Supportive care in patients with acute leukaemia: historical perspectives

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Introduction

Although most treatments for leukaemia were ineffective until the middle of the 20th century, it seemed of interest to review some pertinent examples of the evolution of supportive care from its origin to its current use in this disease. Indeed, patients with leukaemia often have associated life-threatening disorders such as severe anaemia, bleeding or serious infections, and improvements in the management of these complications has totally transformed the outcome of the underlying disease. Modern therapy of acute leukaemia began in the 1960s. The major development in the treatment of acute leukaemia came from St. Jude Children’s Research Hospital in Memphis, with Don Pinkel introducing a four-phase treatment plan for acute lymphoblastic leukaemia, which he called "total therapy". The principles that were applied took into account the advantage of combination chemotherapy in overcoming initial drug resistance and inhibiting acquired resistance, as well as the superiority of some drugs for inducing remissions and others for maintaining the remissions. All available antileukaemic agents were incorporated into the plan, including vincristine and prednisone as induction therapy, 6-mercaptopurine and methotrexate for maintenance chemotherapy, and then radiotherapy to the central nervous system to prevent meningeal relapse. This schedule resulted in a tremendous increase in the survival rate of children with acute lymphoblastic leukaemia with more than 50% becoming long-term survivors.

Concomitantly to the development of more intensive chemotherapies, the need for supportive care increased. Leukaemia and its treatment can cause a number of complications and side effects. Patients receive supportive care to prevent or control these problems and to improve their comfort and quality of life during the leukaemia treatment. Advances in the control of infections and bleeding have been major factors in the improved outcome of leukaemia therapy. Blood transfusions are an important part of haematological care: transfusions of red blood cells may be used to reduce the symptoms of anaemia and platelet transfusions to reduce the risk of serious bleeding. Transfusions have long been associated with some risk to patients. Blood transfusion technology advanced rapidly during World War II, leading to national systems of blood banks.

Prior to 1954, blood transfusions were performed with rubber tubes and glass bottles. Progress in technologies and materials led to significant improvements. The development of plastic phlebotomy equipment has made the procedure of plasmapheresis feasible as an alternate form of blood donation. A platelet replacement programme initiated in children with leukaemia in 1958 demonstrated that platelets had an effect on haemorrhages and mortality.

In this brief historical review, we examine the major advances in the history of blood transfusion and the impact of transfusions on the treatment of acute leukaemia. Targeted more specifically at the young haematologist and the general educated reader, this review aims to communicate accounts of scientific and medical discovery and practice in this domain. It relies upon chronology as an organising framework, while stressing the importance of themes.

Blood transfusions

Blood transfusions represent one of the most important forms of supportive care for patients with leukaemia. Cancer is the major cause of transfusion. One third of transfused patients have a malignant disease, with acute leukaemia being the malignancy in a large part of them. More than 50% of transfused blood products are used in this setting. While the first case of blood transfusion to a patient with leukaemia was carried out in 1873, it was not until 1900 that the most important advance towards safe and effective transfusion took place with the discovery of the human blood groups. However, blood transfusion had a long history in medicine prior to the introduction of chemotherapy and the use of blood products as supportive care in the treatment of leukaemia.

First successful transfusions

After the first historical attempts at blood transfusion between animals or between animals and humans, the technique of transfusion was developed through the 19th century, with alternating periods of enthusiasm and periods of frustration and discouragement (Table I). Blood transfusions came back to the fore with the Franco-Prussian War at the end of the century and the identification of the first blood groups at the beginning of the 20th century.
Table I - Milestones in transfusion medicine from the first successful transfusions to the modern era.

