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Review

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Beyond the Blood Cell: The Emerging Role of Cell-Free DNA in Transfusion Medicine

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Abstract

Cell-free DNA (cfDNA) consists of fragmented nuclear and mitochondrial DNA circulating in the bloodstream, primarily originating from hematopoietic cells. While cfDNA analysis has transformed diagnostic medicine, its presence in transfused blood products introduces emerging clinical concerns. Donor-derived cfDNA may persist in transfusion recipients and contribute to transfusion-associated microchimerism, defined as the long-term presence of donor genetic material in recipient tissues or circulation. These fragments have potential to integrate into the host genome, modify DNA methylation and histone structure, and activate innate immune pathways such as Toll-like receptors. In addition to nuclear and mitochondrial sources, cfDNA in transfused blood may include environmental or dietary DNA acquired by donors, further influencing immune regulation. Current leukoreduction methods do not eliminate cfDNA or prevent microchimerism. This review synthesizes current evidence regarding the persistence, genomic integration, and immunologic impact of cfDNA in transfusion recipients. The findings highlight an urgent need for further investigation and refinement of blood processing practices to ensure transfusion safety and protect recipient health.

Keywords: Cell-free DNA; Microchimerism; Allogeneic transfusion; PRBCs; Epigenetics; Gene transfer; Immune activation

Introduction

Cell-free DNA (cfDNA): origins and biological properties

cfDNA consists of small and large fragments of double-stranded DNA or chromatin, typically ranging from 50 to 200 base pairs long, released from cells throughout the body [1, 2]. These fragments can originate from both mitochondrial and nuclear DNA and may be derived from either healthy or diseased cells [3]. Recent studies have demonstrated that cfDNA in the bloodstream predominantly exists in nucleosome-associated form, reflecting the physiological processes of chromatin degradation (breakdown of DNA-protein complexes during cell death) and nucleosome stabilization (DNA wrapped around histone proteins, protecting it from enzymatic degradation) [4-8].

While initially discovered in blood plasma, cfDNA has been found in various bodily fluids. In healthy individuals, the majority of cfDNA comes from the hematopoietic system, with around 55% derived from white blood cells (WBCs), 30% from erythrocyte progenitors, and 10% from vascular endothelial cells [1, 9, 10]. Non-hematopoietic sources, including hepatocytes, heart, and lung tissues, contribute smaller but detectable amounts (1-5%) [11]. The body utilizes mechanisms such as phagocytosis of apoptotic and necrotic cells to remove cellular debris and limit cfDNA release, though clearance mechanisms remain incompletely understood [12]. Beyond these passive pathways, viable cells actively release cfDNA through mechanisms including neutrophil extracellular traps (NETs) [13, 14]. When the rate of cfDNA release exceeds the capacity of enzymatic degradation, cfDNA may persist in circulation and accumulate systemically [15, 16]. Histone-bound cfDNA further resists enzymatic clearance, as nucleosome packaging enhances its structural integrity and shields it from DNase activity [16, 17].

NETs are expansive, DNA-protein lattices extruded by activated neutrophils to immobilize and neutralize invading pathogens, including bacteria, fungi, and viruses [13, 14, 18]. The DNA released during NETosis contributes significantly to the circulating cfDNA pool and exerts immunomodulatory effects, particularly in the setting of organ transplantation and immune rejection [19, 20]. For example, in liver transplantation, NET-derived cfDNA correlates with ischemia-reperfusion injury and coagulation activation [13]. Mitochondrial cfDNA (cfDNA-mt) contains cytosine-phosphate-guanine (CpG) sites that exhibit inter-individual methylation variability, with hypomethylated CpG-rich regions linked to transcriptional activation [21-23]. Trauma patients and individuals with critical illness exhibit elevated levels of CpG-rich cfDNA-mt, which have been correlated with systemic inflammation and adverse clinical outcomes [13, 24].

cfDNA serves as a biomarker of chromosomal instability and tissue damage, reflecting pre-existing genomic alterations

