***BLOOD TRANSFUSION***

Definition:-

-Transferring whole blood or blood products from the donor to the recipient intravenously

-Process of receiving whole blood or blood products into one’s circulation intravenously

BTU (Blood transfusion unit) sources, processes and stores blood.

The procedure to donate blood is as follows:-

-In order to donate blood the recommended Hb is >12.5 and the recommended weight is >50kg.

NB:- Females can donate every 4 months while males can donate every 3 months.

-There is a donor’s questionnaire to fill prior to donating blood. Part A asks about chronic illnesses and bleeding disorders, and part B asks if the donor has been exposed to any risk factors.

-The donor is then prepared for bleeding i.e. is given a summary of what is going to happen. A toniquet is applied and the site is sterilized with 70% alcohol. The preferred site is the median antecubital vein. The blood is then stored in blood bags.

The blood bag has an anticoagulant which is also a preservative too and this is called CPDA (citrate phosphate dextrose adenine)

STICKY NOTE:-

Citrate – anticoagulant

Phosphate – maintains blood pH

Dextrose – provides energy

Adenine – preservative of RBCs i.e. increases life span of RBCs

The preservative anticoagulant works for 35 days hence after 35 days the blood expires.

There may be a triple bag i.e. primary bag and additional transfer bags. One pilot tube attaches the primary bag to the needle and another pilot tube attaches the primary tube to the transfer bag.

There can be up to 3 transfer bags.

It is sometimes necessary to manually shake the bag and mix the anticoagulant with the blood.

The blood bag can store 400-500 ml of blood which is equivalent to 1 unit of blood.

There is 63ml of CPDA in one bag

On the bag there is an identification of the donor’s number on the pilot tube, date of collection, date of expiry (date of collection + 35 days), and blood group (ABO and Rh)

The blood is screened for transfusion transmitted infections i.e. HIV 1 and 2, Hep B and C, syphilis and sometimes malaria and these details are stuck on the bag.

Storage of whole blood – refrigerated at 2-8˚c and with CPDA lifespan is 35 days.

The blood is refrigerated to reduce cellular mechanism and thus increase lifespan and reduce bacterial growth.

The other blood bags are used to get blood products from whole blood.

***BLOOD PRODUCTS***

Blood products can be prepared by the automated method or manual method.

In the automated method (anopheresis), the blood from the donor goes through the machine which is preset with the blood products we want, the blood products are extracted and then the blood goes back into the donor’s circulation.

In the manual method centrifuge blood and separate into separate bags ensuring a closed system to prevent infection.

1. Fresh frozen plasma (FFP) – This is used to correct bleeding disorders due to coagulation factor deficiencies (coagulopathies). There are hereditary causes (deficiency of F8 {haemophilia A} and deficiency of F9 {haemophilia B}) and acquired causes (deficiency of vitamin K, liver dx, kidney dx and DIC). Bleed into blood bag and centrifuge at high speed for a long time in order to ensure that all cellular products are tightly packed and then transfer into a transfer bag. Store frozen at -20˚c and this has a lifespan of 1 year.
2. Platelet concentrate, platelet rich plasma – Plt concentrate is used to treat bleeding disorders due to low plt count (below 100\*10/L) and thrombocytopenia. Bleed into triple bag and centrifuge. Lifespan of 5 days and store at room temperature in a platelet aggitator
3. Cryoprecipitate – It contains F8, vWF, F1 (fibrinogen) and F13. These are high molecular weight proteins and normally ppt once you thaw FFP at 4˚c for 24 hrs. Leave about 15-20ml of plasma to resuspend the ppt. Used for patients with classical haemophilia, von willebrand dx and hypofibrinogen. Store at -20˚c and this has a lifespan of 1 year.
4. Packed RBCs – Centrifuge whole blood. Get rid of plasma and buffy coal to get the RBCs. Note that the manual method doesn’t produce pure packed RBCs. This is used to treat chronic anaemia, increases red cell mass and is also used in sickle cell crisis. Store by refrigerating at 2-8˚c and preserved using SAGM (saline adenine glucose mannitol) for 42 days.

***BLOOD GROUPING***

*ABO*

The antigens are a and b – one can have either both, only one or no antigens.

To determine ABO you can use RBCs or serum.

We use commercially prepared antibodies for the test:

Antibody A – coloured blue, contains known antibody A and detects if cells have Ag a and causes agglutination if Ag a is present

Antibody B – coloured yellow, detects Ag b and causes agglutination if Ag b is present

Cell grouping is done using the tile method and test tube method

Serum grouping is done to determine ABO type

The standard cells are a, b and o. Add standard cells and test serum, mix, sit for 10 mins and check for agglutination. A cells will detect the A Ab and will agglutinate.

