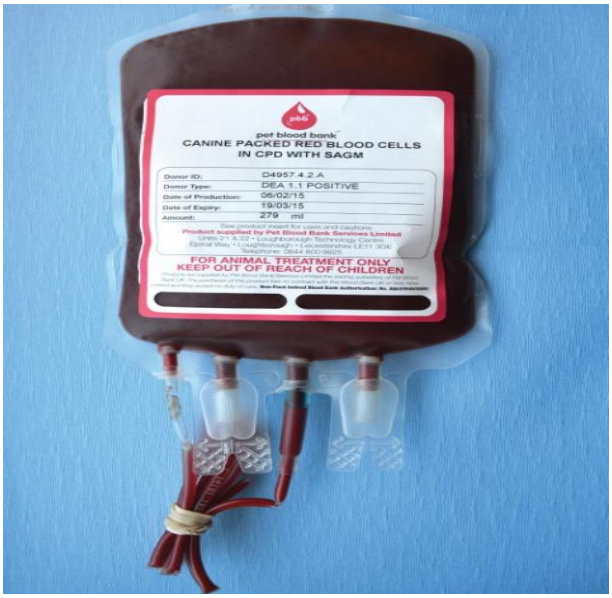

Fig 3: A unit of Canine Packed Red Blood Cells[8]

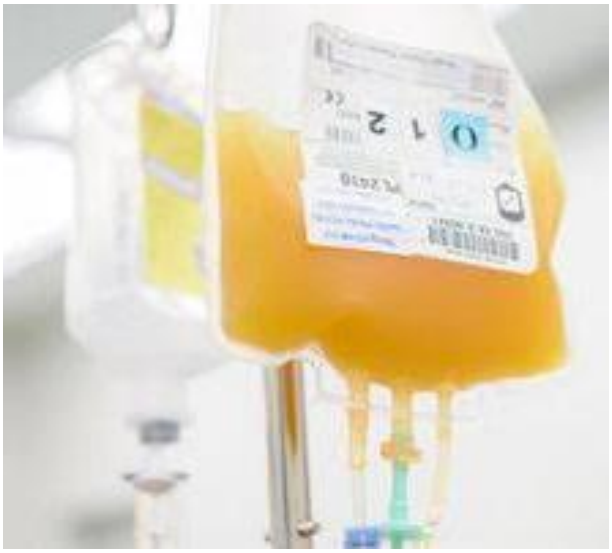

Fig 4: Human Platelet[7]

Fig 5: A Unit of Human Blood Plasma[9]

Fig 6: A Unit of Human Whole Blood [10]

Reason for Blood Transfusion

- Blood lose through accidents, gunshots and surgery
- Illnesses such as fevers,
- Sickle cell diseases
- Anemia
- Hemophilia
- Kidney and liver diseases
- Cancer
- Surgical treatment

Requirement

1. For human donors, the donor should be of good health, physically and mentally and should be between the age of 18 – 60 years (World Health Organisation). Haemoglobin level should be normal; minimum of 12.5g/dL for males and minimum of 11.5g/dL for females, a normal blood pressure of systolic: 100-140mm Hg; diastolic: 60-90mm Hg and pulse rate of between 60 to 100 beats per minutes (World Health Organisation).
2. Compatibility must be ascertained between the donor and the reciepient. This is done by ABO and Rh blood testing for humans. Other blood type testing should be done in animal transfusion. For instance, there are six blood types in dogs belonging to the "DEA" haemoglobin antigens. The testing antigens for the six blood groups are commercially available.
2. World Health Organisation recommends that all blood donors should be screened of infectious diseases such as HIV I and II, Hepatitis B and C and Syphilis.

