Developing a Belgian Emergency Blood Collection Protocol to fill the gap in resource-limited settings: the right product, at the right time, in the right place.

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INSTITUT ROYAL SUPERIEUR de DEFENSE

“Il faut toujours connaître les limites du possible. Pas pour s’arrêter, mais pour tenter l’impossible dans les meilleures conditions. »

Romain Gary

A Dina…

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ABBREVIATIONS TABLE

ADP	Adenosine Diphosphate
AFMPS	Agence Fédérale pour les Médicaments et les Produits de Santé
ANOVA	ANalysis Of VAriance
ASPI	Arachidonic Acid
AWB	A Whole Blood
BB	Blood Bank
BE	Base Excess
BeQuint	Belgian Quality in Transfusion
BFF	Blood Far Forward
BP	Blood Pressure
BTC	Blood Transfusion Center
CMV	CytoMegalo Virus
CPD	Citrate-Phosphate-Dextrose
CPDA	Citrate-Phosphate-Dextrose-Adenin
CPG	Clinical Practice Guidelines
CS	Clot Stiffness
CSWB	Cold Stored Whole Blood
CT	Clotting Time
	Components Therapy
d’	Cohen's d
DCR	Damage Control Resuscitation
DCS	Damage Control Surgery
DIC	Disseminated Intravascular Coagulation
DOW	Dead Of Wound
EDP	Emergency Donor Panel
EDQM	European Directorate for the Quality of Medicines and Healthcare
ER	Emergency Room
ETP	Endogenous Thrombin Potential
FACS	Fluorescent Activated Cell Sorting
FCS	Fibrinogen part of the Clot Stiffness
FDP	Freeze-Dried Plasma
FFP	Fresh Frozen Plasma
FPS	Federal Public Service
FWB	Fresh Whole Blood
Glu	Glucose

Hb	Haemoglobin
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HR	Heart Rate
HTLV	Human T-Lymphotropic Virus
ID	IDentification
IED	Improvised Explosive Device
INR	International Normalized Ratio
JTS	Joint Trauma System
K	Potassium
KIA	Killed In Action
LT	LagTime
LTOWB	Low Titer O Whole Blood
MCF	Maximum Clot Firmness
MHQA	Military Hospital Queen Astrid
MMLC	Military Medical Laboratory Capacity
MTF	Medical Treatment Facilities
NATO	North Atlantic Treaty Organization
OR	Operating Room
OWB	O Whole Blood
PBM	Patient Blood Management
PCS	Platelet part of the Clot Stiffness
PLT	Platelets
PoCT	Point of Care Testing
POI	Point Of Injury
pRBC	packed Red Blood Cells
PT/PTT	Prothrombin Time/Partial Thromboplastin Time
PVT	Psychomotor Vigilance Task
QTT	Questionnaire Triage Tool
RBC	Red Blood Cells
RD	Royal Decree
RDCR	Remote Damage Control Resuscitation
RT	Reaction Time
	Room Temperature

SF	Special Forces
SFGP	Special Forces Group
sO_2	Oxygen Saturation
SPSS	Statistical Package for the Social Sciences
TACO	Transfusion-Associated Circulatory Overload
TCCC	Tactical Combat Casualty Care
TF	Tissue Factor
THOR	Trauma, Hemostasis and Oxygenation Research
TIC	Trauma Induced Coagulopathy
TRAP	Thrombin Receptor Activator
TTD	Transfusion Transmitted Diseases
TTE	Time To Exhaustion
Type sp.	ABO type specific
VAS	Visual Analog Scale
VelInd	Velocity Index
VET	ViscoElastometric Testing
VO_2Max	Maximal Oxygen uptake/consumption
WB	Whole Blood
WBB	Walking Blood Bank
WBC	White Blood Cells
WWB	Warm Whole Blood
η2	Partial Eta-Squared

SUMMARY

Transfusion is a highly regulated activity, and Belgium is no exception. This activity, which saves lives, especially in the case of haemorrhagic casualties, must be integrated into the medical support system for operations organised by the Defence. However, the management and delivery of sensitive products such as blood can present significant challenges in austere environments. In such cases, emergency protocols are essential for deployed medical personnel to ensure the continuity of care and make up for shortfalls. For transfusion, this involves a protocol for the collection of whole blood from donors who are fellow military personnel, who have been previously screened and deemed eligible for donation. The aim of this study is therefore to develop a Belgian protocol adapted to national needs and ambitions, which considers the associated risks.

A meticulous analysis of the risks was carried out during the development of this protocol, based on a systematic review of the literature. This analysis highlighted the logistical and clinical indications for activating the protocol, as well as the risk-mitigation measures reported by the authors. The analysis revealed a convergence in the needs and identification of risks among the nations represented. In addition, the potential risks to donors were assessed. It was determined that a standard donation does not have any adverse effect on the donor's performance or ability to fulfil the mission safely. Conversely, if a standard donation is deemed insufficient, and a double donation is required, it has been demonstrated that the donor's ability to perform their duty is compromised. In such a scenario, the donor would become another patient. In the final phase of the research, the issue of whole blood conservation was addressed with the objective of ensuring the safety of the patient. When whole blood has been collected and not used because a patient who has been evacuated or who has died, it is important to have recommendations to ensure its preservation enabling it to be used for the next patient. A key factor influencing the decision was the probability of utilisation within 48 hours.

The Belgian protocol has therefore been developed, along with recommendations to ensure the safety of all stakeholders. This enables Belgium to comply with its obligations as a member state of the North Atlantic Treaty Organization (NATO) by providing military personnel with the same standard of care that they would receive in their home country.

RESUME

La transfusion est une activité hautement règlementée, la Belgique ne fait pas exception dans ce domaine. Cette activité, qui permet de sauver des vies, d'autant plus dans le cas de blessés hémorragiques se doit d'être intégrée dans le système d'appui médical aux opérations organisé par la Défense. Cependant, la logistique médicale en milieux austères peut se trouver dépassée dans la gestion et la livraison de ces produits sensibles. Il est alors essentiel que le personnel médical déployé puisse disposer de protocoles d'urgence pour pallier aux manques. Dans le cas de la transfusion, il est fait référence à un protocole de collecte de sang total en théâtre à partir de donneur qui sont des collègues militaires colocalisés, précédemment dépistés et éligibles au don. Le but de cette thèse est donc de développer un protocole Belge adapté aux besoins et aux ambitions nationales en tenant compte des risques qui y sont associés.

Lors de la recherche associée au développement de ce protocole, une analyse minutieuse des risques a été effectuée sur base d'une revue systématique de la littérature. Celle-ci a permis de souligner les indications d'activation du protocole qui sont d'ordre logistiques ou cliniques mais aussi de répertorier les mesures de réduction du risque qui étaient rapportées par les auteurs. Il s'avère que toutes les nations représentées convergent vers les mêmes besoins et l'identification des mêmes risques. Sur base de cette analyse, une adaptation du protocole de collecte a pu être amorcée. D'autre part, nous avons intégré la sécurité du donneur qui doit continuer à effectuer sa mission. Il s'avère qu'un don standard n'impacte pas significativement ses performances. Cependant si un don standard ne devait pas suffire et qu'un double don doit être envisagé, il a pu être montré qu'il n'était alors plus en mesure d'assurer sa mission. Il sera alors considéré comme patient. Dans la phase finale de la recherche, la question de la conservation du sang total a été abordée dans le but d'assurer la sécurité du patient. Lorsque du sang total a été collecté et non utilisé parce qu'un patient a été évacué ou est décédé, il est important de disposer de recommandations pour assurer sa conservation afin qu'il puisse être utilisé pour le patient suivant. Dans la recherche menée, il semble que la variable la plus importante à prendre en compte dans ce choix est la probabilité d'utilisation dans les 48heures.

Le protocole belge est donc enfin développé, accompagné de recommandations assurant la sécurité des différents acteurs. Il est disponible pour permettre à la Belgique d'assumer ses responsabilités imposées par l'OTAN qui sont de fournir aux militaires le même standard de soin que celui qui serait fournis dans le pays d'origine.

GENERAL INTRODUCTION

"The only certain result of your plan will be casualties - mainly the enemy if it is a good plan, yours if it's not. Either way, foremost in your supporting plans must be the medical plan."

Maj Gen Rupert Smith

The treatment of war wounded often requires the use of transfusions. Even in an operational context, Defence must comply with Belgian and European laws, including those governing transfusion. However, the often-extreme operational military context, encompassing geographical isolation and restricted access to resources, forces military medical support to constantly adapt to provide an adequate level of care for its personnel. Consequently, the development of exceptional and adapted protocols must be considered to meet the ambitions and fulfil the duties of Defence. Regarding the specific context of transfusions, it is imperative that the military develop a system capable of providing transfusion support, even in hostile environments.

