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Editors

Blood Substitutes and Oxygen Biotherapeutics

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To my wife Dr. Kim Kaye for being the best spouse a man could ask for in his life.
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Foreword

Blood Substitutes and Oxygen Biotherapeutics

Is the Final Destination in the Long, Winding, Bumpy Road Finally in Sight?

It is a tremendous honor to be invited to offer some introductory comments to this comprehensive treatise on blood substitutes and oxygen biotherapeutics. I have interacted in the past with many of the chapter authors, and have studied with admiration the offerings of many of the distinguished colleagues who have endeavored with admirable persistence in this important but slowly and erratically developing field. The editors have been diligent in selecting experts who have pursued the ambitious goal of developing new therapies, and the result is a review of where the field has been, moving to discussions of where the opportunities to achieve licensed therapeutic products will need to proceed. Most of us are familiar with the early encouraging work in the field, perhaps epitomized by the often-featured view of a rodent in a beaker filled with a blood substitute that sustained its life. With retrospective naivety, it might have appeared to the readers and probably to me that therapeutic products would become available in a short interval. The many offerings in this book demonstrate our unrequited optimism but hopefully the synthesis demonstrates that the goal remains cogent and that our endeavors have progressed with knowledge and experience gained along the treacherous path.

The editors have provided extensive coverage to blood substitutes in a logical progression through five sections. The first reviews transfusion science and describes the development of transfusion therapies based upon the physiology of blood and in particular hemoglobin. It discusses evolving transfusion therapies with emphasis upon where blood substitutes might meet a continuing unmet medical need. An important chapter reviews the role of nitric oxide, an area of red cell physiology largely unknown during the early stages of blood substitute development, but now recognized to be an impediment to satisfactory clinical products if its involvement in vasoactivity is not addressed.

The second section provides background on pharmacology of oxygen therapeutic through iterations such as perfluorocarbons and hemoglobin-based oxygen carriers. It reviews previous attempts to limit the recognized toxicities of these products through maneuvers such as cross-linking, conjugation, polymerization, and encapsulation, with limited success in addressing the issues of vasoactivity. Although these product modifications supported early clinical trials, they helped to focus on the ultimate design of a potentially successful product, which has enabled our growing knowledge of molecular biology to add more specific compounds to hemoglobin, which will enable oxygen delivery, reduce vasoactivity, and reduce the inflammation that often accompanies the clinical problems being addressed by these agents. The third section builds upon this experience base to describe products in current and past development, building upon the interpretation of the documented failures of these early products. These developing therapies include further manipulation of hemoglobin, novel additives that should increase the efficacy of these products, and strategies to develop therapeutic agents that might include artificial red cells or oxygen carriers from untraditional sources.

Section four describes a number of proposed products in various stages of development. Many of these formulations are now of historical interest, seemingly attractive approaches at

previous times that failed in operational development or more extensive clinical trials. These presentations all emphasize "lessons learned," which will hopefully lead to successful products in the future. Some of these products with substantial persistence have already undergone extensive trial experience and clinical use with evidence of benefit but remain unapproved due to high regulatory hurdles. Others have not yet been definitively tested or failed to be provide approvable results despite early promise in preliminary studies. The fifth and final section discusses specific indications for future studies and highlights the regulatory requirements they must address to achieve clinical availability and commercialization. Proposals are provided that suggest that the goal of these therapies is not to replace red cell transfusions but treat severe anemia cases where transfusion might not be the best option

The offerings of this treatise provide a comprehensive history of previous attempts and failures for blood substitute development and suggest some new ways of thinking that might be helpful to achieve the ultimate goal. There has been considerable discussion about the indications for blood substitutes. In consideration of how these products could be used, the obvious issue is to balance the clinical benefits versus the recognized toxicities. In the early 1980s, blood substitutes generated widespread public enthusiasm as a means to avoid the known infectious risks of AIDS and hepatitis. Regulatory agencies applied understandable caution for approval for this indication, as other means to avoid these transfusion complications became available. It was also appreciated that the perceived benefits were not justified by the toxicities of vasoconstriction and the clearly recognized shortcoming of blood substitutes with transient in vivo survival. On the other hand, most observers now recognize that red cells do not address all medical needs in some instances of acute anemia and that blood substitutes could be lifesaving for patients in whom blood is not available or the safest option. In these cases, the potential benefits would justify the acceptance of some potential adverse effects. Specific examples would include religious objectors who refuse red cell transfusions but often accept blood substitutes as a matter of conscience. Another cadre of patients who would benefit are those who have antibodies due to alloimmunization or autoimmune hemolytic anemia where compatible red cells are difficult to find so that a blood substitute can provide a bridge until the immunohematologic difficulties have been addressed. Other indications were not foreseen by early investigators. A transfusion complication called hyperhemolysis has now been identified, where transfused patients begin to hemolyze the transfused cells and their own autologous red cells and frequently develop profound anemia. This condition can complicate management of patients with sickle cell anemia but has been described with other diagnoses. Continued red cell transfusions in these cases are ineffective and futile, suggesting a temporizing therapy with a blood substitute could be a critical stabilizing therapy. For these indications for patients with severe anemia, it is now appreciated that clinical trials with randomization and blinding are difficult to develop and have ethical challenges. It is also becoming clear that the study target and the ultimate goal of therapy are not red cell transfusion replacement or avoidance but preventing the ravages of life-threatening severe anemia.