Physiology of Blood Transfusion

The success of blood transfusion is determined by many factor; the most important being the compatibility of the antigens present in the erythrocytes of the donor and the recipient. The discovery of ABO blood group, over 100 years ago, caused great excitement; until then, all bloods were thought to be the same [11]. People with blood type A has antigen A on the surface of their RBCs, people with type B has antigen B, people with AB type has both antigen A and antigen B while people with type O blood has no antigen. Also, type A blood makes anti B antibody, type B makes anti A antibody, type AB makes no antibody while type O blood makes both anti A and anti B antibodies. Another consideration is the Rhesus factor, an antigen also found in the surface of the RBC. People with the Rhesus antigen on their RBCS are Rh positive while people without the antigen are Rh negative. This is the basis of transfusion compatibility.

Hemolytic reaction occurs due to the clash of antigens and antibodies. For instance, if an A group blood receives from a B group type, the antibody of the type A blood (anti B antibody) will begin to fight the antigen B of the received B type blood. The result is agglutination in the blood vessels of the recipient which is always fatal. Also a negative Rh individual receiving blood from a Rh positive individual will cause the production anti Rhesus antibodies in the Rh negative recipient. So the ABO blood group antigens remain of prime importance in transfusion medicine [11].

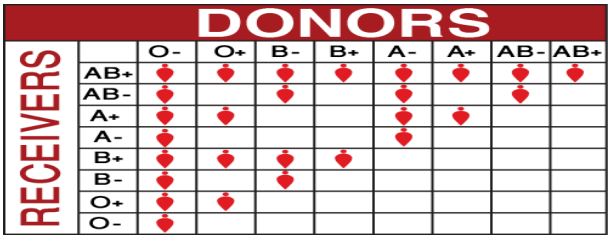

Fig 7: Blood compactibility

Mammalian Blood Group Systems

International Society of Blood Transfusion has recently recognized 33 blood group systems in humans [13]. So, the ABO and Rh blood types are not the only blood group types present in humans; though, they are the most relevant in transfusion medicine. Of the 33 blood group systems, 5 are defined by their carbohydrate structures (ABO, H, P1PK, I, GLOB), 2 are obtained from the plasma (LE, CH/RG) while the remaining 23 are characterized by the protein sequence of the RBC membrane protein [13]. The ABO and Rh blood groups which are the most relevant in transfusion are found in humans and mammals.

Porcine blood group systems

The pig blood group system is very much related to that of human. AO blood types have been identified in pigs. The B-type has not been identified but pigs have blood antigen that is structurally similar to the human B-type antigen. The A-antigen in pigs is identical to the A-antigen of the human ABO blood group and is synthesized by the same enzyme [14]. The O-type pigs lack the A-antigen but the antigen structurally similar to the human B-antigen is present. Pigs also have a single Rh gene which dos not represent any blood group antigen [15].

Feline blood group system

Cats have A, B and AB blood types in the AB blood system which is identical to the human ABO blood group system. A-type cats have A-antigen similar to A-antigen of humans. B-type cats have B-type antigens similar to B-type antigens in humans and AB cats have equal A and B-antigens on the surface of their red blood cells. Just like in human, the A-type cats make anti-B antibodies and B-tpye cats make anti-A antibodies. AB makes both antibodies. The anti-A antibodies are strong agglutinins and hemolysins, especially of the IgM class; in contrast with the anti-B antibodies which are weaker agglutinins and hemolysins and belong to the IgG and IgM class [16].

Canine blood group systems

There are 8 blood types in dogs, all belonging to the DEA blood systems. The acronym DEA stands for "Dog Erythrocyte Antigen", followed by numerical designation of the blood group classified with polyclonal alloantibodies [17]. Of the whole numerical antigens, DEA 1.1 and DEA 1.2 which could be positive or negative.