1. THE TRANSFUSION OF BLOOD PRODUCTS ON BELGIAN NATIONAL TERRITORY

The practice of blood transfusion in Belgium is highly regulated. The responsibility for its supervision falls under the Federal Public Service (FPS) Health. The organisation of this activity is clearly defined and detailed in the Law of 5 July 1994 on blood and blood derivatives of human origin[1]. This legislation is accompanied by Royal Decrees (RD) that provide the legal framework for its implementation. One of these relates to activities associated with blood transfusion centres, namely the Royal Decree of 4 April 1996 on the collection, preparation, conservation and supply of blood and blood derivatives of human origin[2]. The other describe blood bank activities, namely the Royal Decree of 17 February 2005 setting out the standards that a hospital blood bank must meet to be approved[3].

Activities are divided into two main sectors in accordance with the two Royal Decrees: the first relates to blood transfusion centres (BTC), which are designated as "suppliers", whereas the second pertains to blood banks (BB), which are designated as "users". The BTCs are responsible for managing the donor population, organising blood collection, and preparing blood components to make them available to blood banks. They must be approved by the Ministry of Health. Currently, there are only four agreed BTCs in Belgium. Blood banks, which are in hospitals, serve to store and preserve blood, carry out compatibility tests and facilitate blood deliveries by name to the patient. It is of paramount importance that both entities can demonstrate the traceability of the product from donor to recipient to ensure the quality and integrity of the blood supply.

Most donations are made from whole blood, which in Belgium has not been used for transfusions since the end of the 20th century. Whole blood, considered

as "raw material", is therefore separated by the BTC into its components: red blood cells, plasma, and platelets. The development of preservation solutions has enabled the shelf life of each component to be extended, thereby optimising its therapeutic potential. Furthermore, transfusion indications have led to the medical community's implementation of a selective component therapy protocol, whereby only the component required for each pathology is transfused[4].

To obtain the necessary components, BTCs must first recruit and then retain enough donors. According to data published in 2020, Belgium has approximately 300,000 blood donors[5]. To minimise the risks associated with transfusion, donors must meet certain strict criteria. These criteria are designed to ensure the safety of both the donor and the recipient. The primary risks associated with blood donation relate to the health of the donor. The suitability of potential donors is determined by an interview and a medical examination. This process includes inquiries regarding the donor's general health status, including whether the donor is taking medications that could potentially alter the quality and composition of their blood. Furthermore, the risk of transmission of a disease by transfusion is considered. This assessment is based on several health parameters for endemic diseases, including periods of travel to areas endemic for certain blood-borne diseases, such as malaria or Chagas disease. Additionally, questions regarding risky behaviour are included to reduce the risk of transmission of blood-borne infectious agents, including hepatitis viruses and HIV, which may be caused by new piercing or a recent change of sexual partner. It is obvious that prophylactic measures, such as vaccination, can effectively reduce the risk of contamination for certain blood-borne infections, such as hepatitis B[6]. All contraindications are reviewed by a physician during the pre-donation interview. Generally, individuals who are at least 18 years old, in good health, weigh more than 50 kg, and are not at risk of transmitting a disease through blood are eligible to donate. To ensure the safety of the donation procedure, it is essential that the donation remains voluntary, and that no remuneration is offered in return.

Each type of donation is associated with an authorised annual frequency to protect the donor. This frequency is linked to the component taken and the time required for it to be reconstituted by the human body. This approach ensures the donor's immediate and long-term safety, as well as the quality of the collected blood product.

2. IMPACT OF BLOOD DONATION ON PERFORMANCES

It is important to recognise that whole blood donation is not a trivial process, and that the effects of donation should be taken into consideration. The average circulating volume of an adult is estimated to be approximately 5 litres. A standard donation of 450ml would therefore result in approximately 10% of this volume being removed. Furthermore, the oxygen-carrying capacity of red blood cells is diminished because of the donation process. In the general population, the impact of blood donation on the daily life of donors is not significant. However, the possibility of certain adverse effects following a donation cannot be ruled out, and these may have an influence on the donor. Furthermore, there is a correlation between body weight and the occurrence of adverse events[7]. Such adverse effects may be classified into several categories, including lower exercise tolerance[8], exercise-induced tachycardia[9], and increased HR[10]. In the general population, donating blood can also lead to fatigue, especially in cases of iron deficiency[11]. The occurrence of syncope, orthostatic hypotension and hypotension may be reduced by ensuring the availability of salty snacks and water, and by implementing strategies to engage the muscles of the lower extremities[12]. Although these effects may potentially have a noticeable impact on sporting performance, it is unlikely that this will be a limiting factor for the general population. However, for those engaged in high-level competitive activities and seeking to enhance their performance, such limitations may be more pronounced. In alignment with these considerations, the American Red Cross recommends that donors refrain from engaging in strenuous or exhaustive physical exertion on the day of donation[13]. Given that oxygen transport capacity is linked to the availability of red blood cells, it follows that a donation of red blood cells, as opposed to other components of the blood, will impact on oxygen transport capacity and therefore the performance of top-level athletes. These competitors require all their resources to achieve optimal performance, and therefore any limitation in the supply of red blood cells, or indeed any reduction in oxygen transport capacity, could have a significant knock-on effect on their ability to perform at their best. The variables most closely monitored in the assessment of oxygen transport capacity are those related to the quantity of red blood cells (RBCs), the concentration of haemoglobin, an iron-rich carrier protein present in RBCs, or haematocrit, the volume occupied by RBCs in whole blood[14].

A comprehensive review written by Van Remoortele et al. was conducted to elucidate the impact of a standard blood donation on exercise capacity and oxygen uptake[15]. The review covered both maximal and submaximal exercise and set out the effects on haematological variables[15]. It was found that a blood donation had an immediate impact on oxygen transport capacity, with effects observed 24, 48 and 72 hours later in relation to Hct. The effects on haemoglobin (Hb) and red blood cells (RBCs) were sustained for up to 14 days following the donation. The analysis revealed that these haematological effects exert varying influences on physical performance—specifically, VO_2 maximum and exercise capacity or time to exhaustion—depending on the nature of the exercise performed on the heels of the donation. Moreover, a reduction in VO_2 max and exercise capacity was also observed at 24- and 48-hours post-donation, exclusively in relation to maximal exhaustion[15]. These findings were also corroborated by Panebianco et al.[9] in studies involving amateur and competitive cyclists and by Meurrens and colleagues[16] in assessments of endurance capacity among athletes with moderate training levels. Judd and colleagues demonstrated that the time to fatigue remained consistent despite a reduction in maximum oxygen consumption (VO2peak). This suggests that there was potential compensation between the anaerobic and aerobic systems, enabling exercise performance to be maintained after the donation[17]. With respect to submaximal exercise, Van Remoortele et al. demonstrated that this reduction in oxygen transport capacity did not affect the observed variables, including VO_2 at the exercise threshold to 180 bpm[15]. These findings suggest that for high-level competitive athletes, the physiological effects may be significant[15]. Considering these findings, it is recommended that different guidance should be provided for athletes engaged in competitive and recreational activities[15]. This is precisely the approach adopted by the Australian Red Cross. Its guidelines for athletes stipulate that those competing at the highest level should refrain from donating blood during their competitive season, as the donation may affect their performance[18]. It may therefore be advisable to encourage athletic donors to consider plasma donation, thereby conserving their red blood cells (RBC) and ensuring that a substantial quantity of plasma is also collected. Nevertheless, the existing literature suggests that plasma donors may also experience effects on high-intensity performance, despite the absence of alterations to oxygen transport capacity[19]. Indeed, studies have demonstrated that regular and highly regular plasma donors exhibited increases of 10-20% in time to exhaustion, lactate level,

and oxygen deficit, while maximal oxygen consumption (i.e., VO2Max) remained unaffected[20]. Regular plasma donations can also induce iron deficiency[11]. It is recommended that athletic donors are provided with guidance and assistance to enable them to plan their donations in a way that is consistent with their respective training programmes. This approach should help to minimise any potential impact on performance while maintaining their ability to donate blood. In addition, iron supplements may be provided to help limit any impact on muscle function and preserve muscle function[21,22].