Patients with severe hemorrhage in military or civilian settings have long been a prime candidate for blood substitute implementation. Much of the clinical investigation with blood substitutes has targeted trauma, in part due to the size of the potential market. Clinical trials in this area have been difficult, however, because of the distant settings for the military and many civilian cases with limited access to the necessary measures in these complicated studies. In a large trial of blood substitutes in civilians, the transit time to acute trauma centers was too short to allow sufficient infusions of blood substitutes. In recent studies of plasma infusions in trauma settings, it is interesting that benefit was shown in patients with long transport times without benefit in a centralized trauma program with short transit times; studies of blood substitutes in patients with long transit times might have shown different outcomes but are difficult to perform. Another complication of trauma studies is the evolution of therapy that is occurring while trials of blood substitutes are performed or contemplated. Early approaches to trauma emphasized fluid resuscitation at the site of injury where the patient is stabilized prior to transport. Current programs of damage control resuscitation avoid fluids and tolerate hypotension,

with rapid transport to a trauma center to avoid "popping the clot." The role of blood substitutes in evolving trauma care is the subject of current debate. Although the potential patient population is large and the risk of mortality in these situations is high, it remains a difficult group to study in a controlled clinical trial suitable for regulatory approval.

The editors are to be congratulated for amassing this review of the long and arduous road that has yet to provide a licensed blood substitute in the United States. It is anticipated that even skeptics who disparage the need for these therapies will recognize that toxicities can be overcome, and a small group of patients with currently accepted indications will benefit in the near future. It is also possible that continued development may expand the utility of biotherapeutics to other patient groups such as solid organ transplant recipients and patients with uncontrolled inflammatory states. This book, recognizing the failures of the past but providing insights into paths to move forward, is an important contribution to the impetus to continue this important work.

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Part I

Transfusion: Science and Practice

Erythrocyte Transfusion: Brief History and Current Practice

1

George P. Biro

Make thick my blood…

Shakespeare: Macbeth, Act 1, scene 5.

Introduction

Since ancient times blood has been thought to possess mystical and healing properties and the notion of changing personal characteristics, by means of a transfusion of blood from another species or person with desirable attributes, has been attempted perhaps many times in the sixteenth century. In the seventeenth century a better appreciation of the circulation of blood and that blood loss from hemorrhage could be reversed by a transfusion. William Harvey's revolutionary experiments and the publication of "*Exercitatio Anatomica De Motu Cordis"* in Frankfurt in 1628 introduced the concept of experimentation and direct observation initiating the scientific approach to medicine. Animal-to-human and human-to-human transfusions followed, the first in 1666 and 1818, respectively. Not all these attempts were successful. A French physician and naturalist was tried for murder after some unsuccessful animal-to-human transfusion attempts. Subsequently, transfusion attempts were prohibited in both England and France [1–3]. In much of the nineteenth century blood transfusion was not accepted as a safe medical procedure, except for the work of James Blundell, a prominent London obstetrician, who recognized that certain circumstances necessitated human transfusions. He developed devices for collecting and administering blood to treat obstetrical hemorrhage and established a donor base. More widespread use of transfusions was hindered by a multitude of "technical" barriers, the absence of methods of sterilizing devices, of appropriate anticoagulation and preservative media. Despite the carnage of the American Civil War and European wars in the second half of the nineteenth century, the use transfusions was insignificant. The introduction of saline infusion in 1884 improved the treatment of hemorrhage and dehydration [1].

The use of transfusions in the early decades of the twentieth century were helped by the discovery of the major blood groups. The outbreak of World War I did not see extensive use of transfusions. With the outbreak of the World war II, transfusions of blood and of plasma and albumin became strategic endeavors [4, 5]. Soldiers in the German SS had their blood group tattooed in their armpits but battlefield transfusions were rare.

The approach taken in this chapter is not a conventional chronological narrative of the history. Rather, it will highlight milestones of the surgical and critical care use of erythrocyte transfusions only and will refer to those as "transfusion". Blood products and components and the technological aspects of blood banking will not be included. The overriding theme in this chapter is dealing with blood as a *scarce and expensive resource* that is handled with a view to *risk management, whereby expected benefits and hazards are balanced.* It must be emphasized that compelling evidence by clinical trials of the benefit of transfusion against its known risks was not available.

Milestones in Erythrocyte Transfusion

Karl Landsteiner and Discovery of Major Blood Groups

The "coming of age" of blood transfusion began with the revolutionary contribution of the Austrian-born, American physician and immunologist, Karl Landsteiner.

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The Early Years of Transfusion: The First Milestone

Karl Landsteiner

Karl Landsteiner (1868–1943) is celebrated for his landmark discovery of the ABO blood groups in 1901 and, together with Alexander S. Wiener, for the discovery of the Rhesus factor in 1937. Landsteiner received the Nobel Prize in Medicine and Physiology in 1930 [6–8] (Fig. 1.1).

These discoveries have made it possible to infuse another person's blood to someone in great need of it. The ABO blood group system was discovered by Landsteiner by testing samples of erythrocytes with the addition of samples of serum from other individuals, using the methods of immunology in which he had been trained. Some serum samples caused the blood cells to clump, or agglutinate, while others did not. By repeated testing, he intuited that there must have been some element, an *antibody* in some serum samples that reacted with an *antigen* on the surface of red blood cells, causing agglutination; whereas the serum of others contained a different antibody that did not react with cells from the same person. And that a person's blood contained the same *type* of antigen on the red cells as the antibody in their serum. He categorized these blood types as A, B and C. Erythrocytes of type A would be agglutinated when mixed with serum from a person having type B antibodies in their serum, but not when mixed with serum from a type A person. Erythrocytes from a person with Type A antigen, when mixed with serum from another person with type A erythrocytes, did not agglutinate. A third type, that he named type C, erythrocytes of a person of either type A or type B, were not agglutinated when mixed with serum from Type B or type A person, either. This third, type C, had neither type A nor type B antibody in their sera. Such a person could receive a blood transfusion from either a Type A or Type B donor. The type C was later renamed Type O (or zero, for the original German word "*Ohne*", without).