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>1795</td>
<td>Philip Sying Physik performed the first known human blood transfusion.</td>
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<tr>
<td>1816</td>
<td>John Henry Leacock established the principle that donor and recipient must be of the same species.</td>
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<td>1818</td>
<td>James Blundell performed the first documented transfusion of human blood.</td>
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<td>1833</td>
<td>Alexander Wood invented the hypodermic syringe, leading to the development of new devices to carry out transfusions.</td>
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<tr>
<td>1845</td>
<td>Joseph Roussel first employed a direct arm-to-arm transfusion method.</td>
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<tr>
<td>1870s</td>
<td>Period of enthusiasm for transfusion of blood substitutes (milk, etc.), particularly in the USA.</td>
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<td>1870-71</td>
<td>Franco-Prussian War: use of blood transfusions on the battlefield.</td>
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<tr>
<td>1878</td>
<td>Georges Hayem perfected a saline solution, which can serve as a substitute of blood.</td>
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<tr>
<td>1901</td>
<td>Karl Landsteiner documented the first three blood groups.</td>
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<td>1907</td>
<td>Ludwig Hektoen: improvement in safety of transfusion by cross-matching blood between donors and patients.</td>
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<tr>
<td>1914-15</td>
<td>Albert Hustin, Luis Agote, Richard Lewisohn: use of sodium citrate that can be mixed to donor blood to prevent coagulation.</td>
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<td>1916</td>
<td>First blood transfusion using blood that has been stored and cooled after Francis Peyton Rous and J.R. Turner used a glucose additive to improve red cell preservation.</td>
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<td>1918</td>
<td>Oswald Robertson, a medical researcher and USA Army officer, established the first blood depot while serving during World War I.</td>
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<td>1925</td>
<td>Alexander Bogdanov founded the first academic institution devoted to the science of blood transfusion in Moscow.</td>
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<td>1927</td>
<td>Landsteiner and Levine proposed two new blood group systems: MN and P.</td>
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<td>1930</td>
<td>Charles R. Drew discovered that blood could be separated into plasma and red blood cells.</td>
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<td>1936</td>
<td>During the Spanish Civil War, the development of electrical refrigeration resulted in the first blood banks set up by Federico Duran Jorda in Barcelona and Norman Bethune in Madrid.</td>
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<td>1939-40</td>
<td>Karl Landsteiner, Alexander Wiener, Philip Levine, and R.E. Stetson discovered the Rhesus blood group system.</td>
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<td>1941</td>
<td>Isidor Radvil successfully used albumin in transfusions in victims of the Pearl Harbour attack.</td>
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<td>1942</td>
<td>John Freeman Loutit and Patrick L. Mollison introduced acid-citrate dextrose (ACD) solution, which reduces the volume of anticoagulant.</td>
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<td>1944</td>
<td>Use of dried plasma in the treatment of wounded soldiers during World War II.</td>
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<td>1944</td>
<td>Edwin Cohn developed cold ethanol fractionation, a process to break down plasma into its different fractions.</td>
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<td>1945</td>
<td>Robin Coombs, Arthur Mourant and Robert Race described the use of antihuman globulin to identify “incomplete” antibodies.</td>
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<td>1947</td>
<td>Bernard Fantus established the first hospital blood bank of the USA, in Chicago.</td>
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<td>1950</td>
<td>Carl Walter and W.P. Murphy introduced the plastic bag for blood collection.</td>
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<td>1952</td>
<td>Audrey Smith successfully froze red blood cells using glycerol cryoprotectant.</td>
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<td>1958</td>
<td>Jean Daussel discovered the first human leucocyte antigen (HLA) on the surface of blood cells.</td>
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<td>1960</td>
<td>Alan Salomon and John L. Fahey developed plasmapheresis, a procedure for separating whole blood into plasma and red blood cells.</td>
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<td>1969</td>
<td>Scott Murphy and Frank Gardner developed a method for storing platelets at room temperature.</td>
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<td>1971</td>
<td>The practice of testing donated blood for hepatitis B began.</td>
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<tr>
<td>1972</td>
<td>Apheresis, the process of separating out only plasma or one specific type of blood cell from donated blood, came into use.</td>
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<tr>
<td>1979</td>
<td>Introduction of CPDA-1, a new anticoagulant preservative, which increased the blood supply and facilitated resource-sharing among blood banks.</td>
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<td>1980s</td>
<td>Additive solutions extended the shelf-life of red blood cells to 49 days.</td>
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<tr>
<td>1980s</td>
<td>The practice of testing donated blood for HIV began.</td>
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<tr>
<td>1999</td>
<td>The use of nucleic acid amplification testing for active viruses in donated blood was introduced.</td>
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Identification of human blood types and groups

In the latter part of the 19th century, physiologists demonstrated that blood from one species could destroy the cells of transfused subjects of another species. Similarly, blood cells from different individuals of the same species could agglutinate when mixed. In 1901, Karl Landsteiner, an Austrian physician, documented the same species could agglutinate when mixed. In 1901, Karl Landsteiner, an Austrian physician, documented the first three blood groups, based on substances present on red blood cells, A, B and O. The following year, Alfred von Decastello and Adriano Sturli defined a fourth group, AB. Other blood types were described by Jansky in 1907 and by Moss in 1910. In 1927, Landsteiner and Levine proposed a new blood group system after the identification of two new genes, M and N. The system was later extended after Sanger and Race identified the related S and s genes in 1947. Additionally, in 1927, Landsteiner and Levine discovered the P blood group system. In 1907, Ludwig Hektoen suggested that the safety of transfusion might be improved by cross-matching blood between donors and patients to exclude incompatible mixtures. Reuben Ottenberg, who performed the first blood transfusion using blood typing and cross-matching at Mount Sinai Hospital in New York in 1907, also observed the "Mendelian inheritance" of blood groups and the "universal" utility of group O donors. Despite these advances, transfusions continued to be performed without any preliminary "cross-agglutination" testing.

In 1908, Alexis Carrel, a French surgeon, devised a way to prevent blood clotting. His method involved joining an artery in the donor, directly to a vein in the recipient with surgical sutures. The Kimpton-Brown transfusion apparatus, consisting of a paraffin-coated gradient glass cylinder with a horizontal side tube for suction, was commonly used from 1913 before using citrate. The practice of blood transfusion advanced with the outbreak of World War I. Pre-transfusion testing did not become normal practice before 1915 with popularisation of sodium citrate anticoagulation and collection of blood donors. Prior to this, transfusion was only possible using defibrinated blood and by direct donor-to-patient techniques. In 1939 and 1940, the Rhesus blood group system discovered by Karl Landsteiner, Alexander Wiener, Philip Levine, and R.E. Stetson was the most significant advance in blood grouping and transfusion and was found to be the main cause of transfusion reactions up to that time.