The Tier 1 Special Operations Forces (SOF) unit represents the pinnacle of military capability within the Belgian Defence. To draw an analogy with a sporting competition, the unit could be on a par with high-level athletes, in terms of both the exceptional abilities and the rigorous training and selection process that they undergo. Indeed, some within the field have referred to them as "tactical athletes"[23]. However, the context of the soldiers' performance differs from that of athletes. In contrast to athletes, who can be defined by a restricted spectrum of performance linked to their preferred discipline and whose training is geared towards performing optimally for well-defined objectives, these soldiers must demonstrate proficiency across a multitude of domains and excel under a variety of circumstances all year round[23]. Special Forces operators are required to demonstrate excellence in a range of competencies and to maintain optimal performance at all times, while accounting for the unforeseen circumstances inherent to their assignments. Furthermore, the missions of these operators are not solely characterised by the execution of intricate tasks; they also entail the maintenance of cognitive and psychological capabilities. These abilities are crucial for enabling adaptation and optimal functioning in contexts of instability, where the capacity for judgement and analysis is of paramount importance for survival. Nevertheless, despite the lack of empirical research on the impact of blood donation on cognitive functioning, existing evidence suggests that blood donations do not impair the cognitive abilities of blood donors[24].

A few studies have already been conducted on a military population, encompassing both physical performance and cognitive assessment[25–27]. A Norwegian research team demonstrated that performance levels in pull-ups, sit-ups and shooting tasks remained consistent, and no significant difference was observed in VO2Max following the standard donation of 450mL[27]. Lactate values and heart rate did not exhibit any notable changes following the donation,

according to the findings of Strandenes et al.[27]. By contrast, a further study conducted by the same research team demonstrated a notable reduction in VO_2 max levels in elite military personnel engaged in strenuous physical activities following a blood donation[25]. A reduction in both maximal and submaximal performance was observed in subjects tested 24 hours after the donation[25]. However, no difference was observed in the time spent engaging in physical exercise following the donation, indicating that this activity was not impacted by it[25]. For the first time, they demonstrated an increase in heart rate. The discrepancy can be attributed to the study setting. Notably, the post-donation assessment was conducted following a rigorous exercise regimen that included prolonged physical activity, along with dietary and hydration protocols. The researchers propose that compensation mechanisms may be impaired under conditions of stress, resulting in a decline in performance[25]. Additionally, Nadler and colleagues have conducted a study of this nature, utilizing military tasks to illustrate clinically significant changes associated with combat[26]. This study presents a more detailed cognitive analysis of the effects of blood donation than that carried out by the Norwegians, which was limited to a shooting exercise. It is evident that a decline in physical performance can have deleterious effects for soldiers who must donate blood. However, as previously stated, it is also important to consider the preservation of their cognitive potential as this can be an important weapon in combat situations. A battery of tests was employed, including shooting tasks, reaction time tasks, a rapid calculation task and a self-assessment of fatigue and exhaustion[26]. It is therefore evident that the aforementioned tasks are reflective of the performances considered crucial for combat. No significant differences were observed in the cognitive tests after the blood donation. In terms of physical performance, no significant differences were noted following the donation, as evidenced by the HR data obtained and monitored before and after the donation[26]. Upon conclusion of the analysis of these test results in a military context, it can be asserted that the participants' combat skills remained intact.

3. TRANSFUSION IN THE BELGIAN MILITARY CONTEXT

The Belgian Ministry of Defence, which is subject to the same legislation, no longer has an approved BTC as of 2019. As a federal entity, the Ministry relies on the supply of blood products from the French-speaking and Dutch-speaking BTCs of the Belgian Red Cross. These products are used in the military hospital's blood

bank, which is dedicated to territorial and operational support, the latter including support during operations and exercises. Consequently, an agreement outlining the respective roles of all parties involved is binding to both the Belgian Red Cross and the Belgian Ministry of Defence.

The medical component of the Defence is responsible for the physical and mental health of personnel. This responsibility must be fulfilled prior, during, and after military missions, whether at home or abroad. The two principal missions of the component are to ensure the medical readiness of the forces and the operational readiness of the medical forces[28]. Transfusion support is organised and managed within this component.

The Military Hospital Queen Astrid (MHQA) has a Military Medical Laboratory Capacity (MMLC), which serves as a clinical biology laboratory for its territorial part and as a Point of Care Testing (PoCT) and transfusion support department for its operational part. Blood products intended for use in operations are separated, prepared, and packaged on national territory by BTCs before being sent on operations by the MMLC, where medical support is organised. It is essential that blood components intended for transfusion in operations are managed with great care in the medical logistics system, given their sensitivity to temperature and short shelf life. Leukoreduced red blood cells (RBC) have a shelf life of 42 days from the day of collection and must be stored between 2 and 6°C, with the most optimum storage temperature being 4°C. Fresh frozen plasma stored at -20°C for a period of up to three years represents the most utilized option. It is, however, not advisable to send fresh frozen plasma in operation due to the logistical difficulties of storage in a frozen state. An alternative, freeze-dried plasma, is therefore recommended as a more robust solution. The freeze-dried version of plasma can be stored in a powdered form at temperatures between 2 and 25°C, protected from light. This method reduces the product sensitivity to temperature and allows for longer storage periods. In contrast to plasma, platelet storage times are limited to only 5 days' post-collection, with the requirement that they be stored in a shaker at a controlled temperature of between 20 and 24 degrees Celsius. This explains why platelet availability in operational settings is frequently constrained by the time required to deliver them in operational theatres and by storage conditions that cannot be met.

In accordance with the bilateral agreement, the Defence Department is responsible for promoting blood donation and organising the collection of blood

within its own military quarters. Recruiting donors among its staff and retaining them is one of the Department's commitments. The Defence Department employs personnel from a variety of different job profiles, including combat unit personnel, combat support unit personnel and support unit personnel. Each job requires specific skills that must be guaranteed both for the safety of the personnel and for the accomplishment of their mission. The military population is a distinct demographic group that does not reflect the average Belgian citizen. Its members are recruited according to rigorous standards. From initial recruitment to retirement, physical, medical, and psychological criteria are considered. Those who are posted abroad must obtain a certificate of medical fitness, which attests to their ability to meet the minimum medical standards required for their specific job profile. This typically entails a Type A medical qualification. To that end, military personnel are subjected to an assessment at the MHQA before each mission. This process ensures that they are in optimal health and possess the requisite physical and psychological abilities. As a result, military personnel are deemed to be excellent potential donors due to their good physical and mental health, athleticism, and well-trained status. However, specific qualifications (e.g., those involving diving or high-altitude jumping) or functions (e.g., those of pilots or operators of heavy machinery) are associated with greater risk-taking, and individuals engaged in these activities are not considered as potential donors due to safety concerns regarding the performance of their duties[29].

In addition to the recruitment of donors, the Defence Department is responsible for the supply, storage, and issue of blood products during operations. This role is comparable to that of a blood bank. However, some operational theatres are more challenging to reach than others. This is explained by both geographical factors and the availability of appropriate evacuation means. This is particularly pertinent when considering the personnel on Belgian ships or special forces units. The logistics provided by the navy are robust, but the sailors are frequently on the move and far from the logistical infrastructure required for the supply and/or evacuation of personnel. In contrast, the special forces are employed in small, isolated teams, operating deep within enemy territory, which significantly restricts the possibility of evacuations within a reasonable timeframe.

4. MILITARY CONTEXT: TACTICAL MEDICAL SUPPORT CONCEPT

The role of the medical component within the armed forces is to maintain or restore the combat capability of troops from a medical perspective. Given the ever-changing nature of military operations, medical support must be highly flexible to respond to the constantly evolving needs of the battlefield. This flexibility is reflected in the increased mobility of medical teams, which are deployed closer to the frontlines to provide support to the troops in the field. To make the system as resilient as possible and given the distances that must be covered during operations, effective pre-positioning of resources represents a critical element of planning. The most fundamental principle of medical care is that the patient must receive treatment at the earliest possible opportunity, with the goal of improving the chances of survival. This principle is well-established in Belgian emergency services, where the objective is to provide "the fastest appropriate help". It is a principle that is equally applicable when deployed abroad.

This concept is fully integrated into military practice under the term "medical planning timelines," which is defined in the document dated 16 September 2011[30]. It functions as a planning instrument that supports tactical command in providing the requisite amount of medical support for its personnel and adapting it to its mission, considering logistical limitations. To this end, the command has established a chain of medical entities or medical treatment facilities (MTFs), which are collectively referred to as "roles". Each of these roles is responsible for providing a specific set of capacities and possesses varying levels of authority and capabilities.