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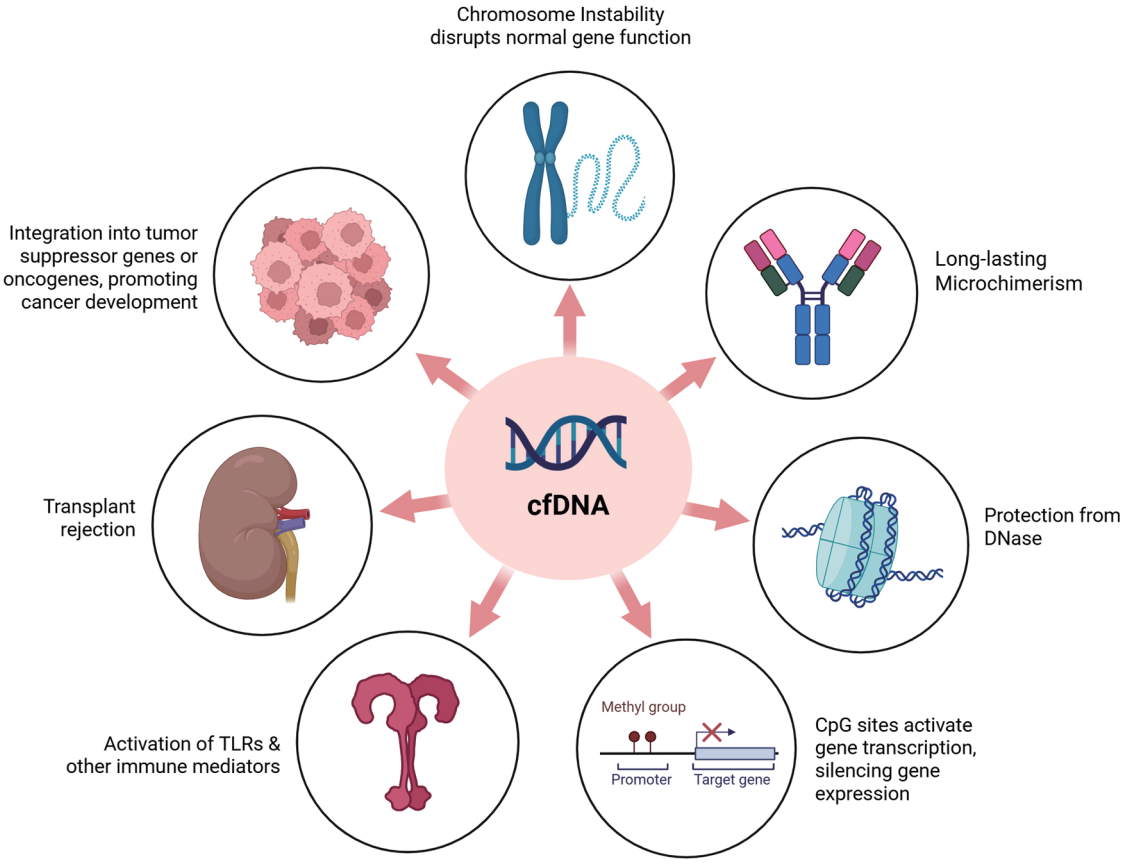

Figure 1. Theorized effects of cfDNA on the transfusion recipient. The impact of transfused cfDNA on the recipient may vary widely depending on factors such as cfDNA concentration, the recipient's genomic background and lifestyle, and the cellular origin of the cfDNA. It is hypothesized that cfDNA bound to histones may evade DNase degradation and potentially integrate into the host genome. This integration could influence gene regulation by promoting transcriptional activation or silencing. cfDNA has also been associated with genomic instability in theoretical models. If not effectively cleared by the immune system, donor cfDNA may contribute to enhanced immune activation, potentially triggering responses linked to transplant rejection or the activation of pathways involving tumor suppressor genes, oncogenes, or Toll-like receptors. cfDNA: cell-free DNA; CpG: cytosine-phosphate-guanine; TLR: Toll-like receptor.

such as double-strand breaks or chromosomal rearrangements in parent cells (e.g., tumors or apoptotic leukocytes) [25-27]. Integration into recipient genomes occurs at fragile sites via non-homologous end joining (NHEJ), a process observed in cancer and transfusion-associated microchimerism (TA-MC) [11, 28]. Transfused cfDNA may persist as microchimerism, activate Toll-like receptor 9 (TLR9)-mediated inflammation via CpG motifs [13, 22], or modulate immune responses [28]. These potential effects of cfDNA on the host including genomic integration, immune activation, epigenetic modification, and microchimerism are summarized in Figure 1.