Development of blood banking

The main events in the development of blood banking are summarised in Table I. In the 1910s, it was discovered that by adding anticoagulant and refrigerating blood it was possible to store the blood for several days. Following the work of Francis Peyton Rous and J.R. Turner, the first blood transfusion using blood that had been stored and cooled was performed in 1916. The first blood depot was established in France during World War I. The Spanish Civil War also gave rise to a major initiative to increase the number of blood donors and to establish large-scale blood depots to ensure adequate supplies. This resulted in its introduction to civilian medical practice. The first hospital blood depots were established by Sergei Yudin in Russia in 1932, and by Bernard Fantus, who originated the term "Blood bank", in the USA in 1937. Within a few years, hospital and community blood banks were established across the USA and blood storage became widely practiced in the most important nations. The International Society of Blood Transfusion (ISBT) was founded in 1935. With the prospect of World War II, the War Office made the decision to determine the blood group of every member of HM Forces and issued all medical units with the equipment required for transfusion. The Army Blood Transfusion Service was set up, as was an equipment depot - the Army Blood Supply Depot. After World War II, it was realised that the structure, which had proven successful for blood collection, should be preserved, leading to the creation of National Blood Transfusion Services.

The modern era

Important developments in transfusion medicine have been achieved in the last 80 years. The main ones are summarised in Table I. Blood can be separated into plasma and red blood cells, and plasma can be frozen separately. Plasma can also be broken down into its different fractions: fibrinogen, gamma globulin, and albumin. This led to the production of novel blood products, including a durable substitute for liquid plasma. The development of plastics enabled the evolution of a collection system capable of safer and easier preparation of multiple blood components from a single unit of whole blood. In 1953, the development of the refrigerated centrifuge began to further expedite blood component therapy. The introduction of acid-citrate dextrose (ACD) solution reduced the volume of anticoagulant necessary and enabled transfusions of greater volumes and longer term storage. Additive solutions extended the shelf-life of red blood cells to 49 days. Technical progress by equipment manufacturers and the advent of computer-based technologies produced an avalanche of new equipment from the 1970s onwards, and many manual techniques and procedures became automated.

Up to the period just after World War II, virtually nothing could be done for patients with acute leukaemia. Nitrogen mustards had been introduced as a by-product of World War I but were not effective.
for the management of leukaemia. In the 1940s, greater understanding of the mechanisms of action of certain agents required for blood production, such as folic acid, led to the development of the first anti-folate drugs, notably amethopterin\textsuperscript{58}. This opened a new era for the management of acute leukaemia. Before that time, the only form of treatment had been blood transfusion\textsuperscript{39}. Without any treatment, patients with acute leukaemia died within a few weeks. When such patients were supported with only transfusions and antibiotics, they died in about 3 months\textsuperscript{40,41}. The first blood transfusion in a patient with leukaemia was carried out by George Callender in 1873 at St Bartholomew’s Hospital in London\textsuperscript{42}. Exchange blood transfusion was first attempted for the treatment of acute leukaemia in the middle of the 19th century, but the first complete blood exsanguination transfusion in a case of leukaemia was given by Jean Bernard and Marcel Bessis in 1947\textsuperscript{43}. The result was a temporary remission of a few months (remissions of acute leukaemia being almost unknown at that time). Work on purine and pyrimidine metabolism led to the discovery of another important agent, 6-mercaptopurine\textsuperscript{44}. Further developments followed rapidly, such as the discovery that corticosteroids had some effect in controlling some types of leukaemia, the discovery of derivatives of antibiotics, notably daunorubicin, and the discovery of the vinblastine and vincristine alkaloids derived from plants. There followed an active period of clinical trials using single or multiple agents in different combinations\textsuperscript{4}. The prognosis of leukaemia, particularly the childhood forms, progressively improved\textsuperscript{45}. Although progress was slower in adults, genuine advances were made\textsuperscript{46-48}. Intensification of therapy, enabled by prophylactic transfusion of platelets, was resulting in long-term survival. As a result of more intensive procedures, by the end of the 1970s, 20% of patients with acute myeloid leukaemia were beginning to live 3 to 5 years\textsuperscript{49}. In the mid-1970s bone marrow transplantation was introduced, and further dramatic advances were made\textsuperscript{50}.

Blood transfusions remain an important part of haematological care. Anaemia is an invariable consequence of acute leukaemia due to a reduction of erythropoiesis caused by the infiltration by leukaemic cells in the bone marrow and the suppressive effect of cytotoxic drug therapy. In one study, it was demonstrated that, on average, a patient suffering from acute myeloid leukaemia requires 41 units of red blood cells, 6 of fresh frozen plasma, 15 of single donor platelets, and 33 of random donor platelets\textsuperscript{51}. Based on these data, it could be estimated that the average blood transfusion support requires the generous gifts of about 100 blood donors per patient\textsuperscript{52}. During induction and consolidation, another study showed that patients received a median of 18 units (range, 3-44) of red blood cell concentrates\textsuperscript{53}. It is common practice to administer red blood cells based on a threshold haemoglobin value of 7-9 g/dL, depending on the patient’s ability to tolerate anaemia. Studies have evaluated restrictive\textsuperscript{44} and augmented\textsuperscript{55} red blood cell transfusion policies in leukaemia. Although the restrictive policy was well tolerated, no bleeding reduction was documented in the augmented policy. Transfusions are given at approximately weekly intervals during intensive therapy. One unit of red blood cells increases the haemoglobin level by approximately 1 g/dL.