Fig. 1.1 Portrait of Karl Landsteiner on an Austrian Postal Service commemorative stamp issued on the 100th anniversary of his birth

Ironically, the revolutionary observation was first reported by Landsteiner in a *footnote* in a paper (1900) on pathologic anatomy, describing the agglutination occurring when blood of one person is in contact with that of another person [7, 9]. The actual description of the discovery of the ABC blood groups was published a year later, in 1901. Landsteiner, at first, did not appreciate the importance of his discovery, writing that "I hope that this will be of some use to mankind" [7].

In 1922 he accepted the invitation by Simon Flexner to join the staff of the Rockefeller Institute where he continued to make major discoveries [8].

The discovery of the Rh, or rhesus factor came about from the case described by Bodner and McKie [10]. The obstetrical patient's physician was Dr. Philip Levine who had been an assistant to Landsteiner for several years.

The patient had a first normal pregnancy, but her second pregnancy ended in the loss of her baby and she suffered a massive hemorrhage. Since both her and her husband had Type O blood, Dr. Levine decided to transfuse her from the husband. To his dismay, she had a violent transfusion reaction. Dr. Levine reasoned that there must have been an alternative blood group type antibody involved in the reaction. It turned out that when the patient's serum was tested against her husband's erythrocytes, agglutination occurred. Moreover, the loss of the baby was due an antigen antibody reaction. The mother's antibody had leaked across the placenta and entered the fetal circulation and caused massive lysis of the fetal erythrocytes which were of a different type inherited from the father. This single case was reported by Philip Levine and Rufus Stetson in 1939 in the Journal of the American Medical Association. They noted the similarity of this first detected case with the then few reported cases of iso-immunization after repeated transfusions [11].

Since the mother's serum caused agglutination of erythrocytes of rhesus monkey, and those of other animal species' erythrocytes, the antibody became known as the rhesus, or Rh factor, subsequently renamed type D antibody. A D-negative mother having a D-positive fetus in her first pregnancy has not yet developed antibodies to the fetus' antigens but will do so when the D-positive fetal

cells leak across the placental barrier during delivery. Subsequent pregnancies may be complicated by the mother's anti-D^+ antibodies entering the fetal circulation. The consequences of the presence of anti- D^+ antibody in the mother and its absence in the fetus, the intrauterine hemolysis, became known as the Hemolytic Disease of the Fetus and Newborn (HDFN) [8].

Landsteiner's many contributions have involved the detection of similar patterns of reactions with rhesus blood. In 1940 he and Wiener immunized rabbits and guinea pigs with erythrocytes of rhesus monkeys. This anti- rhesus (anti-Rh) reacted with 85% of human erythrocytes, indicating the frequency of Rh+ phenotype. It is now known that the type D appellation involves many other different agglutinin subtypes detected by cross matching and phenotyping. After the original discovery of the major blood groups, Landsteiner and coworkers and many followers discovered at least 36 other systems of minor subgroup types with weaker isoreactions [8].

In addition to these important discoveries, Landsteiner also made many others, including the recognition of the viral origin of poliomyelitis, and the diagnostic test for paroxysmal cold hemoglobinuria [6, 7].

Ottenberg (1882–1959) was the first to perform the earliest form of a pretransfusion cross match in 1907, recognizing the clinical significance of avoidance of hemolytic transfusion reactions. This rigorous typing and cross matching have contributed greatly to the safety of early transfusions, however, transfusions remained cumbersome and little used, because of the lack of adequate anticoagulation and storage methods, so that most transfusions were direct donor-to-recipient.

The history of the development of anticoagulant and storage technologies, as well as of those of blood banks, donor bases, and of the introduction of component separation is beyond the scope of this chapter.

The next, second, milestone in this narrative is the recognition that parallel to the risks of anemia, transfusion's benefits may have to be balanced by the recognition that risks are also inherent in transfusions.

Balancing the Risks and Benefits of Anemia and Transfusion: The Second Milestone

The immunologic investigation of blood group types and antigens accelerated in the 1940s as testing technologies improved and became more routine in blood banks. As a result, the use of transfusions of whole blood, and then that of red cell units and components, accelerated, both in cases of acute blood loss (surgery and trauma), and in "chronic "cases (postoperative anemia and in "medical anemia", such as in malignant disease).

With increasing use and availability of blood for transfusion, the prescribing of transfusion became a more common medical treatment where the decision was based on the *expectation of a benefit to the patient* by increasing oxygen carrying capacity and transport. However, there was little objective evidence supporting the expected benefit, especially in the case of single-unit transfusions, that generally result only in a 10 g/L[1] increase in Hb concentration.

Since transfusions had long been in use when the use of clinical trials of establishing efficacy and safety was introduced, transfusion of blood was not subjected to rigorous trials evaluating its efficacy. One of the few medical interventions that remains without rigorous safety and efficacy testing by clinical trials. More recently questions have been raised about when a transfusion is appropriate and the notion of *balancing risks and benefits* of both *the transfusion and of anemia* has become a dominant consideration, but without evidence-based support. The balance is not simple because the transfusion is expected to provide a medical benefit, BUT there are no benefits of severe anemia. On the other hand, both have risks.

The Risks of Anemia

There are no known benefits of severe anemia; its risks need to be considered first.