Table 1: Canine Blood Group Systems[17]

DEA group	"old" name	Population incidence	Natural antibody	Transfusion significance
1.1	A_1	40-60%	No	Acute hemolytic reaction
1.2	A_2	10-20%	No	Acute hemolytic reaction
3	B	5-20%	Yes	Delayed hemolysis
4	C	85-100%	No	None
5	D	10-25%	Yes	Delayed hemolysis
6	F	98-99%	No	Unknown
7	Tr	10-45%	Yes	Delayed hemolysis
8	He	40%	No	Unknown
Dal		100%**	No	Acute hemolysis
Kai-1		94%***	Not as yet	Unknown
Kai-2		1%***	Not as yet	Unknown

Blood group systems of other mammals

There are 8 major blood types in horses of which 7 have been recognized internationally (A, C, D, K, P, Q and U). In addition to these, there are about 22 other minor blood types recognized in horses. Sheep have seven blood types: A, B, C, D, M, R and X. The B-type is highly polymorphic and has many antigens. Goats have Similar blood types as sheep with five major types (A, B, C, M and J). Cattles have 11 blood types (A, B, C, F, J, L, M, R, S, T and Z). Like in sheep, the B-type is highly polymorphic and has more than 60 antigens [16].

Comparative Mammalian Haematology

At site, different mammals look entirely different but the physiology of mammals have lots in common. In most mammals, the blood components and functions are similar. This is relevant in xenotransfusion in that a blood taken from a species and introduced into another species should produce the same result as the blood of the recipient species. Blood is made of cells and plasma. In human, plasma accounts for 55 % of which 92 % are water, and the contents of the remaining 8% are glucose, hormones, proteins, minerals, fats and vitamins [18]. The remaining 45% of the blood is made of cells: red blood cells, white blood cells and platelets. Pig is considered the best potential donor in human Xenotransfusion due the closest haematological relation with humans. Therefore, as a case study in comparative mammalian haematology and with respect to xenotransfusion, elaborate emphases on the haematological parameters of pigs and humans are made below.

Table 2: Haematology and Clinical Chemistry Parameters in Pigs and Humans: Normal Ranges [19]

Parameter	Pigs	Humans
Haematology		
Hemoglobin (g/dl)	10-16	12-18 (M); 11-15 (F)
Hematocrit (%)	32-50	32-52 (M); 34-45 (F)
Red blood cells (10^{12}/L)	5.0-8.0	4.3-5.8 (M); 3.9-5.1 (F)
Mean corpuscular volume (μm^3)	50-68	82-99
Mean corpuscular hemoglobin (pg)	17-21	28-34 (M); 27-34 (F)
Mean corpuscular hemoglobin concentration (g/dl)	30-34	33-35
Platelets (10^9/L)	320-520	130-400
White blood cells (10^9/L)	11-22	3.8-10.9
Neutrophils (10^9/L)	3.1-10.5	2.0-7.2
Lymphocytes (10^9/L)	4.3-13.6	1.1-3.0
Monocytes (10^9/L)	0.2-2.2	0.06-0.75
Eosinophils (10^9/L)	0.06-2.4	0.03-0.35
Basophils (10^9/L)	0-0.45	0.01-0.16
Chemistry		
Sodium (mmol/L)	140-150	140-150
Potassium (mmol/L)	4.7-7.1	3.5-5.0
Chloride (mmol/L)	100-105	104-111
Bicarbonate (mmol/L)	18-27	24-30
Calcium (mg/dL)	11.0-11.3	8.7-11.0
Phosphate (mg/dL)	4.0-11.0	2.2-4.7
Magnesium (mg/dL)	1.9-3.9	1.8-2.4
Iron (µg/dL)	73-140	50-160 (M); 60-140 (F)
Urea nitrogen (mg/dL)	8-28	5-25
Creatinine (mg/dL)	1.0-2.7	0.5-1.4
Glucose (mg/dL)	65-95	65-105
Cholesterol (mg/dL)	117-119	140-220
Total biluribine (mg/dL)	0-0.2	0.1-1.0
Alkaline phosphatase (IU/L)	60-269	40-130
Aspartate aminotransferase (IU/L)	25-87	7-27
Alanine aminotransferase (IU/L)	11	0-40
Creatinine phosphokinase (IU/L)	65	30-220
Total protein (g/dL)	3.5-6.0	6.0-8.0
Albumin (g/dl)	1.9-2.4	3.2-4.8
Globulin (g/dl)	1.0-1.3	2.3-2.8
Uric acid (mg/dL)	0.25-1.95	3.5-8.5 (M); 2.5-7.0 (F)
Triglycerides (mg/dL)	0-145	40-160 (M); 35-135 (F)
Lactate dehydrogenase (IU/L)	211	80-200
Fibrinogen (mg/dL)	100-500	200-400

Close similarities in the haematology and blood chemistry of pigs and human suggest that the porcince erythrocytes may perform the oxygen transport properly in human tissues and organs. Possibility of xenotransfustion can therefore be established with comparative haematology.