**Platelet transfusions**

Platelet transfusions are indispensable for supportive care of patients with haematological diseases. Unlike red blood cells, the history of platelet transfusions has only developed over the past 40 years. The existence of platelets and their contribution to haemostasis was described in the 1870s\textsuperscript{56}. At first, whole blood was used to raise the platelet count in patients with thrombocytopenia. It was in 1910 that William W. Duke first noted that platelets from whole blood reduce the bleeding time and then evoked a potential role for platelet transfusion in therapy\textsuperscript{57}. In the 1950s, platelets were collected and transfused, but only for diagnostic purposes. During World War II, observations were made that plastic surfaces and silicone-coated surfaces preserved the morphology and function of platelets. In the 1960s plastic blood bags became available which enabled platelets to be separated from blood collections by centrifugation. In 1961, the role of platelet concentrates in reducing mortality from haemorrhage in cancer patients was recognised\textsuperscript{58}. Before the 1970s, thrombocytopenic bleeding was the major cause of serious morbidity and mortality in patients undergoing intensive chemotherapy.

Platelet transfusions became available following the vision of Isaac Djerassi in Philadelphia\textsuperscript{59,60} and of Emil J. Freireich in Bethesda\textsuperscript{61}. A platelet replacement programme initiated in children with leukaemia in 1958 showed that platelets had an effect on haemorrhage and mortality\textsuperscript{62-66}. It was demonstrated that platelets stored for longer than 48 hours produced very markedly decreased post-transfusion increments\textsuperscript{67} and that platelets could be concentrated under certain conditions at an appropriate pH and transfused as platelet concentrates\textsuperscript{68}. In the 1970s platelet transfusion became routine practice when Scott Murphy and Frank H. Gardner provided evidence that platelet function was best preserved when platelets were stored at room temperature with agitation, revolutionising platelet transfusion therapy\textsuperscript{69}. This resulted in the introduction of prophylactic platelet transfusions for patients with
leukaemia, and dramatically changed the causes of death among patients with this malignancy, such that haemorrhage as a major cause of death was virtually eliminated. Furthermore, the practice of prophylactic platelet transfusions enabled therapy intensification. The trigger platelet count of 10×10^9/L has been adopted while a threshold of 20×10^9/L is used for patients with bleeding, fever, infection, or a rapid fall in the platelet count. These recommendations do not apply to patients with acute promyelocytic leukaemia, since their bleeding risk and platelet transfusion requirements remain higher. Additional risk factors, such as sepsis, use of drugs, or other abnormalities of haemostasis, are indications for higher thresholds.

New methods for platelet transfusion were introduced in the 1980s using cytapheresis techniques, which led to an increase in platelet transfusion therapy to support myeloproliferative therapies including intensive chemotherapy and stem cell transplantation currently used in the treatment of leukaemia. This was refined in the 1990s with methods of collecting leucocyte-reduced platelet products. Leucocytes present in allogeneic blood components have a number of untoward side effects, such as HLA immunisation, non-haemolytic febrile transfusion reactions, virus transmission and infections. Currently, platelet transfusions are of low immunogenicity. Platelets have a shelf-life of up to 7 days, and even matched platelets can be routinely delivered. Further improvements can be expected from uniform "type & screen" approaches for immunised patients and cross-matching by computer.

Approximately 0.6×10^11 platelets can be harvested from a single unit of blood. Ten times this amount can be collected from one donor using a cell separator. Platelet refractoriness was identified in the 1960s as a major complication of chronic platelet transfusions and linked to complement-fixing isoantibodies. Refractoriness to platelet transfusions is most frequently due to the presence of alloantibodies in the recipient. Alloimmunisation is defined as occurring when an adequate platelet count increment (10 to 20×10^9/L) is not achieved 1 hour after transfusion. Anti-human leucocyte antigen (anti-HLA) alloimmunisation, which is associated with febrile reactions and refractoriness to random donor platelet support, is more frequent in female patients as a possible consequence of primary sensitisation during pregnancy. However, alloimmunisation to platelet antigens accounts for only approximately 20% of cases of refractoriness, and results from exposure to contaminating leucocytes in platelet products. This has been confirmed by controlled trials, particularly the Trial to Reduce Alloimmunization to Platelets (TRAP).

There are a number of methods used to select HLA-matched platelet products. Commonly recipient and donor are matched for HLA A and B antigens. A grading of the quality of matches has been defined. HLA A and B antigens can be organised into cross-reactive groups on the basis of which public epitopes they share. Platelets with one or two mismatches can be used as long as these antigens fall within the same cross-reactive groups. The antibody specificity prediction method can also identify the specificity of HLA antibody and antigen-negative platelet products. Recently, the software tool HLAMatchmaker has been used to predict HLA compatibility by identifying immunogenic epitopes represented by amino acid triplets in antibody-accessible regions of HLA molecules. Despite the lack of convincing evidence, provision of HLA-matched platelets for patients suspected or known to have alloimmune refractoriness remains a standard of care.