In an anemic subject oxygen delivery may be impaired, depending on its severity, to an extent that physiological functions may deteriorate, activity may be limited, and organ dysfunction may supervene. This may be explained by the concept of supply dependence when the supply is so limited that a substantial mass of body cells are hypoxic and oxygen consumption falls [12]. There are occasional instances observed when an individual may survive such low hemoglobin concentration[2] as 10-20 g/L. However, retrospective aggregated data from Jehovah's Witnesses who refuse transfusion on religious grounds, reveal how dangerous severe anemia is. At persistent hemoglobin concentration of 11 g/L in-hospital mortality was 100% at 30 days. For every 10 g/L reduction of hemoglobin concentration from 50 g/L, the probability of adverse outcomes, such as myocardial infarction, respiratory and renal failure, etc., doubled [13–15].

Thus, the threat to life represented by severe anemia in compromising oxygen delivery was thought to mandate medical intervention that intended to *prevent,* if possible, such hypoxia (e.g., a case of continuing blood loss). If prevention is not feasible, *amelioration* is required as soon as possible. Thus, a transfusion would be prescribed, in the absence other effective interventions. The expectation of benefit would only be tempered by the then recognized dan-

[1]The international unit of g/L will be used throughout.

[2]Hemoglobin will be abbreviated as Hb; its concentration is abbreviated as [Hb].

ger of *transfusion reactions* (see below). This desired goal is hampered by the lack of objectively definable, universally applicable *thresholds* to facilitate a *rational c*linical decision [12, 16].

The Target Organs of Anemia-Induced Injury

The organs most vulnerable to hypoxia are those of obligate aerobic metabolism, the *brain* and *heart*. Healthy volunteers, subjected to isovolumic hemodilution to a hemoglobin concentration of 50 g/L, exhibited reversible cognitive and memory impairment that was improved by oxygen breathing, indicating the mechanism to be hypoxia [17, 18]. Clinical studies have identified cerebral injury in anemic perioperative patients [16, 19]. Jehovah's Witness patients who refuse transfusion even in the face of severe anemia and/or continuing blood loss (hemoglobin concentration < 80 g/L) have been found in an 11-year review to suffer all-cause mortality rate of 19.8%, and at a hemoglobin concentration ([Hb]) <50 g/L, are very likely to die [15]. These findings strongly suggest that in patients who may have underlying coronary artery disease, severe anemia represents a real threat. In view of the belief that severe anemia is a threat, transfusion had been used in the *expectation of benefit and avoidance of harm.*

An excellent experimental study on rats has shown that anemia induced tissue hypoxia occurs at different levels of [Hb] in different vital organs [20]. The study subjected rats to isovolemic hemodilution to [Hb] concentrations of 90 g/L, or 70 g/L, or 50 g/L and was compared to the baseline of 130 g/L. Tissue hypoxia was indicated by increases in HIF-1α-luciferase[3] activity and NOS[4] expression. Whole body HIF activity increased progressively as the [Hb] was decreased, indicating the presence of tissue hypoxia somewhere in the body even at [Hb] of 90 g/L. In the kidney HIF activity was like baseline at [HB] both at 90 and 70 g/L but became significantly increased at [Hb] = 50 g/L., suggesting a relative degree of tolerance of modest hypoxia. In contrast, the liver exhibited increased HIF expression at [Hb] = 70 g/L, suggesting a higher threshold of hypoxia.

The next, third, milestone in this narrative is the recognition that parallel to the risks of anemia, transfusion's benefits may have to be balanced by the recognition that substantial risks also attend transfusions.

Benefits and Risks of Erythrocyte Transfusion

The Benefits of Transfusion

How do transfusions benefit a patient facing the risks of anemia?

Transfusion is intended to prevent or ameliorate the signs and symptoms of anemia of significant severity that interferes with the supply of oxygen sufficient to the physiological demands of effective functioning. The "physiological benefits" of a two-unit transfusion were described in a study on ICU patients undergoing invasive hemodynamic monitoring [21]. The transfusion's effects included a rise in hematocrit ratio, from 0.22 ± 0.2, to 0.28 ± 0.03, and [Hb], from 76 ± 8 to 94 ± 9 g/L. It is not clear whether the average pre-transfusion [Hb] of 76 g/L would be associated with the need for increased oxygen capacity to ameliorate critical organ hypoxia. There was also significant improvement in hemodynamic variables and oxygen flux and a reduction in the heart rate. However, it is not clear, whether the documented improvements represented a physiologically significant degree of tissue hypoxia or, whether an improvement in blood volume also contributed. The study did not provide definitive evidence of *efficacy*.

A related aspect of transfusion's efficacy is *the timing* of the benefit. Banked erythrocyte units are well documented to have properties different from those of native erythrocyte: the well-known phenomenon of the "*storage lesion*" [22]. This consist of changed biomechanical properties of the erythrocyte that significantly impair perfusion in the microcirculation [23–25]. Animal experiments have shown that the impaired biomechanics of stored erythrocytes' adherence to capillary walls and rigidity represent impaired flow and clinical risk [23, 24, 26]. Finally, the breakdown of cells and the release of their fragments and hemoglobin interfere with NO-mediated vasodilator regulation [22]. These effects are reversible within about 24 hours and the transfused erythrocytes become functional, but their circulating half-life is shortened.

Effectively demonstrating the benefits of transfusion in individual cases is also subject to uncertainties. Not every patient with a given [Hb] is the same as every other patient with same [Hb]. This is due to the variability of individuals' *physiological adaptation* to the anemia that include:

- Duration of anemia: chronic vs acute. Physiological adaptations developed to anemia.
- Increase in cardiac output. Potential redistribution of available blood flow.
- Modification of erythrocytic 2,3 diphospho-glycerate (2,3 DPG) modulating oxygen unloading.
- Presence of comorbidities that may affect or limit the physiological adaptations.