The presence of cfDNA in blood products: sources, implications, and concerns

Packed red blood cells (PRBCs) are among the most frequently used blood transfusion products. These preparations primarily contain red blood cells (RBCs) and a relatively low number of donor-derived WBCs. Leukoreduction, a filtration process designed to remove WBCs before transfusion, is widely implemented to reduce the risk of immunologic complications [29]. While leukoreduction techniques can help limit the WBC count in PRBC products, they are not always universally applied, and acceptable WBC levels in blood products vary based on different standards [30]. Notably, studies have shown that leukoreduction has not been shown to affect the presence or duration of TA-MC [30].

A key concern in transfusion medicine is the presence of cfDNA in blood products. While the primary source of cfDNA is donor WBCs, additional contributions may come from apoptotic cells derived from various tissues, NETs, stem cells, and even dietary or environmental DNA [1, 9, 10, 16, 31-34]. While mature RBCs lack nuclei and mitochondria, and thus do not contain DNA, they are rich in various RNA species [35]. However, studies have shown that the predominant nucleic acids in blood products are double-stranded DNA rather than RNA [16, 30, 31]. These potential contributors to the circulating cfDNA pool are summarized in Figure 2.

Beyond cellular origin, several logistical and techni-

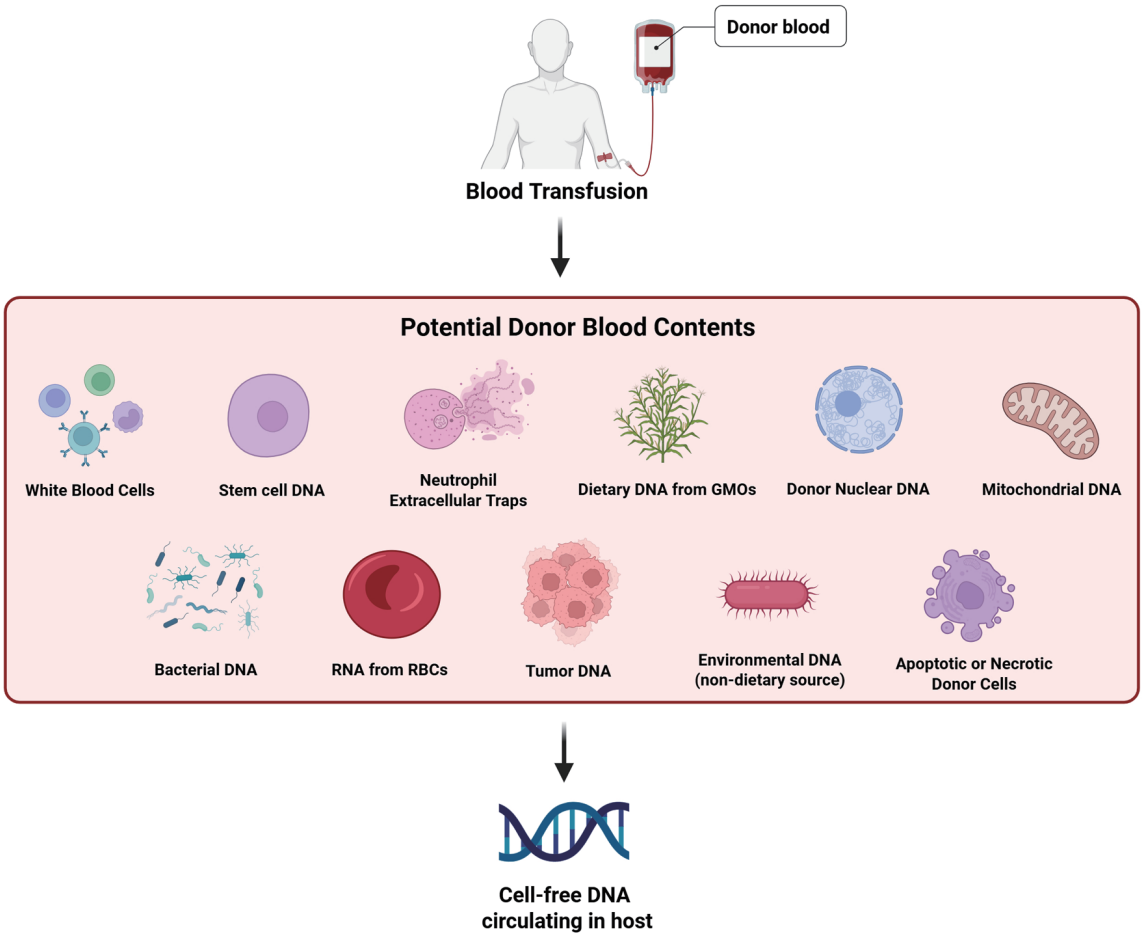

Figure 2. Potential sources of donor-derived cfDNA in transfused blood. Donor blood may contain various sources of cfDNA, including DNA from white blood cells, stem cells, NETs, dietary DNA (e.g., from GMOs), nuclear DNA, and mt-cfDNA. These fragments can persist in the recipient's circulation post-transfusion, despite leukoreduction, and may contribute to downstream immunologic or epigenetic effects. cfDNA: cell-free DNA; cfDNA-mt: mitochondrial cell-free DNA; GMOs: genetically modified organisms; NETs: neutrophil extracellular traps.

cal variables influence the presence of cfDNA in transfused blood. Although PRBCs are stored for variable durations to meet clinical demand, studies show that neither storage time nor processing significantly affects cfDNA levels [36, 37]. Consequently, modifications to storage and processing techniques are unlikely to prevent cfDNA transmission during transfusion. While cfDNA detection has advanced noninvasive prenatal testing, transplant monitoring, and cancer diagnostics [38-42], its inadvertent transfer through transfusion remains a matter of concern.

Methodology