**Granulocyte transfusions**

Despite antibiotic therapy, severe neutropenia remains an important and severe complication of intensive chemotherapy and haematopoietic stem cell transplantation, and can be complicated by serious infections with bacteria, yeasts, or fungi. This led to the concept of granulocyte replacement by transfusion therapy. Granulocyte transfusions were first explored in 1934, but it was not until 1953 that the first promising experiments with leucocytes were reported. In the 1960s, enthusiasm for granulocyte transfusions was fuelled by the ability to collect granulocytes from patients with chronic myeloid leukaemia and the early success in patients with severe granulocytopenia. Cell collections yielded as many as 1×10^11 phagocytic cells and transfusion led to improvement in septic neutropenic patients. However, this practice was abandoned due to the unavailability of donors and concerns about transfusing malignant cells. Sufficient granulocytes could be obtained from normal donors after stimulation with corticosteroids and rapid leukaemapheresis. In the mid-1990s, the advent of recombinant granulocyte-mobilising cytokines (granulocyte colony-stimulating factor [G-CSF]), which recruit large numbers of granulocytes into the blood, the use of haemapheresis instruments and starches to increase the efficiency of the separation of red cells from granulocytes have been combined to make granulocyte transfusion feasible. Combining glucocorticoids and cytokines results in even greater efficacy. It was demonstrated that the neutrophils induced by G-CSF to circulate have a different transcriptional profile and a much longer life-span.
Despite technical problems, toxicity, and undefined therapeutic advantage, some controlled studies have shown benefit when using granulocyte transfusions, particularly when more than $1 \times 10^{10}$ cells were transfused\textsuperscript{105}. A meta-analysis of randomised controlled studies\textsuperscript{111-116} concluded that there were significant survival benefits in the following settings: low survival rate of controls, adequate dose of granulocytes, timely marrow recovery, and pre-transfusion assessment of compatibility of granulocytes\textsuperscript{117}. However, all the studies included in the meta-analysis had been conducted prior to the availability of new-generation antibiotics and anti-fungal drugs. Granulocyte therapy was not efficacious when buffy coats containing less than $0.5 \times 10^9$/kg granulocytes were transfused.

Apheresis procedures are performed with a special centrifuge\textsuperscript{118}. The leucocyte-containing layer is harvested to form the granulocyte concentrate, and red cells and plasma are returned to the donor. The ability of granulocytes to adhere to nylon in the presence of divalent cations was also initially exploited to separate the cells, but was later abandoned\textsuperscript{119}. The addition of a rouleaux-inducing sedimenting agent enhances the separation of red cells from white cells and increases yields as much as two-fold. The most common rouleaux-inducing agents employed are hydroxyethyl starches\textsuperscript{99-101}.

The benefit of granulocyte transfusions also depends on compatibility with any alloantibodies present and matching might have a role in selected conditions\textsuperscript{120}. The indications for granulocyte transfusion are not well defined. Patients may benefit from granulocyte transfusions when serious infection is present and if neutropenia is expected to last for more than several days. When possible, attempts should be made to match for leucocyte antigens and transfuse cytomegalovirus-negative, irradiated cells.

Despite more than 15 years of experience with G-CSF-stimulated granulocyte transfusions, it is still unclear whether this therapy really affects the resolution of infection and reduces mortality\textsuperscript{121,122}. Only one randomised phase III study of therapeutic granulocyte transfusions in neutropenic patients has been reported\textsuperscript{123}. However, the data presented were of no use for rejecting or proving the clinical value of granulocyte transfusions. Prophylactic granulocyte transfusions did not improve the outcome of patients\textsuperscript{124,125}. There were only reductions in the percentage of patients with fever, median number of febrile days, days on antibiotics and the percentage of patients with bacteraemia\textsuperscript{126}.

Granulocyte transfusions have also been used as secondary prophylaxis against fungal infections. In a study of patients given granulocyte transfusions, none of the patients included had reactivation of their previous infections\textsuperscript{127}, but voriconazole alone was equally successful\textsuperscript{128}. Furthermore, one major drawback to prophylactic granulocyte transfusion is the increased likelihood of transfusion reactions, alloimmunisation, lymphocytotoxic antibodies, and refractoriness to future transfusions\textsuperscript{129,130}. Severely immunosuppressed patients are at risk of serious complications, such as Graft-versus-Host disease\textsuperscript{131,132}. Granulocyte transfusions should be given in specific situations, according to well-established and standardised operational procedures for both the donor and the patient\textsuperscript{33}.

**Apheresis**

Apheresis refers to any procedure during which blood is withdrawn from the donor or patient and separated \textit{ex vivo} into some or all of its components\textsuperscript{134}. The first experimental procedure was performed in 1660 by Richard Lower on dogs. Plasmapheresis, corresponding to removal of plasma and return of red cells, was first performed in France in 1902 and used in artificial kidney research at Johns Hopkins University by Roundtree and Turner in 1914\textsuperscript{135}. In 1960, Alan Salomon and John L. Fahey reported the first therapeutic plasmapheresis procedure. In 1964, plasmapheresis was introduced as a means of collecting plasma for fractionation. In 1972, apheresis was used to extract one cellular component, returning the rest of the blood to the donor. In the 1980s, automated on-line cell separation devices were developed\textsuperscript{136}. Granulocytes, platelets, and fresh-frozen plasma were the original components collected by means of apheresis technology. Leucostasis, manifested by headache, blurred vision, dyspnoea and hypoxia, constitutes a medical emergency, and efforts should be made to lower the white blood cell count rapidly. This can be achieved by leukapheresis. Historically, leukapheresis has been used in this setting in patients with white blood cell counts $>100 \times 10^9$/L in those with acute myeloid leukaemia and $>100-200 \times 10^9$/L in those with acute lymphoblastic leukaemia and neurological and pulmonary symptoms suggestive of leucostasis\textsuperscript{137}. However, it remains controversial as to whether reducing circulating blasts by leukapheresis results in less mortality, and improvement in asymptomatic patients\textsuperscript{138,140}.

**Stem cell harvesting**

Recently, novel apheresis procedures have been developed for the collection of different combinations of blood components from healthy donors\textsuperscript{141}. Details on the methods of preparation and content of blood components can be found in the North American "Technical Manual" of the American Association of Blood Banks (AABB)\textsuperscript{42} and in the European "Guide to the preparation, use and quality assurance of blood components"\textsuperscript{91,142}.