Searching for objective markers of tissue hypoxia lead Hare and colleagues [27] to the kidney as a vulnerable organ during cardio-pulmonary bypass. Acidosis and increased plasma lactate concentration were indicative of some tissue hypoxia. Actual measurements in animal experiments of

[3]Hypoxia Inducible Factor

[4]Nitric Oxide Synthase

renal medullary pO_2 by polarographic electrodes, has shown the presence of tissue hypoxia during cardio-pulmonary bypass [28]. Erythropoietin (EPO) is released to the plasma in the presence hypoxic injury to the kidney. A rise of this hormone was correlated with the onset and severity of anemia, suggesting that EPO could be a potential biomarker for the need for transfusion to avoid hypoxic injury to the kidney during cardio-pulmonary bypass [27]. The potential of EPO being a biomarker for the need of transfusion requires further exploration.

Recognizing the need for objective evidence-based markers for the need of transfusion, significant efforts have been directed at developing clinical trial-based guidance on the expected benefit of transfusion. The introduction of physiological, rather than [Hb] – based ones have been used as surrogates (e.g., heart rate ECG changes, mixed venous oxygen saturation, plasma lactate, etc.) [29]. A transfusion-attributed 10 g/L increase in [Hb] resulted in reduction of lactate clearance by >10% and increased central venous oxygen saturation by >5% in a third of the subjects [29]. Thus, there were putative physiologically meaningful benefits in *some but not all of the subjects*. There may be three conclusions from this study. First, that objective, physiological indicators can be applied to assess transfusion "efficacy" , and that a 10 g/L increment in a subject's [Hb] may offer a marginal benefit, and, lastly, that it confirms that not all individuals are alike in their responses and hypoxia tolerance.

This desired goal is hampered by the lack of objectively definable, universally applicable indicators to facilitate a *rational c*linical decision [12, 16].

The *expectation of benefit and of the efficacy of the transfusion* were important contributors to a degree of chaotic and individualistic approach to the use of transfusions, especially in surgical settings. Transfusion practices were variable, both among specialties and institutions, as well as within institutions. Many transfusions had been prescribed based on practitioners' personal values and expectations, as the true magnitude of the hazards of transfusion itself were not fully appreciated.

The Risks of Transfusion

Transfusion Reactions

Transfusion reactions as risk factors for adverse outcomes: these are adverse outcomes of a specified nature and had been well recognized.

(Chapter 6 of Part I of this book offers discussion of the nature, frequency, and clinical significance of *transfusion reactions* directly attributable to an incompatible transfusion.)

Transfusion reactions are identified *post facto* and their frequency, severity and their putative causes are monitored by national hemovigilance programs in most countries.

Transfusion reactions include [2, 3]:

- Incompatibility reactions to major or minor antigen mismatch, with or without hemolysis.
- Anaphylactic or allergic reactions [30].
- Accidental mismatch or preventable errors: wrong unit given to wrong patient.
- Transfusion Mediated Immune Modulation (TRIM) [31].
- Transfusion-Related Acute Lung Injury (TRALI) [32] and Transfusion-Associated Circulatory Overload (TACO) [33].
- Adverse reactions initiated by inflammatory mediators potentially derived from residual white cells remaining in transfused erythrocyte units.
- Febrile non-hemolytic transfusion reaction. Delayed serologic reaction.
- Post-transfusion purpura.
- Transfusion-Associated Graft vs Host reaction (T-A GVH) most likely affecting immunocompromised patients [34].

Fatal transfusion-related events occurring in the USA and reported to the FDA in the 5 years between 2012 and 2016 totaled sixty-five, of which one-half were hemolytic transfusion reactions. Despite the relatively low incidence of fatal transfusion reactions that should theoretically be preventable, these do happen and are a cause for concern [35]. The prevalence per 100,000 units transfused is reported yearly. This reporting is a great benefit in decisions of the statistical probabilities of assessing risk tolerance by both the prescriber and the patient.

Transfusion Reactions, TRALI, TRIM and T-A GVH) are rare but serious complications of transfusions.

In the surgical setting the immune suppression due to transfusion may be aggravated by immune suppression due tissue injury. In such cases the compelling argument favoring a transfusion are the consequences of the blood loss. Immune modulation is a well-known contributing risk factor for nosocomial infections in postoperative patients. Amelioration of the immune suppression may be a consideration for possible *avoidance* of transfusion, if feasible. Thus, the balancing of expected benefits and known and anticipatable risks is the *sine qua non* of a transfusion decision.

Residual leukocytes in erythrocyte units are thought to be a contributing risk factor to the pathogenesis of TRIM. Hence, increasing attention is directed at producing *leukoreduced* erythrocyte units. Comparison of transfusions of leukoreduced and non-leukoreduced units has shown true superiority of the former [36–39]. The ongoing universal implementation of leukoreduction and introduction of other specialized erythrocyte units (e.g., CMV-free units) became available and ameliorate these risk factors.

Transfusion Transmitted Infectious (TTI) Pathogens

In addition to the risk of transfusion reactions, another category of risks is the transmission of infectious pathogens present in donors, since blood cannot be sterilized [40]. The first infectious disease recognized to be transmissible by transfusion was syphilis and the Serologic Test for Syphilis (STS) was introduced in blood testing in 1935. The actual usefulness of this test is questionable, but it has remained in use. As refrigeration kills the *T. pallidum,* the clinical risk of syphilis has been overtaken by the more prevalent hepatitis viruses, transmissible by both blood and blood products, starting in 1965, which are not affected by refrigeration [1, 41].