This narrative review synthesized current and foundational literature on cfDNA in transfusion medicine. A comprehensive search of PubMed, Scopus, Web of Science, and Google Scholar was conducted using combinations of the following terms: "cell-free DNA," "cfDNA," "circulating free DNA," "blood transfusion," "packed red blood cells," "PRBCs," "microchimerism," "donor-derived DNA," "horizontal gene transfer," "epigenetics," and "cfDNA integration." Studies were selected through title and abstract screening, followed by full-text review. Research from the last 10 years was prioritized, with earlier seminal works included for context. Only peer-reviewed *in vitro*, *in vivo*, and observational studies were considered; conference abstracts and preprints were excluded. No formal analysis was performed due to the narrative nature of this review. Where findings conflicted, differing perspectives were included with attention to methodological quality. The evidence quality was assessed by considering study design, sample size, methodological rigor, and consistency with other published findings.

cfDNA Transfer and Microchimerism

Microchimerism is defined as the long-term persistence of genetically distinct cells or cfDNA within an individual, originating from another organism. This phenomenon occurs bidirec-

tionally through natural processes (e.g., pregnancy, twinning) or artificial routes (e.g., blood transfusion, organ transplantation) [42-45]. In transfusion medicine, TA-MC refers specifically to donor-derived genetic material (cells or cfDNA) that persists in recipients, even when leukoreduced blood products are used [45-47].

The integration of cfDNA and its impact on gene expression and genomic stability are considered crucial factors contributing to these long-term effects [43, 48]. For example, when Khaki Campbell duck DNA was injected into Pekin ducks, unexpected modifications occurred in nine out of 12 treated ducks, while no changes were observed in 24 control ducks [49]. These modified traits were passed down to descendants for up to three generations, suggesting that the DNA-induced changes were both stable and transmissible [49]. Although conducted decades ago, this experiment offers early proof of principle for horizontal DNA uptake leading to stable, transmissible genetic changes.

Building on these foundational insights, recent research has highlighted the clinical relevance of cfDNA in modern medicine. In transplantation, donor-derived cfDNA (dd-cfDNA) is now recognized as a sensitive biomarker for early detection of allograft injury and rejection, outperforming traditional markers such as serum creatinine. For example, dd-cfDNA levels > 1% demonstrate high diagnostic accuracy for acute rejection in pediatric renal transplants [50], while elevated dd-cfDNA in kidney allograft recipients strongly correlates with antibody-mediated and T-cell-mediated rejection, independent of standard clinical parameters [51]. In transfusion medicine, *in vitro* studies demonstrate that transfused cfDNA may alter recipient cell behavior and may persist and expand for months or longer, particularly when genomic integration or microchimerism occurs [43, 46, 52]. Despite these advances, the long-term clinical consequences of dd-cfDNA in transfusion recipients remain poorly understood [53].