Peripheral blood stem cell harvests

Peripheral blood stem cell transplantation was introduced in 1986 as a transplant modality, and has now replaced bone marrow as the source of stem cells in almost 100% of autologous transplants and approximately 75% of allogeneic transplants. Since the hypothesis in 1909 that haematopoietic stem cells circulate and subsequently the discovery of dividing non-leukaemic DNA-synthesising cells in peripheral blood, the demonstration that stem cells from shielded haematopoietic tissue areas entered the circulating blood and subsequently repopulated the irradiated bone marrow, the demonstration of haematopoietic reconstitution following myeloablative irradiation and parabiosis in rodents and cross-circulation experiments in large animals, the applications of haematopoietic stem cells in humans have emerged. The next step was to harvest circulating white blood cells and, among them, putative stem cells. "Blood stem cells" were then introduced as a transplantable cell population in the early 1960s. The first attempts at haematopoietic stem-cell transplantation into lethally irradiated large animals were not pursued at a clinical level because of the rarity of circulating stem cells at steady state. Improvements came from the development of the continuous-flow apheresis technology in the 1960s and the early 1970s.

Peripheral blood autologous stem cell harvests

The modern era of autologous blood stem cell transplantation began in the 1980s with the documentation that, using multiple leukaphereses, there were sufficient numbers of autologous haematopoietic stem cells in steady-state blood to ensure engraftment after myeloablative therapy. The autologous transplant modality also benefited from improvements regarding cryopreservation technologies. The first successful autologous stem cell transplant in a patient with chronic myeloid leukaemia was performed in 1979. After cell engraftment was demonstrated in a patient receiving granulocytes derived from a donor with chronic myeloid leukaemia. The first successful hematopoietic reconstitution after autologous blood stem cell transplantation was described in 1986. Initially autologous stem cells were used to shorten the period of pancytopenia following myeloablative chemotherapy.

Peripheral blood allogeneic stem cell harvests

The first mobilisation study on healthy donors was published in Japan in 1993. The first attempt to transplant non-mobilised T-cell-depleted peripheral blood stem cells into a HLA-identical sibling was performed in 1989. G-CSF-mobilised stem cells were used in this setting 4 years later. Allogeneic blood stem cell transplantation results in rapid, multi-lineage engraftment following successful mobilisation of donor stem cells with G-CSF. Nevertheless, early enthusiasm was offset by reports of chronic Graft-versus-Host disease, which occurred despite the low numbers of CD3+ cells present in the blood stem-cell suspension following CD34+ cell selection. Diminishing the Graft-versus-Host effect while retaining the graft-versus-leukaemia effect remains a challenge. Results of the first successful trials were published in 1995. Allogeneic blood cell collection and reinfusion (donor lymphocyte infusion) may be of benefit to bone marrow transplant patients with recurrent leukaemia. This form of immunotherapy uses the inherent immune reactivity of apheresis-derived allogeneic leukocytes from the original donor either to prevent recurrence or to re-induce remission. With strategies that manipulate the timing and the dose of T lymphocytes infused following transplantation, this therapy attempts to exploit the beneficial graft-versus-leukaemia effect while minimising the risks of Graft-versus-Host disease. More recently, the role of photopheresis in managing Graft-versus-Host disease after haematopoietic stem cell transplantation has been developed. Cord blood transplantation, an extension of peripheral blood stem cell harvesting from a neonate's cord blood, was first introduced in 1989. It has now become a widely used stem-cell transplantation option in children and in adults.

Non-mobilised stem cell harvests

Circulating haematopoietic stem cells have been harvested in steady-state by apheresis without specific mobilisation strategies. Engraftment times are comparable to those obtained after transplantation of bone marrow. However, apheresis-derived blood stem cells offer several benefits over marrow transplants. The disadvantages include a longer time to obtain an adequate harvest, a greater volume to infuse, and, in the autologous setting, the possibly greater risk of Graft-versus-Host disease.

Mobilised stem cell harvests

Mobilisation strategies have significantly improved the efficiency of peripheral blood stem cell collection by apheresis and have decreased the number of procedures necessary to collect target amounts of stem cells. Stem cells can be harvested during the recovery phase following myelosuppressive chemotherapy, a period associated with increased concentrations of circulating haematopoietic progenitor cells. Since their first descriptions in 1988, mobilisation regimens have incorporated cytokines, such as G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF).
in addition to chemotherapy, in order to increase progenitor cell concentrations over steady-state blood levels. Repeated daily collections over 2 to 5 days were used to reach an adequate threshold of stem cells. Follow-up studies showed a mobilisation advantage of G-CSF over GM-CSF. More recently, plerixafor, a small molecule antagonist which reversibly inhibits the interaction of the chemokine stromal cell-derived factor 1 with its cognate receptor, CXCR4, was used for stem-cell mobilisation. The first clinical study with plerixafor used for the mobilisation of haematopoietic progenitor cells in healthy donors was published in 2003, after studying the drug's pharmacokinetics in healthy volunteers. The combination of G-CSF and plerixafor has become the standard treatment regimen in difficult-to-mobilise patients after finding that the combination has a synergistic effect on the mobilisation of CD34+ cells. The number of circulating CD34+ cells on the day of leukapheresis is an accurate predictor of the optimal time of stem cell harvest. The adequacy of the collection is dependent on the mode of mobilisation, the timing of the collection with respect to the recovering leucocytes, the volume of whole blood processed, and the amount of pre-treatment the patient has received.