Comparative Mammalian Immunology of Transfusion

The immune system is the defense system of an organism. If incompatible blood is given in a transfusion, the recipient immune system will see the donor cells as foreign invaders and the immune system will launch an attack against the cells accordingly [11]. Because blood cells carry a lot of antigens, immune response is a vital aspects of transfusion. Some of the severe immunological side effects of transfusion include: haemolytic reactions, TRALI (acute lung injury) and PTP (post-transfusion purpura).

Dean [11] wrote that the general formation of immune response occurs in three stages.

- The immune system detects the foreign antigen
- The immune system processes the antigen
- The immune system mounts a response to remove the antigen from the body

Immune response to foreign blood antigens in humans

The immune system uses either antibodies or immune cells (such as macrophages, lymphocytes, etc) to launch immune attacks. Macrophages on encountering any red blood cell with a foreign antigen in the blood stream, will engulf and digest the cell. The fragments of the antigen will then be deposited and mix with the Major Histocompatibility Complex II (MHC II) on the surface of the macrophage. When a T helper cell sees the antigen fragment with MHC II, it will bind to them and this causes the macrophage to release cytokines (an immune messenger), which stimulate the production of T cells. In the presence of T cells, the T helper cells will be fully activated. Being activated, the T helper cell activates the B cell. The B cell will be stimulated by the T cell to mature and divide. On division, the B cell produces daughter B cells, some of which forms the plasma cells. The plasma cells are responsible for production of antibodies (belonging to IgG and IgM class) against the particular antigen that initiated the immune response. Some of the daughter B cells however do not form plasma cells, rather they form the memory cells that remembers the antigen that initiated the immune response. This is a primary immune response, which occurs if the immune system just encountered the foreign antigen for the first time. However, if the immune system has encountered the antigen before, a secondary immune system is initiated by the memory B cells which is more aggressive than the primary immune response.

Porcine blood group antigens

A sugar, galactose-α (1,3)-galactose (α-Gal) is a blood antigen present in porcine and bovine red blood cells. Unfortunately, anti-Gal is a natural antibody produced in most primates including humans. This is the major issue with human xenotransfusion and xenotransplantaion using pig. α-Gal antigen is however, structurally similar to the human B-type blood antigen. The antigen α-Gal is present in all pigs, however, the antigen-A is expressed in only A-type pigs and this antigen is identical to the human A-antigen of the ABO blood group and is even synthesized by the same enzyme, A-transferase.[20] Pigs can easily be modified genetically to produce only O-type blood which lacks the A-antigen.

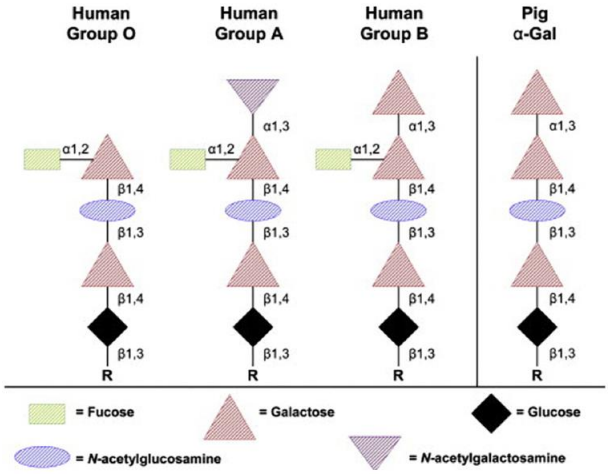

Fig 8: Structure of Human ABO Antigens and Pig Gal Antigen[15]