The different roles correspond to the levels of treatment capacity required for war casualties. It is therefore necessary to place and distribute these capacities in an appropriate and efficient manner over the territory to be covered by the medical support to best meet the deadlines mentioned in the timeline: the 10-1-2 concept. This necessitates the deployment of a non-medical military personnel trained in Tactical Combat Casualty Care[a] (TCCC)[31]. He or she must take actions at the Point of Injury (POI) within 10 minutes of the injury to control the haemorrhage and clear the airways. It is then necessary to transfer the injured person to a medical facility

[a] Prior to deployment, military personnel undergo training in the Tactical Combat Casualty Care (TCCC) programme, which encompasses several techniques and tactics designed to facilitate immediate medical care or buddy aid in the event of enemy fire. This enables non-medical soldiers to assist their colleagues in need.

within an hour, which must have resuscitation capabilities, including blood products. This level corresponds to Damage Control Resuscitation (DCR) and is performed at "role 1" level. DCR (or RDCR in its remote version[32]) represents a strategic concept for prehospital resuscitation, with the aim of reducing morbidity and mortality through transfusion[33–35]. This systematic approach to the management of trauma patients is designed to limit blood loss and prevent coagulopathies. This process should begin in the prehospital setting and should be initiated with the early administration of blood following the implementation of massive transfusion protocols at the site where the soldier sustains their injury, or the POI[36]. Within two hours of the incident, the injured soldier should have been transferred to a first surgical unit, also known as "role 2". This is where basic surgical procedures directly related to the patient's survival will be performed, with the aim of controlling the haemorrhage. This is the Damage Control Surgery (DCS) stage. At this point, the patient is deemed stable and may be evacuated over long distances to a hospital on national territory or the equivalent in an inter-allied deployment, in which case it will be classified as a "role 3". The subsequent role must possess the capacity to provide a level of reconstructive surgery to patient before his transfer to a role 4 or an environment conducive to rehabilitation before returning to the operational theatre to continue the mission. Throughout the patient's evacuation from one medical facility to another, continuity of care must be ensured. This concept is referred to as the continuum of care (Figure 1). The proposed approach entails alternating between "scoop and run" emergency care provided at MTFs and "stay and play" care at the MTF level. It also allows for the decision to skip certain roles to evacuate a patient to an appropriate MTF, considering the patient's condition and the availability of MTFs at the time of evacuation.

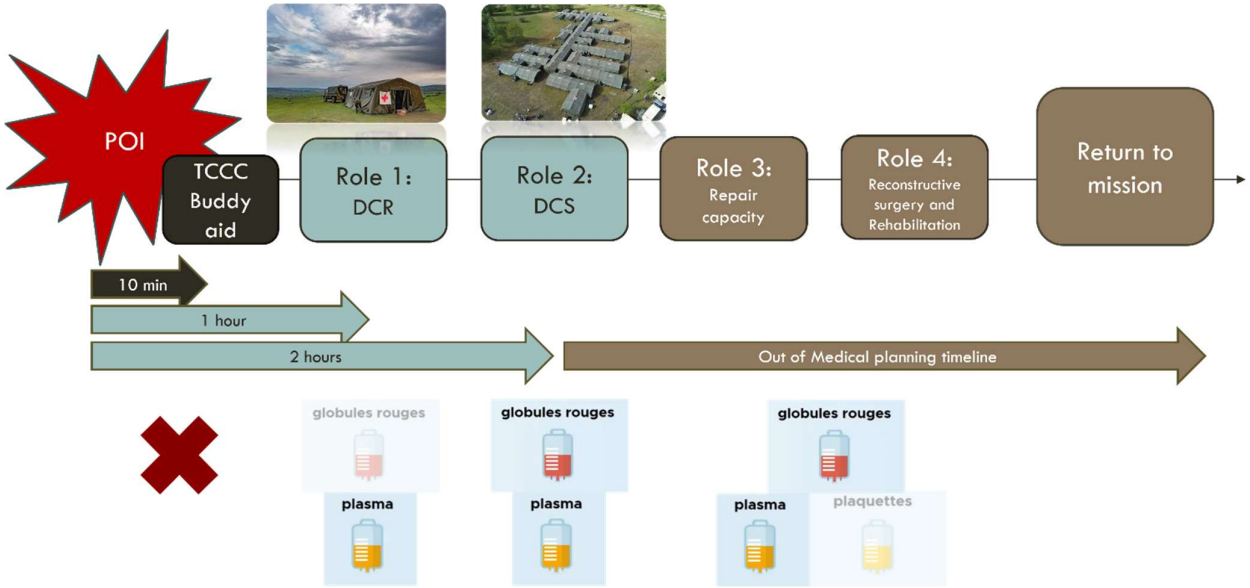

Figure 1: This schema represents the continuum of care organised by military medical support and is structured according to increasing medical capacities. It is designed to ensure that the war casualty receives care at the earliest possible point, at the point of injury (POI). Tactical combat casualty care (TCCC) is a technique taught to military personnel to enable them to take care of their colleague until he is evacuated to be treated by medical personnel specialised in the role or medical capacity. The care structures are organised in increasing medical capacities. The first role is represented by the damage control resuscitation (DCR) capacity. This requires the presence of blood products and is managed by an emergency physician. The second role has a basic surgical capability (i.e., DCS) which enables the stabilisation of the patient before evacuation. The role 3 provides repair surgery and the role 4 reconstructive and rehabilitative surgery. These ultimately enable soldiers to return to combat within a timeframe appropriate to their injuries. The timeframes required by NATO for each type of treatment are indicated by the grey arrows at the bottom of the diagram. (Figure retrieved from Allied Joint Doctrine for Medical Support AJP-4.10)[37].

5. BATTLE WOUNDS AND AVOIDABLE DEATHS

War-related injuries are characterised by specific causes, including firearms, artillery fire effects, blast, stab wounds, shrapnel, and the systematic perforation of soft tissue and organs, fractures, amputations, and burns, which frequently require the administration of massive transfusions[38].

The Eastridge team demonstrated that, of casualties who died on the battlefield (Killed in Action, KIA), a quarter of deaths could have been prevented if medical care had been more rapid and appropriate (Figure 2). Nearly 90% of these so-called avoidable deaths were caused by massive haemorrhage following injury[39].

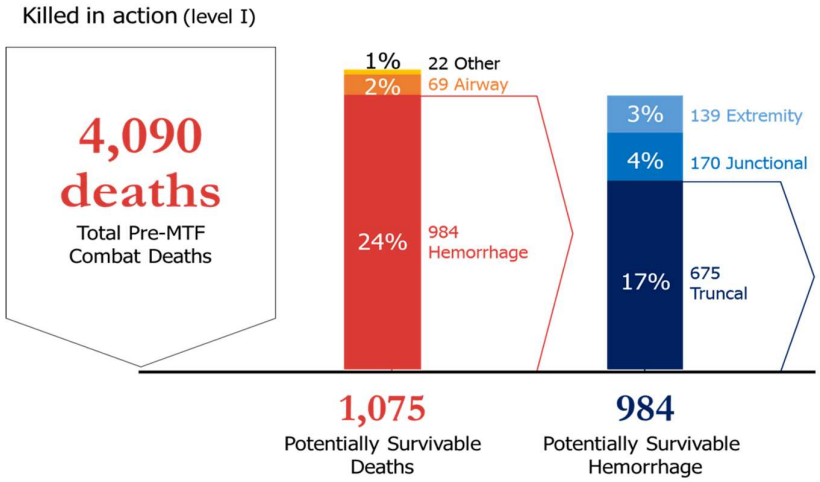

Figure 2: The figure illustrates the proportion of deaths from injuries sustained in combat (Killed in action = 4090) that could have been avoided if they had been treated in a pre-hospital setting (1075). Most of these deaths are due to haemorrhage, as indicated by the proportion in red (984). Of these, the most affected are those involving the upper body, as shown in the dark blue inset (675). The figure is based on data presented in Eastridge et al. (2012)[39].

TCCC Training for non-medical personnel to perform essential actions such as placing tourniquets or maintaining compression points has been shown to improve survival rates in soldiers awaiting medical assistance[40]. However, it has also been demonstrated that access to transfusion management prior to arrival in an MTF has a positive impact on survival rates[41]. The utilisation of blood products in the pre-hospital environment or in the pre-MTF military context has been demonstrated to considerably reduce the risk of death. However, this study goes further and

demonstrates that the time taken to administer the product can influence the mortality rate. Indeed, the earlier the product is used, the greater the chances of survival. Consequently, it is imperative that blood products are made available as close as possible to the point of injury (POI) to minimize mortality in the field. Massive haemorrhage is the primary cause of potentially avoidable deaths. By treating these injuries as close to the POI as possible, the administration of blood products has the potential to significantly improve the vital prognosis of casualties. This highlights the importance of the DCR concept and, in conjunction, the Golden Hour principle[42].

Theoretical principle of the Golden hour

The concept of the Golden Hour is outlined by Trauma, Haemostasis and Oxygenation Research (THOR). The NATO Blood Panel, a NATO-affiliated working group involving leading national transfusion experts[43], emphasises the significance of speed in managing haemorrhagic casualties and the necessity of ensuring the availability of blood within one hour, which is known as the Golden Hour. This principle entails an optimal transfusion management timeframe that is expected to enhance the recovery of war casualties, as evidenced in Iraq and Afghanistan[6,44]. Nevertheless, subsequent studies have demonstrated that the Golden Hour may not be as effective as previously thought, suggesting that the current approach may still be too slow to reduce mortality (Figure 3).