The salient events in this regard in the USA were [1]:

- Hepatitis B surface antigen (HBsAg) discovered in 1965.
- Testing of blood donors for Hepatitis B surface antigen introduced in 1972.
- Transfusion-Associated Acquired Immunodeficiency Syndrome recognized in 1982.
- Donors deemed at high-risk behaviors were excluded in 1983.
- Human Immunodeficiency Virus (HIV) identified in 1984.
- HIV antibody testing introduced in 1985.
- Surrogate testing for hepatitis, (liver enzyme alanine transaminase ALT), hepatitis B antibody testing introduced in 1987.
- HTLV antibody testing introduced and hepatitis C virus identified in 1989.
- Hepatitis C testing introduced in 1990.[5]
- HIV 2 testing introduced in 1992.
- Nucleic acid testing and increasing numbers of rigorous virus testing introduced in the years following [1].

By the 1970s, the risks to the blood supply of potentially infectious *paid* donors were recognized, just as the demand for transfusions was increasing with the soaring number of coronary artery bypass graft (CABG) operations, starting in 1960. Many countries have made the shift away from paid to volunteer, unpaid, donors to protect the safety of their blood supply.

The HIV/AIDS Catastrophe

The arrival of the Human Immunodeficiency Virus (HIV) and its presence in the blood supply became the *third milestone* event.

The safety of the blood supply and of the erythrocyte and blood products became questioned with a panicked response by the population in most countries. People were unwilling to accept transfusions and untrue rumors circulating caused donations to plummet.

The tragic toll of potentially preventable illness and death became the stimulus for many countries to undertake rigorous and wide-ranging examination of the causes and the failures of national policy and response to the tragedy.

The Response to the Aids Crisis in Blood

The tragic toll exacted by the HIV and hepatitis viruses in the blood supply focused a searchlight on Transfusion Transmissible Infections (TTI) [41, 42], as a transfusion risk, distinct from transfusion reactions.

In the USA in the early 1980's 10,000 hemophiliacs and 12,000 other patients were infected by the HIV virus by blood and blood products and about 300,000 additional persons were infected with the HCV virus. To quote [43]:"The lessons from these tragedies compel greater vigilance and higher regulatory standards to protect the Nation's blood supply from emerging infectious agents and blood borne pathogens" [43–45]. Several policy recommendations were made to establish sub-Cabinet level Committees and Agencies to be responsible for protecting the safety of the blood supply, and these were to be established by statute. The introduction of new safety measures and policies to safeguard the blood supply was soon justified by the challenge of the emergence of a novel infectious agent, the Zika virus [46]. All donors, as of 2018, are screened by a high-performance Nucleic Acid Amplification Test (NAT) test. This newly emerged threat emphasized the importance of vigilance and horizon scanning to prevent the recurrence of a new HIV-like crises [47].

The introduction of new technologies for rigorous screening of blood, e.g., NAT testing, also introduced additional costs to the provision of blood for transfusion, to maximize blood safety.

NAT testing was first introduced as a sensitive and specific identifier of viral RNA or DNA to blood screening of donated units in the "window period" before infection could be detected by serological tests. They can be performed either on a single sample, or as multipacks combining a multiple of samples. NAT testing was first introduced in Germany in 1997, followed by the Netherlands in 2000. Approximately 33 countries use NAT testing on their blood collections.

NAT tests performed on multiple combined samples have the advantage of having the lower cost of fewer tests than tests performed on individual samples from all donations. Their disadvantage is that once a multipack is identified as positive, all units in the multipack sample need to be quarantined until further tests are completed to identify the one positive unit and the rest can be released [48]. The alternative to testing multipacks is testing of single units; this will

[5]The 2020 Nobel prize in Physiology or Medicine was awarded to H.J Alter, H Houghton and C.M. Rice for the discovery of the Hepatitis C Virus: https://www.nobelprize.org/prizes/medicine/2020/press-release/

increase the test's sensitivity, but also multiplies the total costs, while avoiding delays in releasing non-reactive units.

Cost-effectiveness in pharmacoeconomic analysis uses the metric of incremental cost of Quality Adjusted Life Years (QALY) achieved. Pharmaco-economic analysis was performed in several countries following the implementation of NAT testing. In the United States, [49] the study found that, using minipool samples, NAT testing would avoid an estimated 37, 128, and 8 cases of HBV, HCV, and HIV, respectively, and would add 53 additional years of life, and 102 additional QALY, compared with single samples tested at a net cost of $ 154 million. For relative scale, note that approximately eight million units are transfused annually in the USA. The incremental cost ratio estimated was $1.5 million per QALY gained. The authors concluded that the cost-effectiveness of adding NAT screening in the US blood system would be outside the typical range of most health care interventions, but not for established blood safety measures.

Following the introduction of NAT testing in Germany in 1997, the German Red Cross reviewed its experience with NAT testing the German blood supply [50]. In the eight-year period (1997-2005) 30.5 million donations (representing about 80% of the total blood collected) were tested. A total 27 HCV, seven HIV-1, and 43 HBV positives had been detected that would have been missed by serological methods only. Thus, NAT testing applied in the "window period" found that the residual risk per unit transfused was estimated at 1 in 10.88 million units for HCV, 1 in 4.3 million units for HIV-1, and 1 in 360,000 units for HBV. The authors concluded that the risk avoided by the addition of NAT testing was "very low", at a substantial cost.

A third study conducted in Zimbabwe shows how extreme inequality between high- and low-income countries affects these policy decisions [51]. The estimated prevention of infections by the addition of NAT testing would be 25, six, and nine HBV, HCV, and HIV infections, respectively. The incremental cost was estimated at US $ 17,774 for each QALY achieved. This is three times the gross per-capita income in Zimbabwe and fails the test of a reasonable cost.

Thus, the mandates to maximize the safety of the blood supply in high-income countries come at high cost that is felt to be within their national priorities in maintaining the safety of blood. It is clearly an impossibility in countries with low incomes and failed economies.