Immunological barrier to human xenotransfusion using pigs

Any Xenotransfusion that triggers immune response of the recipient is not a success. The presence of α-Gal antigen in porcine bloods and anti-Gal antibodies in primates is one of the major immunological barrier of human Xenotransfusion using porcine species. Other antigens present in pRBCs that triggers immune response in humans are Neu5Gc (N-glycolylneuraminic acid) and Sd^a (Sid antigen). According to Smood *et al.* [15] when pRBCs were transfused into the old world non-human primates, expression of Gal resulted in almost uniform hyperacute rejection or cell lysis, similar to that seen after ABO-incompatible blood transfusion. Another immunological barrier of human xenotransfusion using pigs is the human macrophages. Human macrophages can be activated and attack the pRBCs without antigenic stimulation. This is so because of the incompatibility of signal-regulatory protein-α (CD47/SIRP-α).

Xenotransfusion Compatibility

Irrespective of the immunological barriers of porcine to human xenotransfusion, pigs are still the best candidates of for human Xenotransfusion due to the following reasons:

- Pigs have the closest haematological similarities with humans.
- Porcine AO blood system is identical to human ABO blood system.
- Pigs can easily be modified to produce only the universal donor O-type bloods.
- The immunological barriers of porcine-human xenotransfusion can be conquered through recombinant DNA technology.
- Pig breeding is not difficult, therefore constant availability of usable RBCs for transfusion is assured.
- Pig is the leading species in xenotransplantation research.

Porcine to Human Blood Transfusion

The current xenotransfusion and xenotransplantation research has shifted from Ape-human to Pig-human. Pigs appear much different to humans but share close physiological similarities to human and can be easily breed. Ape on the other hand is an endangered species and cannot be simply bread like pigs.

Overcoming immunological barriers of pRBCs transfusion to humans

Fortunately, α-1,3-galactosyltransferase gene-knockout (GTKO) can produce pigs which do not express Gal, thereby overcoming the Gal immunological barrier to human Xenotransfusion using pigs.[15] Natural antibodies to the remaining two porcine antigens (Sd^a and Neu5Gc) have been identified in humans. Therefore, Smood *et al.* [15] said "if pRBCs are to be transfused successfully into humans, RBCs from triple-knockout (TKO) pigs are required, in which all the three of these antigens have been deleted. Smood *et al.* [15] also wrote that a comparative analysis of human antibody (of IgG and IgM) binding to pRBC isolated from TKO pigs and allogeneic human RBCs (RBCs from different human donors) were carried out. The result showed that human antibody binds less to TKO pRBCs than to allogeneic human RBCs in 43 % of samples. This shows that TKO pRBCs reduces or eliminates the immunological barrier of human Xenotransfusion using pigs.

The issue of macrophage activation via incompatibility of CD47/SIRP-α can also be conquered. Human CD47 (hCD47) is a protein that interact with SIRP-α to inhibit macrophage phagocytosis. Porcine CD47 does not inhibit macrophage phagocytosis in humans. Therefore, for a successful inhibition of phagocytosis, hCD47 needs to be genetically expressed in pigs. However, expression of hCD47 on pRBCs will be difficult and will require novel techniques of transgenesis [15]. Future outcome in xenotransfusion and xenotransplantation research may yield pRBCs which can be used by humans.

Safety and relevance of xenotransfusion

Safety

Safety of xenotransfusion is a major consideration before any clinical trial of xenotransfusion should be carried out in humans. The past xenotransfusion were done in of ignorance of blood antigens and antibodies. Current xenotransfusion research however, is targeting the complete understanding of any possible adverse effect of a species blood in another species. Scientists have been working to conquer the barriers to human xenotransfusion using porcine species. In 2002, GTKO technology became available which solved the issue gal antigen present in pigs and natural occurring anti-gal antibodies in humans. The development of TKO technology has also improved the possibility of porcine-human xenotransfusion. Apart from immunological barriers, transmission of porcine-related diseases is also an area of consideration. A major concern here is the transmission of porcine endogenous retroviruses (PERV). However, mature pRBCs do not have nuclei and therefore do not harbor PERV. The issues in starting clinical trials of xenotransfusion in humans are the macrophage activation by porcine cells, full understanding of porcine pathobiology and ethical issues surrounding xenotransfusion. There is hope that future outcomes will eliminate the remaining barriers preventing human xenotransfusion using porcine. species. In other species, proper practice of xenotransfusion will take so long to set in. This is because, among all the mammals, the human blood group systems and immunology is most understood. This is why xenotransfusion is just an emergency clinical solution to anaemic animals.