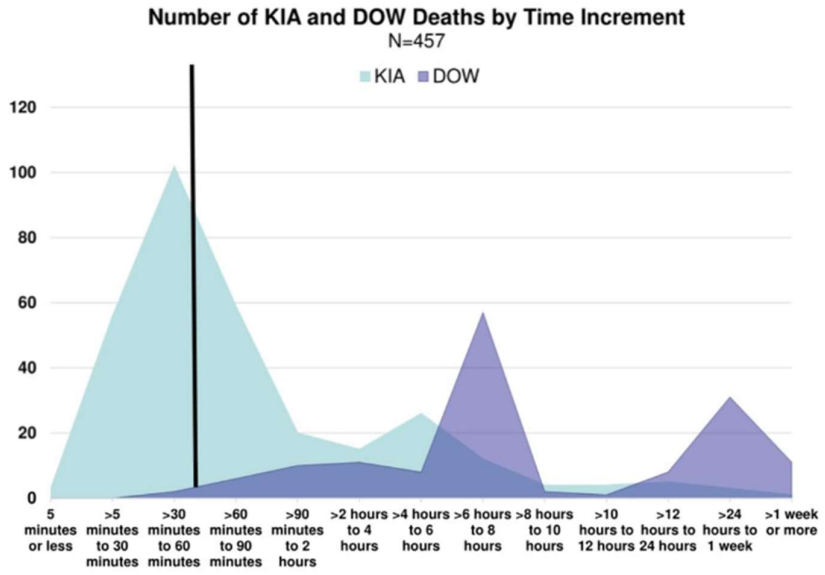

Figure 3: The following graph illustrates the temporal trend in mortality rates resulting from injuries. The green area represents the number of combat-related deaths in a pre-hospital context (i.e., Killed In Action, KIA). The blue area, in contrast, depicts the number of injury-related fatalities during the care process (i.e., Dead Of Wound, DOW). The solid vertical line represents the golden hour limit; therefore, it is proposed to reduce the advised time for access to blood supplies, represented by the golden hour, to 30 minutes. Then, it can be observed that by adhering to the aforementioned delay, a significant reduction in the number of combat deaths represented by the area under the green curve would be achievable. (Figure retrieved from THOR-RDCR)[45]*. The graph includes the following abbreviations: KIA = Killed in action, DOW = Dead of wound, POI = Point of injury.*

From the perspective of war injury typology, these haemorrhages are typically regarded massive and rapidly become life-threatening through the involvement of a lethal triad, which has been described by David et al. (2013)[40] as a vicious circle (see Figure 4). This is a combination of three self-perpetuating phenomena: hypothermia, coagulopathy, and acidosis.

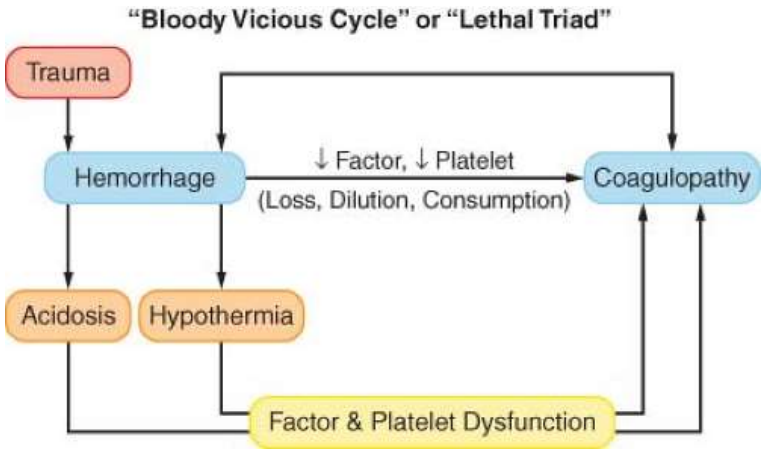

Figure 4: The schematic representation of the lethal triad demonstrates that the massive haemorrhage that occurs following trauma causes hypothermia and acidosis. These two factors induce coagulopathy because of platelet and coagulation factor dysfunction. This ultimately results in an exacerbation of the bleeding, which, in turn, accelerates the vicious circle until the patient died. This cycle is characterised by a loss of coagulation factors and platelets, which in turn increases coagulopathy. However, the only means of avoiding this is to promptly implement measures aimed at controlling the bleeding, rectifying the acidosis, and precluding the development of hypothermia. (Figure retrieved from Capan, Trauma and Burns)[46].

The primary consequence of significant blood loss is hypothermia. This phenomenon occurs due to the blood's inability to serve its thermoregulatory function of continuously circulating a warm liquid. The loss of heat prolongs the clotting process, as evidenced by a reduction in the effectiveness of coagulation factors and a reduction in platelet activation. Furthermore, hypothermia, particularly when associated with acidosis, induces platelet dysfunction, and reduces the activity of coagulation enzymes[47].The combined effect of these factors influences the consistency of the clot and, consequently, the extent of bleeding[48]. In addition to hypothermia, tissue hypoperfusion resulting from the loss of circulating fluid causes acidosis. To restore oxygen transport conditions, it is essential to compensate for blood loss by transfusion[35]. Failing to do so will result in the tissue becoming ischaemic. This deficit can be assessed over time and may be accumulated, resulting in an overall oxygen debt that may be the underlying cause of shock. In combination with a reduction in renal function and therefore the presence of H+ ions, this phenomenon will result in a reduction in pH, which in turn affects the effectiveness of coagulation factors. In addition, coagulopathies—which

may be exogenous, resulting from dilution, or endogenously caused by loss of clotting factors—constitute another significant factor contributing to the risk of shock[49]. This substantial blood loss and hypotension, accompanied by vasodilatation, promote the recruitment of interstitial fluid into the vascular compartment by osmosis, thereby diluting coagulation factors still present in the circulation. The phenomenon of haemodilution may be further enhanced by the utilisation of infusion filling solutions. The phenomenon may be described as exogenous in nature, with its occurrence largely attributable to the extensive utilisation of fluids for the purposes of resuscitation[50]. It has been demonstrated that the administration of any type of infusion, including 0.9% sodium chloride, colloids, and crystalloids, even if it allows for volume maintenance, contributes to the haemodilution observed in the patient. Consequently, these agents facilitate a prompter onset of shock, attributable to platelet dysfunction. They also precipitate trauma-induced coagulopathy (TIC) and disseminated intravascular coagulation (DIC)[51,52] The simultaneous occurrence of these three phenomena will result in such a deterioration in the patient's condition that it cannot be stabilized even with subsequent efforts. This is referred to as haemorrhagic shock. To prevent the development of this state of shock, it is essential that blood products with a lower haemodilution effect and an optimal pH be administered as soon as possible in the evacuation chain.

The initial step in the treatment of a casualty is therefore to identify those at risk of developing shock by making a reliable diagnosis. In the context of operations, this necessitates the utilisation of the available resources, including clinical signs and, in some instances, biological measurements, contingent upon the availability of a field laboratory. Regarding clinical signs, the absence of a radial pulse or a very weak radial pulse, in conjunction with an altered mental state, has been demonstrated to be 91% specific in the onset of shock[48]. It should be noted, however, that there are more precise criteria, which are more specific but also more difficult to measure in an austere environment. The aforementioned criteria include a systolic pressure of less than 110 mmHg, a heart rate of greater than 105 bpm, a haematocrit level of less than 32%, a pH of less than 7.25, or an INR (coagulation test derived from prothrombin time) of greater than 1.4[36]. Biomarker measurement can assist in the refinement of shock diagnosis and severity estimation when combined with observed clinical signs[36]. The measurement of lactate above 4-5 mMol/L has been identified as a promising point-of-care (PoCT)

measurement for predicting high-risk trauma and haemorrhagic shock. This is a direct consequence of acidosis, which can be measured in patients in shock and subsequently monitored every 30-60 minutes[35].Furthermore, this measurement can be conducted at an early point in the management process, as it requires minimal logistical resources and is relatively straightforward to implement and interpret[35].

Should a patient be identified as being at risk or suffering from shock, it is vital that they are treated as quickly as possible with blood products to avoid the development of the lethal triad. In addition to its function as a simple fluid, blood can be considered as a vital organ or system, which is in turn integrated with the endothelium. The three essential components of blood—red blood cells (RBCs)[34,35], plasma[53] and platelets[34]—work together to ensure optimal oxygen transport and haemostasis in the right place at the right time. These components relate to each other as well as with the surrounding endothelium to facilitate their respective functions. In the absence of any of these components, the blood is unable to perform its full capacity. Furthermore, a supply of platelets and coagulation factors has been found to positively impact survival by correcting coagulopathy[54].