Infections transmitted by transfusion

Because patients with leukaemia develop infections very easily, they systematically receive antibiotics and other drugs to protect them from infections. All types of infections can occur; they may be present at diagnosis or arise during the treatment of the leukaemia and subsequently follow-up and can be caused by micro-organisms normally present in the environment or the host which become more pathogenic by reason of changes in the patient's defences. Transmission of infections by transfusion has also occurred. However, current preventive screening methods are becoming increasingly effective. Reliance on single-donor rather than pooled whole blood-derived platelets was proposed to reduce the risk of transfusion-associated sepsis.

Bacteria

Historically, there was concern about transmitting infectious diseases from a donor to a recipient. With regards to safe transfusions, the dangers of infection (both local and systemic) started to be resolved when, in 1865, Louis Pasteur (1822-1895) recognised that bacterial or fungal contamination causes putrefaction, and Joseph Lister (1827-1912), an English surgeon, used antiseptics in 1867 to control infection during blood transfusions. As a result, the sterilisation of instruments and antiseptic methods began to be introduced. During the Spanish Civil War, in an attempt to prevent the growth of anaerobic bacteria, pressurised air was forced into each bottle of preserved blood, converting 99% of the haemoglobin to oxyhaemoglobin. The earliest efforts to prevent a transmission-transmitted infection involved syphilis. The first identified cases of transmission of syphilis by direct transfusion were published after 1915. However, spirochaetes do not survive well in citrated blood stored for more than 72 hours. In 1947, syphilis testing was performed on each unit of blood. While bacterial contamination in re-used glass bottles was among the earliest causes of transfusion-related infections, the introduction of sterile plastic container systems and controlled refrigeration of blood components almost completely eliminated this problem by the 1960s. However, technical developments, including sterile docking devices, allowed prolonged storage of platelets at room temperature and bacterial contamination of platelets remained a significant issue complicating transfusions. Improved donor selection and special screening techniques have minimised the risk of this complication during the last decade.

Viruses

In 1943, Paul Beeson (1908-2006) linked the occurrence of jaundice in seven cases to blood or plasma transfusions, providing the quintessential description of transfusion-transmitted hepatitis. During the Korean War, 22% of those who were transfused developed hepatitis. “Serum hepatitis” transmitted by whole blood or plasma was presumed of viral origin. In 1965, Baruch Blumberg of the National Institutes of Health discovered Australia antigen (HbsAg) in aborigines and showed the presence of the antigen at high frequency in patients with leukaemia and children with Down's syndrome. In 1971, Blumberg identified a substance on the surface of the hepatitis B virus that triggers the production of antibodies. This led to the development of a test identifying infected donors. In the 1980s, investigators from the Centers for Disease Control and Chiron (Emeryville, California) identified the virus of hepatitis C. In 1990, blood banks began screening blood donors for hepatitis C, but it was not until 1992 that a blood test was perfected that effectively eliminated the virus from the blood transfusion supply. In 1981, the first cases of acquired immune deficiency syndrome (AIDS), a syndrome initially called gay-related immunodeficiency disease (GRID), were reported. In 1982, Bruce Evatt suspected that AIDS was blood-borne after the discovery of the disease among haemophiliacs. The virus that causes AIDS was isolated as LAV (lymphadenopathy-associated virus) in 1983 by Luc Montagnier at the Pasteur Institute in Paris (who was awarded the Nobel Prize in Physiology or Medicine in 2008), while Robert Gallo from the National Institutes...
of Health announced its identification in 1984 as HTLV-III (human T-cell lymphotropic virus). A legal battle ensued over who should be credited for the discovery, which ended in 1987 when the United States and French governments agreed to share credit and royalties from the sales of test kits for the virus. The first screening test (an enzyme-linked immunosorbert assay) was licensed in 1985. The human immunodeficiency virus (HIV) epidemic during the 1980s was a major cause of fear, after the suspicion that AIDS could be transmitted by means of transfusion was confirmed. The blood bank community has been chastised for its perceived failure to act during the early years of the AIDS epidemic, and many lawsuits were brought for being late to introduce a surrogate marker for HIV and to introduce inactivation measures for clotting factor concentrates.

One positive outcome of that outbreak tragedy was a new paradigm in blood transfusion, the precautionary principle, which prompted the development of haemovigilance, tracing and tracking transfusion-related adverse events and incidents affecting blood donors and recipients. Since then a series of more sensitive tests have been developed and implemented to screen donated blood for viral diseases: two tests that screen for indirect evidence of hepatitis, i.e. detection of antibodies to hepatitis B core antigen (anti-HBc) and assays of alanine aminotransferase levels (1987); the human T-lymphotropic-virus-I-antibody (anti-HTLV-I) test (1989); the hepatitis C virus test (1990); the HIV-1 and HIV-2 antibodies test (1992), the HIV p24 antigen test (1996); and nucleic acid amplification testing, which directly detects the genetic material of viruses such as HCV and HIV (1999). These developments have greatly increased the safety of blood transfusion. New methods, including novel molecular and micro-array testing, which can identify many infectious agents, will be developed in the near future.