Immunologic and Pathological Associations of cfDNA

Emerging evidence shows that cfDNA can integrate into host genomes and alter gene expression, contributing to durable microchimerism [43, 54]. *In vitro* studies have demonstrated that the internalized cfDNA may influence gene regulation, particularly by inducing pro-inflammatory genes in the cells that have absorbed the cfDNA [54-59]. Though best known in bacteria for facilitating the spread of antibiotic resistance, in human cells, horizontal gene transfer (HGT) has emerged as a relevant model for understanding cfDNA uptake and functional integration [60, 61].

Adding to this growing body of evidence, donor DNA has been detected not only in recipient blood but also in epithelial cells (e.g., buccal mucosa) unrelated to transfusion [62]. Early theories attributed this to stem cell plasticity, but contemporary evidence implicates gene transfer (GT) as the primary mechanism, enabling even non-hematopoietic cells to acquire donor DNA [63]. Seminal work by Garcia-Olmo et al demonstrated that plasma from colorectal cancer patients could transfer human oncogenes such as K-ras and p53 to murine fibroblasts, which subsequently induced tumor formation in immunodeficient mice [63]. This evidence underpins the genometastasis theory, which proposes that circulating tumor DNA has the potential to induce malignant transformation in distant, susceptible cells [63, 64]. More recently, Salazar et al showed that hepatic progenitor cells co-cultured with colorectal cancer cells could acquire the KrasG12D mutation and exhibit tumorigenic behavior, reinforcing the concept that horizontally transferred cfDNA can have lasting functional effects in recipient tissues [64].

Beyond oncogenic transformation, the immune consequences of cfDNA exposure are also significant. Once internalized, dd-cfDNA may engage innate immune sensors such as TLR-9, triggering nuclear factor-kappaB (NF-κB) activation and downstream expression of pro-inflammatory cytokines including interleukin (IL)-1β and C-X-C chemokine ligand 8 (CXCL8) [65, 66]. These immune responses mirror those observed in infectious and neoplastic conditions, further underscoring the immunogenic potential of transfused cfDNA (Fig. 1).

It is further reported that a small portion of cfDNA can circulate as nucleosomes, which consist of DNA wound tightly around histones, that may exert biological effects, including modulation of immune responses and regulation of gene expression [54, 67-69]. Rather than being a passive biomarker, cfDNA plays a dynamic role in maintaining immune balance. For example, cfDNA clearance in plasma is indispensable for maintaining immune homeostasis, and its accumulation can trigger pro-inflammatory pathways and complement activation [68]. *In vitro* studies show that NETs release high-molecular-weight DNA (1 - 30 kbp) that serum nucleases rapidly degrade into mononucleosomal fragments (81.3% of remaining DNA after 24 h), mirroring the size profile of cfDNA in inflammatory diseases [54].

The broader pathological relevance of cfDNA has been demonstrated across diverse clinical contexts. Elevated cfDNA levels have been observed in malignancy, autoimmune diseases, trauma, sepsis, psychiatric and neurodegenerative conditions, and organ dysfunction [70-76]. For instance, Lopes et al reported that cfDNA concentrations rise substantially during and after major surgery, particularly in patients with advanced disease, with shorter DNA fragments predominating [77]. In bacterial sepsis, increased cfDNA is strongly linked to poor prognosis, likely due to TLR activation and subsequent cytokine storm [54, 58]. This is likely due to activation of TLRs and the resulting cytokine storm, a cascade linked to splenic and acute lung injury and prolongation of neutrophil lifespan [60]. As a recognized damage-associated molecular pattern (DAMP), cfDNA can amplify or mitigate disease progression through its interactions with pattern recognition receptors [77, 78].

Although these findings stem from other pathological conditions and non-transfusion settings, they raise important concerns: if endogenous cfDNA can contribute to such profound pathophysiology, the introduction of dd-cfDNA via transfusion may carry underappreciated risks, particularly in susceptible recipients. As transfusion remains a cornerstone of supportive care, these parallels underscore the urgency of evaluating the biological activity and clinical impact of cfDNA in transfusion medicine.