Canada's blood system was severely impacted by the HIV crisis. And the failure of a timely response to introduce testing blood collected for the hepatitis virus, as a surrogate, before the identification of the HIV virus. The panic had been aggravated and the tragedy amplified. Criminal charges had been filed against several individuals deemed to be responsible for the delays in recognition of the threat and failing to act in a timely manner. At trial, those charged were not convicted. Those responsible, however, were confronted by many of the victims, and the participation of victims in the review of the events provided those affected an opportunity to express their grief.

A wide-ranging and clear-eyed examination of all the factors was undertaken by a Royal Commission under Mr. Justice Horace Krever over 3 years and costing CDN $ 17 million [52]. The three volumes and appendix take an enormously expansive look at all aspects of the provision of all blood products and components and the means available for reducing contamination. The policy recommendations were far reaching. Before the crisis, the Canadian Red Cross managed all aspects of donor recruitment, donations and processing of blood and components, except for apheresis collection and processing blood products.

The Commission recommended a complete reorganization of all aspects of Canada's blood system and all its recommendations were implemented by statute. The Canadian Red Cross lost all participation in managing the blood system. Blood, blood products and components were to be treated not as commodities but as taxpayer-funded public goods. A completely new organization, Canadian Blood Services (CBS), was set up on a nation-wide scale, to become the overall manager of blood collection from volunteer donors only, all aspects of processing and supply and to include under its aegis organ transplants and stem cells, as well. Cord blood collection remained in private hands. No blood and blood components are imported to Canada, and blood products are imported only after heat treatment. The CBS encompassed all Canadian provinces and territories, except for Quebec where a similar organization, Hema-Quebec, fulfills a similar mandate. CBS' s global budget is funded from provincial and federal contributions on an annual basis and is overseen by a council of all Health Ministers. Hema-Quebec receives its funding from the Province and the federal government. CBS provides hospital blood banks and other blood users all blood and components free of charge and recipients are not charged for any services. Blood products for hemophiliacs are provided free by the provincial health insurance agencies.

CBS screens all blood collected with NAT testing for HIV-1 and 2, HBV and HCV, as well as for West Nile Virus during the summer season and for Chagas' disease (*T. cruzi*) in travelers.

Volume 3 of the Krever Report provides an exhaustive review of international events and national blood systems, including those of the USA, and comparisons made between systems.

Ten years following the Report, an appraisal concluded that the reform of the Canadian blood system was successful. The public has been kept safe from transfusion transmissible infectious threats by rigorous screening and deferral of potential high-risk donors, by an all-volunteer loyal donor base [53].

Two non-fiction books by Canadian journalists tell the story of those affected in Canada [54, 55].

The World Health Organization (WHO) publishes periodic reports on blood safety and availability in most countries [56].

Thus, the milestone event of the HIV crisis focused attention on transfusion transmissible infections (TTI). This also meant that TTI's came to be recognized as the second category of serious risk of transfusions, in addition to transfusion reactions. In many countries national policies were introduced mandating maximal efforts to safeguard the scarce and precious resource. The public policy to restore the public's trust in the safety of blood by a costly effort has been successful in many countries.

As if to reinforce that the emergence of novel and rare infectious disease threats requires continued vigilance and rapid response, the Zika virus arose from Micronesia and was brought to Brazil by Olympic athletes from French Polynesia. Sporadically reported from Africa and Asia before, this mosquito-borne virus attacked an immunologically naïve population in Brazil and caused the birth of thousands of microcephalic infants. The arrival of the virus in 2015 caused an international public health emergency [47]. Infected adults have viremia, but 80 percent are asymptomatic, spreading the virus widely [57]. Potential viremic blood donors without symptoms would threaten the blood supply if sensitive testing were not introduced promptly. While a NAT based test became available in Brazil, not all blood centers had been required to introduce it universally. Apparently, a few cases of transfusion transmitted infections have been reported, although the overwhelming majority of infections did not enter the blood supply. According to AABB[6] criteria, the virus should be classified as a high-risk infectious agent [46]. Whereas most infections cause no symptoms, the virus is also implicated in rare cases of Guillain-Barre syndrome. Hence, recipients of infected units are at risk of serious but rare complications. The Zika virus is another infectious agent that poses threats to the blood supply in endemic areas, posing challenges to blood collection [58].

In the USA FDA issued Guidance in August in 2016 recommending universal NAT testing for Zika in blood donors. By then, more than 4000 travel related Zika infections had been reported to the Centers for Disease Control and Prevention (CDC) [57].

As health care costs escalate in most countries, the distribution of scarce resources, including financial ones, become important considerations. Pharmaco-economic analysis is being applied to aid decision-making about resource allocation. Among these, the mandate to assure the attainment of best available safety of the blood supply is also constrained by the escalating costs associated with the introduction of newer tests mandated and more expensive technologies introduced. Economic considerations have been applied to blood processing and transfusion-associated costs [59]. As the effectiveness of transfusion has been often overestimated, whereas the risks have been underestimated; cost-effectiveness of transfusion as a frequent medical-surgical intervention needs to be examined [59].