The theoretical basis of coagulation

Coagulation constitutes the principal response to a breach or lesion in a blood vessel. It represents a complex regulatory mechanism involving several coagulation factors and platelets. This process begins with primary haemostasis, which occurs in various interrelated phases, including platelet adhesion, activation, secretion, and aggregation, as shown in Figure 5. Finally, secondary haemostasis may occur, which aims to stabilise the platelet plug by the addition of fibrin produced at the end of the coagulation cascade, as illustrated in Figure 5. In the event of vessel damage, two crucial proteins are exposed: collagen and tissue factor (TF). These act as initiators of the coagulation process through their ability to stimulate platelet activation. Additionally, collagen can trigger the extrinsic and intrinsic pathways of the coagulation cascade. These two cascade activation pathways result in the generation of thrombin, a protein responsible for the transformation of fibrinogen into fibrin. The latter substance solidifies the platelet plug at the site of the breach. Concomitantly, the coagulation cascade continues to expand. Activated platelets secrete molecules which act as recruiters and activators of neighbouring platelets, forming a chain. The recruited platelets, in turn, are activated by the coagulation

cascade and the presence of collagen. Once sufficient fibrin is available, the cascade is stabilised.

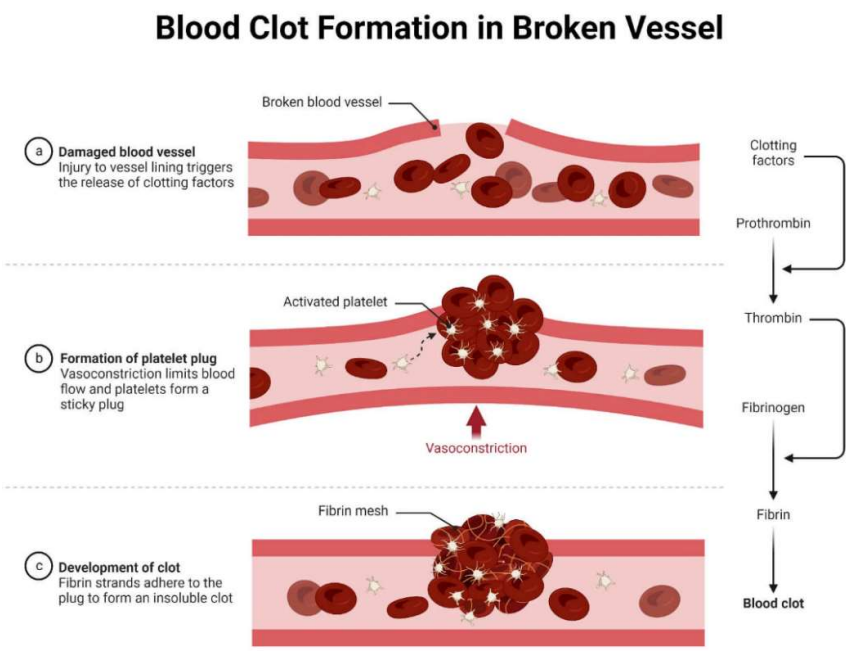

Figure 5: Schematic representation of the sequence of events that lead to platelet activation and aggregation during the initial stages of coagulation, commonly referred to as primary haemostasis (step a and b in the figure), is presented. Primary haemostasis represents the initial phase of coagulation, during which a platelet cluster is formed at the site of endothelial breach. The successive stages of adhesion, activation, and aggregation lead to the formation of the platelet plug. The initial phase of breach appearance is characterised by the emergence of key factors such as tissue factor and collagen, which serve to attract platelets, facilitate their adhesion and engage the coagulation cascade involving clotting factors. Subsequently, platelet activation markers are expressed on their surface, thereby recruiting, and activating other platelets, which represents the activation phase. Finally, during the aggregation phase, other activated platelets around the site of injury aggregate to form a cluster, thereby effectively closing the breach. The coagulation phase (or secondary haemostasis represented in step c), is responsible for the formation of fibrin to hold the clot. The coagulation phase is initiated by the conversion of fibrinogen, a coagulation factor, into fibrin, a protein that acts as a sealant to reinforce and stabilize the platelet cluster. This process is mediated by thrombin, a product of the coagulation cascade. (Figure retrieved from HEMOSTASIS- DEFINITION, MECHANISM, SIGNIFICANCE (MICROBENOTES.COM)[55]

Blood components represent the optimal solution for enhancing the standard of medical care. However, they are temperature-sensitive, and their shelf life is relatively short[2,3,56]. Consequently, it is essential to implement a thorough planning process to ensure their availability at the earliest stage of the care process. This requires the management of the cold chain, the training of staff and the fine-tuning of logistics. It must be acknowledged that the operational theatre medical support chain, even if carefully planned, will inevitably encounter setbacks, and changes due to the inherently volatile and unpredictable nature of the operational environment. This will have an impact on material resources, transportation times, means of evacuation, and ultimately the availability of adequately trained personnel. Nevertheless, Belgium, like other NATO countries, must strive to provide a standard of medical care that is equivalent to that which the casualty would have received in his country of origin[37].

In light of the findings of numerous studies, a variety of suggestions were made regarding the specific management of trauma with haemorrhage. One of these suggestions indicated that component therapy utilising ratios comparable to those observed in whole blood or the '1:1:1' approach for Red Blood Cells (RBC), Fresh Frozen Plasma (FFP) and Platelets (PLT) yielded superior outcomes with respect to patient survival[57]. According to calculations performed in the United States, separate component therapy provides 660 millilitres of fluid at a haematocrit of 29%, 88,000 platelets per microliter, and 65% clotting factor activity (one unit of 335ml red blood cells with a haematocrit of 55%, one unit of platelet concentrate containing 551010 platelets, and one unit of 275mL fresh frozen plasma with an 80% clotting factor activity). By contrast, a unit of fresh whole blood diluted in 70 ml of anticoagulant (500 ml) has a haematocrit of 38 to 50%, containing between 150,000 and 400,000 functional platelets per microliter, as well as active coagulation factors[44,58] . These calculations demonstrate that whole blood is more favourable than component therapy in terms of avoiding the dilution effect and the coagulopathy that can result[44]. Whole blood provides access to the three components in physiological quantities, with the addition of a smaller quantity of a preservative solution. Furthermore, a single temperature for storage will be applied, which will facilitate the conservation of the product. Additionally, it limits exposure to donors, as the three products originate from the same individual.

The recent reintroduction of the use of whole blood in some countries has already demonstrated certain advantages[59–62]. Firstly, the right proportions of

components are available, which simplifies the supply chain. Secondly, clinical outcomes for patients are improved. One of the advantages of this product is that it can be stored in an adenine-free CPD (Citrate Phosphate Dextrose) storage solution for up to 21 days. This period can be extended to 35 days if adenine is included in the CPDA-1 (Citrate Phosphate Dextrose Adenine) storage solution[63]. It should be noted that the military medical supply chain of certain nations, not including Belgium, already has the capability of supplying whole blood in the field[64]. It is collected and prepared on national territory and delivered to the operational theatre or directly collected in the operational theatre from soldiers deployed in the same theatre.

The product is generally leukodepleted when prepared on national territory. It is group O and has a low haemolysin titer, which means that it can be considered a universal product. It is known as Low Titer O Whole Blood[65]. Furthermore, it appears to provide a survival benefit compared with that obtained with component therapy[66]. Leukodepletion has the beneficial effect of reducing the transmission of immunomodulators and viruses transmitted by white blood cells (WBCs), such as cytomegalovirus (CMV). The use of group O allows for the avoidance of significant risks associated with the transfusion of red blood cells, while the selection of donors with low titer of anti-A and anti-B antibodies in plasma helps to mitigate minor risks.

ABO compatibility principle

The ABO compatibility principle is a fundamental tenet of blood transfusion. This principle stipulates that a blood product may be transfused without eliciting a transfusion reaction in the recipient, thereby ensuring the recipient's safety.

The ABO compatibility rules differ for each blood product, and both the presence of antibodies and antigens derived from this system must be considered (Figure 6). Blood groups are identified by the presence of antigens on the surface of cells. The ABO system is the most important one, as it is associated with the presence of corresponding natural haemolytic antibodies. These antibodies lead to haemolysis and death if the compatibilities of this system are not respected. These antibodies are also known as haemolysins. An individual from group A has A-type antigens present on the surface of their red blood cells and antibodies in their plasma that bind to B-type antigens. In contrast, an individual from group B has B-type antigens present on the surface of their red blood cells and antibodies in their plasma that bind to A-type antigens. An individual with group AB has both types of

antigen present on their red blood cells but neither anti-A nor anti-B antibodies are present in its plasma. Consequently, this plasma can be employed universally in transfusions. Indeed, in the absence of antibodies, it will not elicit any minor transfusion reactions. Additionally, group O individuals exhibit the presence of both types of antibodies in their plasma, although they lack antigens on the surface of red blood cells. This is the reason why O RBCs are regarded as universal products. Therefore, they can be administered to all groups without inducing major transfusion reactions.