Dietary and Environmental DNA: Implications for Transfusion Medicine

While much of the focus has been on cfDNA derived from human cellular sources, emerging evidence suggests that environmental and dietary DNA may also play a role in transfusion-related exposures [79, 80]. This broader category of cfDNA introduces new dimensions to consider, particularly when evaluating the origins, persistence, and biological activity of genetic material in transfused blood. The gastrointestinal tract serves as the primary route of natural exposure to foreign DNA through food consumption. Research shows that 1-2% of ingested DNA can survive digestion, penetrate the intestinal wall, and reach the nuclei of leukocytes, spleen, and liver cells and may occasionally be integrated into the host genome [80, 81]. Animal studies have shown that dietary DNA can also traverse the placental barrier and persist in fetal tissues for extended periods, prompting concerns about the long-term effects of environmental DNA exposure [80]. In humans, dietary cfDNA, stemming from donor dietary habits, may resist degradation, cross the intestinal barrier, enter the bloodstream and be present in transfused blood products [80, 82]. A large study of maternal blood samples found plant-derived DNA in circulation, with higher levels observed in patients experiencing systemic inflammation, suggesting that dietary cfDNA may play a role in modulating immune responses [82].

Concerns about genetically modified organisms (GMOs) in the diet have centered on the potential for HGT of antibiotic resistance genes or other transgenic elements to human cells or gut microbiota [83]. While heat treatment of foods might reduce the risk of DNA transfer, it also causes significant DNA base damage, which may increase genotoxicity and DNA repair activity in recipient tissues [77, 80]. Unlike food, blood products cannot be heat treated without destroying their viability, so any cfDNA present in donor blood, including that derived from GMOs, may be transfused directly to recipients [20] (Fig. 2).

Once introduced to the recipient, donor cfDNA may integrate into host genomes via homologous recombination (HR) or NHEJ, both of which may disrupt gene function or genomic stability [43, 84, 85]. Integration of cfDNA has been shown to alter DNA methylation and transcription patterns, potentially driving oncogenic or other epigenetic changes [86]. For example, cfDNA from leukemic cells can induce DNA damage and apoptosis in stromal cells [37, 87]. Moreover, environmental factors such as diet and psychological stress may further influence DNA methylation patterns in recipients, compounding the potential for epigenetic dysregulation [88, 89].

Together, these findings support the plausibility that both dietary and transfusion-derived cfDNA could influence recipient health. This possibility warrants further exploration into the biological fate and downstream consequences of cfDNA exposure in transfusion settings, especially as clinical use of blood products grows more widespread.

Conclusions

The presence of cfDNA in blood products poses significant concerns for transfusion medicine. cfDNA, which includes both mitochondrial and nuclear DNA fragments, originates from leukocytes and possibly donor stem cells in transfused blood. Despite leukoreduction techniques aimed at reducing WBC content, cfDNA levels can persist for extended periods post-transfusion, ranging from minutes to years, contributing to TA-MC. This integration into the recipient's genome can lead to genomic instability, increased mutation susceptibility, and disruption of gene function. Furthermore, cfDNA alters the epigenome by modifying DNA methylation patterns and histone configurations, potentially initiating oncogenic pathways. It also modulates inflammatory and immune responses, stimulating pro-inflammatory gene expression. cfDNA can resist degradation, allowing it to be internalized by cells, translocate to the nucleus, and affect gene regulation. This persistence raises the risk of long-term immune modulation and latent inflammatory responses. Additionally, cfDNA fragments from tumor cells could accelerate tumorigenesis. Current processing methods have limited impact on cfDNA persistence, highlighting the need for improved management strategies. Future research should focus on the mechanisms of cfDNA integration, its epigenetic effects, and the long-term outcomes of transfusion-related cfDNA transfer.

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Conflict of Interest

The authors declare that they have no competing interests.

Author Contributions

Jackson M. Wahman led the narrative review, performed the majority of the literature search and synthesis, and drafted the initial manuscript. Rhoda Hijazi contributed to manuscript development, analysis of sources, and critical revisions. Elizabeth Duncan designed and prepared the figures. Petra Rocic provided expert review and editorial feedback. Dominica Moussoki supported the literature search and source curation. Hosam G. Abdelhady conceptualized the project, served as the corresponding author, and oversaw all phases of development and final approval.

Data Availability

All data generated or analyzed during this study are included in this published article.

Abbreviations

cfDNA: cell-free DNA; cfDNA-mt: mitochondrial cell-free DNA; CpG: cytosine-phosphate-guanine; dd-cfDNA: donor-derived cell-free DNA; DSB: double-strand break; GF: gene transfer; HGT: horizontal gene transfer; HR: homologous recombination; NETs: neutrophil extracellular traps; NHEJ: non-homologous end joining; PRBCs: packed red blood cells; RBCs: red blood cells; TA-MC: transfusion-associated microchimerism; TLR: Toll-like receptor; WBCs: white blood cells

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