**Prions**

Creutzfeldt-Jacob disease is not known to have been transmitted by transfusion. In 1996, variant Creutzfeldt-Jacob disease, caused by the same strain of prion as bovine spongiform encephalopathy, was identified. The first possible transmission of variant Creutzfeldt-Jacob disease by blood transfusion was reported in 1998. Since then, transfusion-transmitted cases have been traced to donors who became symptomatic with variant Creutzfeldt-Jacob disease 3 or more years after the index donation. Because of the long, asymptomatic carrier state and the strong resistance of prions to inactivation procedures, the primary intervention to prevent transfusion-transmitted cases of variant Creutzfeldt-Jacob at present is to defer indefinitely any potential who have a history of visiting bovine spongiform encephalopathy-affected countries, particularly Great Britain, during the years of likely exposure. Universal white blood cell reduction was introduced in Europe in an attempt to prevent transmission of variant Creutzfeldt-Jakob disease by transfusion.

**Other risks of transfusion**

Transfusion-related risks other than infectious diseases should be taken into account, such as post-transfusion reactions. These reactions include transfusion-related lung injury (TRALI) during which the donor's immune antibodies cause breathing problems in the recipient. This condition was first described in 1957, but its specific clinical and radiographic findings were defined in the 1980s. TRALI can be due to white blood cell antibodies, soluble biological response mediators accumulating during the storage of cellular blood components, or other still unidentified agents contained in blood components. Other reactions include transfusion-associated cardiac overload (TACO), and post-transfusion iron overload which is a build-up of iron in the body usually caused by multiple or regular transfusions. Alloimmunisation occurs when the recipient develops a reaction to the donor's red blood cells. This includes transfusion-related immune modulation (TRIM). TRIM effects may be mediated by allogeneic mononuclear cells, white blood cell-derived soluble mediators, and/or soluble HLA peptides circulating in allogeneic plasma. Another complication attributable to leucocytes is a febrile reaction. Passage of blood through a filter containing nylon fibres was introduced in 1962 to prevent such reactions. Modifying the blood before transfusion can also reduce these reactions. Using male plasma and platelets may eliminate the transmission of certain antibodies, which can be found in previously pregnant women and transfused males. Alternatively, new technologies can be used to collect large amounts of red blood cells and platelets from a single donor, decreasing the number of donors to whom a transfused patient is exposed. Reduction of inappropriate transfusions is another, and perhaps better, approach to reducing donor exposure.

**Conclusion**

Transfusion medicine already has a long history and it is continuing to advance into new areas which could potentially have an impact on the treatment of acute leukaemia. The clinical promise of cellular therapies is strengthening rapidly. The field of hematopoietic stem cell transplantation is shifting from cell replacement therapy to adoptive cellular therapy. Lymphocytes collected from haematopoietic stem cell donors have been infused as an adoptive cellular therapy to treat leukaemia.
relapses following allogeneic transplantation. New techniques can isolate specific cell populations from blood. Most importantly, haematopoietic progenitor cells can be isolated and used for haematopoietic stem cell transplantation. In the future, these cells may be grown and expanded for clinical use. Similarly, specialised cell types may be isolated and used in cellular therapy because of their action on proliferation and immune responses during the engraftment of transplanted cells. Epstein-Barr virus-specific cytotoxic T cells, generated in vitro, can mediate antiviral and even antitumour effects in vivo. Cytotoxic lymphocytes of other specificities are also under evaluation. T cells specific to leukaemia antigens such as Wilms' tumour 1 (WT1) and proteinase 3 (PR3) are under investigation to prevent or treat leukaemia relapse following allogeneic stem cell transplantation. Recently, vaccination has been able to induce T-cell responses against cancer-associated antigens. This offers the prospect of combining vaccine strategies with adoptive transfer of specific T cells to achieve optimal T-cell expansion and therapeutic benefit. This offers the prospect of combining vaccine strategies with adoptive transfer of specific T cells to achieve optimal T-cell expansion and therapeutic benefit. Adoptive cellular therapy protocols have also begun to use natural killer (NK) cells. NK cells from the stem cell transplant donors are being administering post-transplantation. The allogeneic NK cells are administered to the recipient at the time of disease relapse. The recipient is immunosuppressed and treated with interleukin-2 to allow in vivo NK cell expansion. This NK cell therapy has resulted in complete remission in patients with acute myeloid leukaemia. Dendritic cells generated by incubating peripheral blood monocytes with the differentiating agents interleukin-4 and GM-CSF to produce immature dendritic cells have been used in clinical trials, but for most trials they have been incubated with maturation agents to produce mature dendritic cells. However, many trials are now using genetically engineered dendritic cells to epitope-load HLA antigens. Another promising use of genetically engineered cells involves arming autologous T cells with T-cell receptors that have a high affinity for cancer antigens, expanding these genetically modified T cells in vitro and infusing them into patients. Chimeric antigen T-cell receptors (CAR) are also being used in adoptive cell therapy. One CAR that has been tested clinically is made up of the antigen recognition portion of CD19, the zeta chain of the T-cell receptor and a portion of the co-stimulatory molecule CD28. Autologous T cells transduced with anti-CD19 CAR are cytolytic to malignant B cells that express CD19. Although clinical trials of these genetically engineered T cells are just beginning, preliminary results have been encouraging. Investigators are working on cell-reprogramming strategies to produce naive and stem T cells for adoptive cellular therapy. Recently, gas-permeable flasks have been used to expand cytotoxic T cells to a much higher concentration than possible with bags or traditional flasks. The progress in adoptive cellular therapy in leukaemia parallels the rapid evolution of immunology, gene therapy and stem cell biology, and the translation of advances in these fields from research to the clinic.

**Keywords:** history, leukaemia, transfusion, supportive care.

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