Ethics, Public Perception and Regulatory Aspects

There are many ethical issues surrounding xenotransplantation already. However, some of the ethical issues in xenotransplantation is not considered in xenotransfusion. Xenotransfusion is less complicated than xenotransplantation and so has raised less ethical issues. That is why Francoise *et al.* [21] said that it much more ethically acceptable to raise pigs for xenotransfusion than xenotransplantation as the former doesn't cause damage to the animal health. The major issues in xenotransplantation is the immunological barriers and transmission of porcine endogenous retroviruses (PERV). In xenotransfusion, the immunological barrier as of today is almost conquered by gene-knockout technologies. There are no ethical issues against using transgenic pigs to produce usable bloods for transfusion. The issue of PERV is not a threat to xenotransfusion because mature mammalian RBC lacks nucleus, therefore, xenotransfusion cannot transfer PERV to humans.

Public perception has a negative impact on xenotransfusion. Some, due to religious believes would prefer to die rather than having an animal blood flowing through their veins. However, it is an ignorance to reject xenotransfusion because the product is from non-human species. Already, there are existing therapeutically products from non-human species. Insulin injected to diabetic patients are synthesized from bacteria. Tetanus antitoxin (ATS) is a purifies antibodies prepared from horse blood. Therefore, rejecting xenotransfusion outside the grounds of safety is not justified.

On the regulatory aspect, the whole practice of xenotransplantation attracted interest from regulatory authorities, particularly after the demonstration of possible pig-to-human transmission of PERV [19]. However, xenotransfusion cannot transmit PERV; hence attracted less regulatory authorities. Once scientists have demonstrated a satisfying safety and efficacy of xenotransfusion in non-human primates and humans *in vitro*, clinical trials of xenotransfusion will set in.

Importance of Xenotransfusion

1. The World Health Organisation estimated an approximate of 112 million units of donated blood each year. This unit is far away from satisfying the global need of blood transfusion; hence, having an alternative to human red blood cells which is clinically safe, available, inexpensive and physiologically effective will be of great advantage [15].
2. The global demand for organ transplantation is overwhelming. Xenotransplantation is a hope to provide solutions for this. Xenotransplantation might not be possible if scientists have not at least achieved xenotransfusion in humans.
3. Pigs can be breed in a pathogenic free environment, therefore provides solution for the risks of infectious human RBCs.
4. Xenotransfusion application in veterinary practice provides therapy for anaemic animals whose same species cannot donate blood to due to small body weight or difficulties in finding a correct cross match.

Challenges of Xenotransfusion

1. Public perception
2. Immunological barriers
3. Pathobiology of donor species

Future of Xenotransfusion

With the recent advances in recombinant DNA technology and xenotransplantation, xenotransfusion may resurface.

However, Smood *et al*., [15] advises that prior considering clinical trial of pRBC transfusion to humans, the pathobiologic barriers must be understood and overcame through both *in vitro* and *in vivo* investigation. Porcine-human xenotransfusion can be followed *in vivo* by transfusion of modified pRBCs into non-human primates and *in vitro* by direct use of human blood in physiological environments.

Conclusion

Xenotransfusion is an important emergency therapy in veterinary practices. Human xenotransfusion was rejected due to ignorance. Current studies have revealed the possibilities of human xenotransfusion using pigs. However, prior the practice of xenotransfusion, all the immunological barriers leading to rejection of xenotransfusion must be conquered and public perception controlled. Future outcome from xenotransfusion and xenotransplantation researches may bring back the rejected xenotransfusion in humans.

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