*RHESUS*

Divided into two i.e. based on presence or absence of Rh Ag.

The D antibody (clear) causes agglutination if the patient is rhesus positive.

Worldwide 15% are Rh- while 85% are Rh+

Du test – confirms absence of Rh Ag, also detects for weak D Ag (then grouped as Rh+)

Antigens in Rh blood group system are 5 i.e. D, C, c, E, e

D is major while the rest are minor

People who are D+ can either be homozygous or heterozygous

Rh- do not form Rh Ab unless exposed to Rh Ab eg an Rh- mother giving birth to a Rh+ baby gets fetal blood containing Ag during delivery

***COMPATIBILITY TESTING***

This is mandatory before transfusion

The purpose is to detect any Abs in patient’s plasma/serum against Ag in donor blood

*COMPONENTS/PHASES*

Phase 1 – SRT (saline at room temp) – put equal volume of patient plasma and donor cells (donor cells from the unit of donated blood) and incubate at room temp for 45 mins

Phase 2 – Saline at 37˚c (S37) – put equal volume of patient plasma and donor cells and incubate in water bath and maintain at 37˚c for 45 mins

Phase 3 – indirect coombs test (ICT) – add equal volumes of pt and donor cells and check for a reaction (agglutination) once time is up

No agglutination = no Abs thus the blood is compatible

NB:- Different temperatures are used because Abs react at different temperatures and in different media

Phase 1 – detect naturally occurring cold Abs

Phase 2 – detect naturally occurring warm Abs

Phase 1 and 2 detect ABO incompatibility or a and b Abs

Any reaction caused here is as a result of a clerical error

Phase 3 – detects immune IgG incompatibility or rhesus incompatibility

IgM vs IgG

-Best reacts at room temperature vs best reacts at cold temperature

-Pentamers vs monomer

-10 binding sites vs 2 binding sites

-High molecular weight protein vs low molecular weight protein

-Cannot cross placenta vs can cross placenta

-Direct agglutination vs indirect agglutination

*COOMBS TEST/ ANTIGLOBULIN TEST*

This is used to detect immune IgG Abs that do not cause direct agglutination

We sensitize cells to bind to active site on the RBCs but do not cause agglutination/ do not cause a visible reaction hence we use special media which bring out the agglutination e.g.

1. Antihuman globulin (AHG) – coombs reagent
2. Bovine serum albumin (BSA) – albumin technique
3. Enzyme papin – enzyme technique

Antiglobulin test – coombs test, indirect antiglobulin test (IAT)

ICT

Test sample serum (red vacutainer) – allow blood to clot then get the serum which has IgG Abs

Detects whether cells are sensitized in vitro

Use standard cells (5% cell suspension) (having the appropriate Ag) to react with the serum

Put equal volume of test serum and o+ cells then incubate in water bath at 37˚c for 45 mins

This will cause sensitization of the cells i.e. Ab-Ag complex but there is no visible reaction

Wash mixture using normal saline at 3000 revolutions for 3 mins 3 times – this gets rid of the excess proteins which are not bound to the cells which would otherwise neutralize the antihuman globulin hence causing a false negative.

Add 2 drops of AHG and spin at 1000 rev for 1 min

In cases of cross match use donor cells (not standard cells)

Place on stand

DIRECT COOMBS TEST/ DIRECT ANTIGLOBULIN TEST

This detects whether the cells are sensitized in vivo or are coated with a complement

The sample is whole blood (RBCs have the Ag)

Wash cells in normal saline (same as ICT) – spin 3000 rev for 3 mins 3 times then add 2 drops of AHG and spin at 1000 rev for 1 min

Check agglutination – if present then it is then DCT positive and if it isn’t then it is DCT negative

Clinical significance:-

1. Diagnosis of haemolysis disease of newborn, autoimmune haemolytic anaemia, drug induced haemolytic anaemia
2. Tx of haemolytic transfusion reaction

HAEMOLYTIC DX OF NEWBORN

Caused by ABO or Rh incompatibility

Rhogam – This is IgG anti-D given to the mother preventing her from forming Rh Abs after exposure to the Ag. It is given within 72 hrs after delivery of an Rh+ baby. Rhogam is also given at 28 and 34 week of gestation. This is 90% effective.

Q. The mother is B- and the baby delivered is jaundiced. Rule out or confirm haemolytic dx of newborn

Determine Rh status of baby

-Rh positive – indirect coombs test on mother (DCT on mother has no value) and direct coombs test on baby

-Rh negative – find another cause of jaundice