[6]American Association of Blood Banks

Transfusion-Attributable Adverse Outcomes

The fourth Milestone event: It is being recognized that those receiving transfusions are at risk for adverse outcomes that occur more frequently than in those who had not been exposed to a transfusion. These adverse outcomes are recognized, based on presumptive evidence, as the *third category of risks affecting transfusion recipients, in addition to transfusion reactions and TTI's.*

Jehovah's Witness patients undergoing cardiac surgery are an instructive cohort to consider, when compared to patients undergoing similar procedures who also receive transfusion. Cardiac surgery patients are good examples, because they are at high risk of needing a transfusion, due to uncontrolled bleeding, anticoagulant use, and coagulation defects. A statistical tool, "propensity matching", enables the selection, from a large cohort, patients who are closely comparable to a smaller cohort when the two cohorts differ in a single attribute, namely, whether or not exposed to transfusion. The study from the Cleveland Clinic [14] reviewed retrospectively in a seven-year period 87, 775 consecutive cases undergoing Coronary Artery Bypass Grafting (CABG). Of this population, 56% (48, 986) received transfusion(s). Using propensity matching, the study selected 322 transfused patients who matched 322 Jehovah's Witness untransfused patients. The matching created two comparable cohorts of equal size, comprising of patients who were like each other with respect to many preoperative and operative characteristics, but differed with respect to transfusion exposure. During the 30-day postoperative period there were 14 deaths in the transfused and 10 deaths in the untransfused Witness patients (14/322; 4.3%, vs 10/322; 3.1%; Not significantly different). However, significantly more *adverse events* of myocardial infarction, respiratory failure and reoperations occurred in the transfused cohort. Indicative of the severity and frequency of adverse outcomes, longer ICU, and operative hospital lengths of stay (LOS) were also seen in the transfused patients. Long term survival of those followed up also favored the Witness patients.

A study deploying similar methodology also found differences in adverse outcomes experienced between transfused and untransfused patients as those in the Cleveland Clinic study above [60]. This study population comprised two cohorts of 857 matched pairs. More of the transfused patients experienced myocardial infarctions, respiratory and renal failure, reoperations and longer ICU and hospital LOS. These

comparative studies, while not definitive, do suggest that the exposure to transfusion may be a contributing risk factor to more frequent adverse outcomes. This introduces the concept that *transfusion avoidance* may be a desirable clinical goal, avoiding some of the excess risks that transfusion recipients may experience, leading to the fifth milestone.

Transfusion Avoidance and Blood Conservation

From the foregoing narrative it is evident that there are benefits and risks to be considered when a transfusion decision is made. Thus far, both have been considered in the abstract, without regard to the severity of the anemia of the patient, and its risks. From the consideration of risks of anemia above, it is evident that a [Hb] less than 50–60 g/L is a significant threat to survival. Even that threshold may be dependent of the patient's physiological reserves and resilience.

To summarize the intertwined risks and benefits of anemia and transfusion:

- SEVERE ANEMIA:
  - If untreated, is a threat to health and survival.
  - Is a risk factor for transfusion,
  - Is potentially improved by transfusion by avoiding anemia threats.
- TRANSFUSION:
  - Has inherent risks: transfusion reactions, transmitted infections, transfusion-attributable enhanced risk of adverse outcomes.
  - There is benefit in avoiding transfusion: avoid above risks to individual.
  - Benefits to community: Conserve scarce resources: blood, financial.

The predictive importance of preoperative anemia was evaluated in a cohort of 33,411 patients undergoing elective cardiac surgery [61]. Thirty-one percent (n = 10, 357) of these patients received transfusion(s) indicating how frequently transfusions are prescribed in these circumstances. The likelihood of transfusion was correlated with preoperative anemia. The adjusted mortality rate and a greater number of adverse outcomes was correlated with receipt of transfusion. This indicates that transfusion is an independent risk factor for additional adverse outcomes [62, 63].

In each case, an additional consideration of cost differences may also apply [64, 65] (see below).

Following the recognition of transfusion associated risks to the patients, the then (1997) available guidelines for the use of erythrocyte transfusions were reviewed [66]. The review found no expert consensus-based guideline or practice recommendation for objective guidance for erythrocyte transfusions.

In 2011 a paper appeared in the British Journal of Anaesthesia with the provocative title, "*What is really dangerous: anaemia or transfusion?"* [67] Shander and colleagues reviewed the physiological mechanisms available to protect from hypoxic tissue and organ injury and called for further research to characterize these risks to better enable rational transfusion decisions that minimize risks and maximize benefits.

The Search for an Objective Transfusion "Trigger"

The fifth milestone:

The Transfusion Requirements in Critical Care (TRICC) Study

The first randomized controlled clinical trial intended to find *objectively definable transfusion "triggers"* in ICU patients, appeared in the New England Journal of Medicine, February 11, 1999 [68]. The study was intended to find non-inferiority between two groups of critical care patients in 22 tertiary care and three community hospitals in Canada and the USA. The two groups were randomized to receive daily transfusions, to maintain their [Hb] either within the range of *70–90 g/L* in the so-called *restrictive* cohort, and within the range of *100–120 g/L* in the so-called *liberal* cohort.

Carefully selected inclusion/exclusion criteria and characterization of each subject's pre-randomization profile (using such as Multiple Organ Dysfunction Scores (MODS)) [69] were recorded, to allow clinical comparison of the two cohorts, as well as daily measures during the trial to compare outcomes between the two cohorts. The enrolled population was randomized one-to-one into either the restrictive or the liberal transfusion cohort (n = 418 and n = 420), respectively. Primary outcome measures were mortality at various time points.

The subjects were successfully maintained at their assigned [Hb] ranges (85 ± 7 and 107 ± 7 g/L, $p < 0.01$). Mortality rates in the ICU and at 30 days were lower in the restrictive than in the liberal groups. The mean number of transfusions received was significantly different between the groups; 2.6 ± 4.1, vs 5.6 ± 5.3 units per subject in the restrictive and liberal groups, respectively. The difference between the groups was also evident in the total number of transfusions *avoided*: 138 of the 418 subjects in the restrictive group (33%) entirely avoided transfusion, whereas all subjects in the liberal group received at least one transfusion. The transfusions avoided by the restrictive group subjects represented a 46% reduction in total number of transfusions. The clinical