The introduction of an unknown antigen to the patient's body, where the patient has the antibodies to neutralise it, results in the development of a transfusion reaction of haemolysis. This reaction is classified as major incompatibility. The body continues to produce antibodies for as long as it can do so, aiming to destroy all non-compatible red blood cells. Conversely, if antibodies are introduced into the body and encounter the corresponding antigens, this causes a minor transfusion reaction. This is referred to as a minor transfusion reaction because it ceases once the antibodies are fixed, thus preventing the complete destruction of red blood cells.

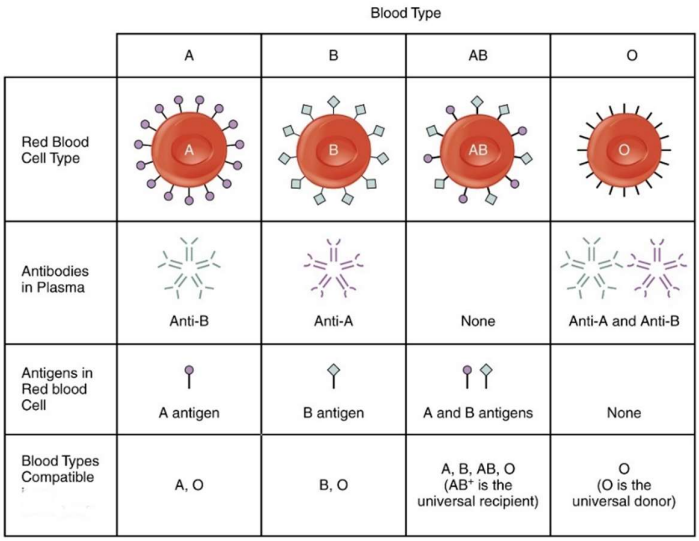

Figure 6: The table illustrates the presence of erythrocyte antigens and natural anti-erythrocyte antibodies to be considered by type of donor (i.e., A, B, AB and O) in order to summarise donor-recipients compatibility for red blood cell transfusions. (Figure retrieved from ABO AND RH BLOOD GROUPS | TRANSFUSION | GEEKY MEDICS)[67]*.*

Moreover, whole blood can also be collected on site from donors deployed on the same site. The blood of a compatible colleague could potentially address many of the issues previously discussed. It will be available at the appropriate temperature and will be able to compensate for the loss of volume, as well as oxygen transport and restore haemostatic capacity. This would reduce the risk of shock and the onset of coagulopathies, which can lead to rapid death. Similarly, whole blood stored at a cold temperature and delivered by the logistics chain would also be beneficial. The onsite collection of blood, designated either a Walking Blood Bank (WBB) or an Emergency Donor Panel (EDP), constitutes an emergency protocol employed in extraordinary circumstances or austere environments, including on ships[68] and for special forces units[69]. Such isolated and/or logistically vulnerable units are inadequately supported in terms of blood product provision. It has been demonstrated that WBBs facilitate the rapid delivery of blood products when evacuation is delayed[70].

This protocol represents a significant advance in military operational support, addressing a critical gap for nations that have yet to reintroduce the use of whole blood, including Belgium. Some advanced nations, responding to evolving healthcare needs, have already developed, and implemented comprehensive protocols for military operational support. Norway is a prime example of such rapid development, showcasing a forward-thinking approach in this field[71]. To ascertain the efficacy and safety of the fastest possible care in austere environments, the Norwegian military has established the research programme 'Blood Far Forward' (BFF)[72]. This initiative encompasses the investigation of donor performance, optimal training techniques to ensure the secure collection and transfusion of blood in such settings and the utilisation of personnel with specific expertise and qualifications. Additionally, it includes the exploration of emergency blood collection protocols. The objective is to demonstrate the efficacy of whole blood, which would serve to provide data and evidence to change attitudes and encourage its wider use, potentially to the detriment of alternatives such as crystalloids and colloids[72]. Consequently, there is a clear lead in the development of WBB, which has already resulted in the development of a protocol, training and educational programmes, and kits for the collection, storage, and administration of whole blood[71]. Furthermore, the Norwegians have collaborated with the United Kingdom to develop an adapted questionnaire for blood donor selection[29]. Other nations, such as the United Kingdom, France, and the USA, have established protocols in

response to conflicts in the Middle East. These protocols have been in place for a considerable period, and feedback has been gathered from their use[38,59,73–75]. Thus, there is evidence of advantages associated with such protocols[60,61,76,77] and of feedback on contamination issues[78]. Furthermore, our French partner is the only country in Europe with an exceptional regulatory article for its Army use[79]. This article covers and provides a framework for blood collection in exceptional situations, despite restrictions set out in a European directive. This framework, which is considered necessary and complementary to the stocks of blood delivered on operations, constitutes an integral part of French operational transfusion support[80]. The various existing protocols exhibit minimal variation in their phases, regardless of national origin. An illustrative protocol is provided in Figure 7. The rationale for this approach is that the steps are comparable to the national blood collection process, which is relatively consistent across countries. Additionally, it is advantageous to avoid deviating excessively from existing protocols when developing a new one, particularly if it can be integrated into a larger system. This interoperability is a key objective in military healthcare, given its inherent expense and the need to deploy qualified personnel, which often results in the pooling of resources during deployment.

Such a protocol does not permit full compliance with all the articles of the Belgian law governing blood transfusion. Certain articles of the legislation that specify the obligation of preparation and testing stages are limiting in a logistically weak environment, such as that encountered in operating theatres. Consequently, the potential risks are reviewed in the light of an exceptional situation. This involves the deployment of exceptional resources and procedures. Even if these risks are increased, they are analysed and calculated to ensure their acceptance. A set of potential preventive measures is also included. Consequently, it can be postulated that the circumstances require different selection procedures for donors and the acceptance of associated risks[29,81].

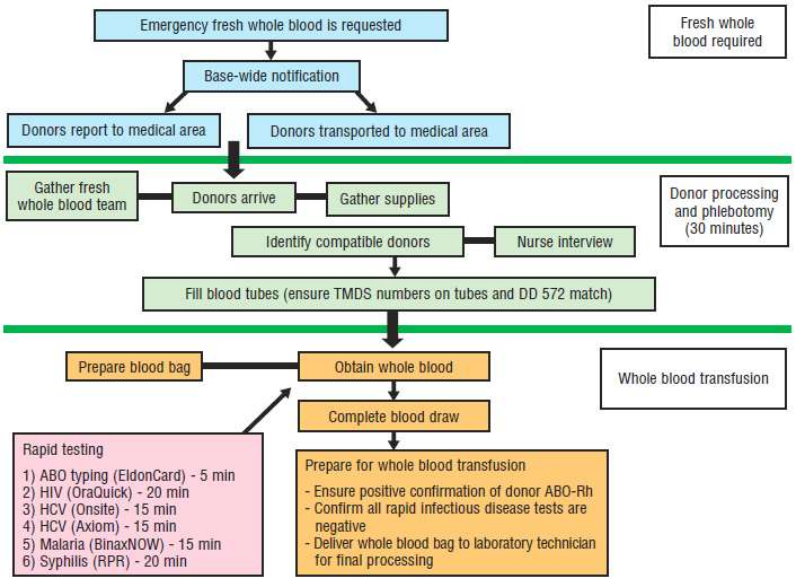

Figure 7: Sequence of events initiated for the collection of fresh whole blood as part of an emergency protocol. The protocol can be divided into three distinct phases: (1) the expression of the need for fresh whole blood; (2) the selection of donors; and (3) the collection and transfusion of blood. Phase 1 includes the steps required to call donors in response to the expressed need. The donors may be located in the vicinity of the medical facility or require transportation to it. In the second phase, the assembly of the personnel who are to implement the protocol and the preparation of the requisite equipment coincide with the arrival of the donors. Among these potential donors, those who are compatible must be identified and an interview conducted. The tubes for the rapid tests are then drawn and used for the rapid tests. The purpose of these tests is to confirm the blood type and to assess the potential risk of TTD (HIV, HCV, HBV, malaria, and syphilis). Details of the rapid tests to be carried out are shown in the pink box. In the third and final phase, the whole blood is collected. This phase includes the preparation for the transfusion, which entails the verification of the results of the rapid tests and their compatibility with the recipient. It is of paramount importance that the traceability of the donor, product, recipient, and the associated results are maintained throughout the process. Abbreviations: DD572 = Department of defence form 572, RPR = rapid plasma reagent, TMDS = Theatre medical data store, HIV = Human immunodeficiency virus, HBV = Hepatitis b virus, HCV = Human hepatitis C virus. (Figure retrieved from Goforth)[82].

6. RESEARCH QUESTION AND OUTLINE OF THE THESIS

In the light of the aforementioned considerations, Belgium is developing its own exceptional protocol with the objective of providing optimal transfusion care for war wounded. Each country is responsible for developing its own protocol in accordance with its national legislation and any higher directives[83,84]. The implementation of this protocol would provide Belgium with an additional capability in its medical support armoury, as well as enhancing its capacity for interoperability. This could facilitate participation in joint efforts to ensure the availability of blood products.

The aim of this research project is therefore to **propose an emergency whole blood collection protocol tailored to Belgium and its needs, its duties, and ambitions.** To achieve this, the following five research questions must be answered (figure 8):

1) What circumstances warrant the implementation of an emergency blood collection protocol in operation?

2) What risks are associated with implementing an emergency blood collection protocol and how are these risks mitigated?

3) What are the immediate effects of a 450mL donation on the physical performance and alertness of elite soldiers?

4) What are the immediate effects of a 900 mL donation on the physical performance, alertness, and decision-making of elite soldiers, and what is the recovery time for these functions to revert to their original state?

5) What is the optimal method for storing surplus blood collected in an emergency blood collection protocol within the first 48 hours that preserves all its therapeutic benefits for bleeding patients? Is there a difference in quality, and therefore efficacy in haemostasis, between cold- or warm-stored blood?

Chapter 1 presents the findings of a systematic literature review which identifies the underlying rationale behind the activation of an emergency protocol. The objective is to establish a framework of criteria to be considered in the formulation of Belgian recommendations with a particular focus on identifying and evaluating risk prevention and reduction strategies associated with this protocol. Additionally, a comprehensive risk management strategy is proposed to facilitate

the incorporation of standardised risk management approaches in the Belgian protocol (research question 1 and 2).

The issue of donor safety (i.e., research questions 3 and 4) is addressed in Chapters 2 and 3. This is a key area of investigation, as it ensures the safety of the mission and good health of our donors. It is essential for the safety of the donor, who is a deployed soldier, that he should still be able to carry out his mission both physically (endurance and resistance) and cognitively, despite the standard donation of 450ml of whole blood. This issue is analysed in chapter 2, using two settings, and focusing on special forces teams operating in isolated and dangerous locations. The initial assessment is conducted in a controlled environment, namely a sports laboratory. Following this, an analysis is carried out in a more ecologically valid environment incorporating tasks carried out by soldiers under comparable conditions of fatigue and climate. Chapter 3 goes further, investigating the impact of a double blood donation on the military performance of SF operators. It should be noted that if the protocol is aimed at a small special forces team, there is a significant risk of running out of resources or even compatible donors. The RDCR management of a casualty may require numerous transfusions. However, it may not be possible to collect enough compatible products. A strong temptation may exist to collect more than one blood bag per donor; this is precisely where the risk lies of endangering the donor to maintain the patient in a stable state. Therefore, it is essential to understand the limits of the protocol by testing the impact of a double donation of 900 mL on performance. This analysis employs a controlled experimental approach, conducted in a standardised environment, to ensure the safety of the participants. The performance analyses of both the standard donation and double donation scenarios are designed to inform the optimal donor management practices to guarantee their safety.

Chapter 4 addresses the quality of collected whole blood (research question 5). Whole blood is either utilised immediately, at a physiological temperature, as part of an EDP or stored at a cold temperature to build up a WBB or replenish an existing one. In the event of a patient's death, transfer or evacuation, the blood collected in the context of an EDP may not be used. It is therefore important to determine the storage requirements in the event of such an occurrence. The analysis provided in this chapter allows us to determine the optimal product for use in the treatment of haemorrhagic injuries, based upon its metabolic properties, haematological

parameters, and haemostatic potential. The products under consideration are either whole blood stored cold or whole blood stored hot.

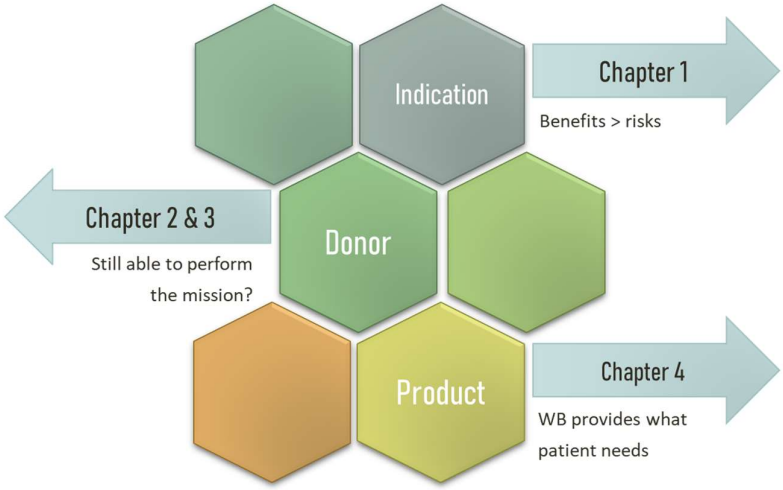

Figure 8: The following figure provides a summary overview of the structure of this research project according to the themes addressed and the chapters associated with the analysis. The objective of each chapter of this thesis is to answer a research question on a specific theme. This is depicted in the hexagons shown in the figure.

The discussion presents the formulation of the most appropriate recommendations for Belgian Defence, based on a comprehensive analysis of the responses to each of the research questions. In addition, the inherent limitations of each recommendation are addressed. Furthermore, a protocol has been developed in collaboration with the clinicians concerned, anaesthetists and emergency physicians and presented in the discussion of the thesis, providing a framework for the implementation of the recommendations. Additionally, a decision-making algorithm is also proposed and discussed in the thesis.

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CHAPTER 1:
A SYSTEMATIC REVIEW OF INDICATIONS WHEN AND HOW A MILITARY WALKING BLOOD BANK COULD BRIDGE BLOOD PRODUCT UNAVAILABILITY.

Published article:
Degueldre, J., Dessy, E., T'Sas, F., & Deneys, V. (2024).
A systematic review of indications when and how a military walking blood bank could bridge blood product unavailability: When and how a walking blood bank can be a life-saving protocol?.
Blood Transfusion.
10.2450/bloodtransfus.603

ABSTRACT

BACKGROUND - Blood supply problems in remote areas are well known. To overcome this shortage, many countries have developed innovative walking blood bank (WBB) protocols. However, no common standards have yet been set for their use and common actions. Given that these procedures involve a certain risk, it would be interesting to analyse the activating criteria that lead to using this unusual protocol. Thus, this review aimed to identify indications for a WBB and the common risk mitigation measures.

MATERIAL AND METHODS - This PRISMA-compliant review only included studies published from 1985 to 25th of January 2023 that describe adult male military casualties requiring blood transfused locally using a walking blood transfusion protocol. All relevant data (i.e., activation and contextual factors and risk mitigation measures) were tabulated to retrieve information from the selected military studies.

RESULTS - Our results indicated that activation criteria were homogeneous across the 12 reviewed studies. Whole blood was collected from a WBB when there was a shortage of blood products and when platelets were needed. In the literature reviewed, the main risks associated with such a protocol, namely hemolytic adverse events and transfusion transmitted diseases, are mitigated by the use of typing and screening measures if they are reported. However, there is less consistency in the implementation of those risk mitigation measures.

DISCUSSION - This unusual protocol needs to be integrated into the medical support plan until conventional transfusion support can take over, and should include on-site blood collection from a donor, whether a WBB or an emergency donor panel. The benefits of such a protocol outweigh the risks in a life-threatening situation, especially since these risks can be anticipated and minimised by planning to pre-screen all potential donors before their deployment. Finally, educating and training the staff who must implement this unusual procedure can also improve the safety and survival rate of future patients.

KEYWORDS: Walking blood bank, whole blood, Emergency donor panel, indications, risk mitigation measures.

1. INTRODUCTION

Over the last decade, transfusion medicine has evolved towards fractionated whole blood (WB) components such as red blood cells, platelets, or plasma, to improve the efficiency of storage and use in a standard hospital environment[1]. However, in austere environment (e.g., combat zones), military medical support must also provide the most appropriate product for the treatment of shock and coagulopathies, as hemorrhage remains a major cause of death among combat casualties[2]. Nevertheless, logistical constraints limit access and/or storage of these blood products[3]. The medical support system has been forced to adapt by developing innovative solutions that improve combat casualty care (e.g., DCR)[4]. They have therefore developed techniques, such as walking blood bank (WBB) protocol, to sufficiently access blood anywhere to support combat casualties until their evacuation[5] and thereby increase their survival rate[6]. A WBB is a pool of donors available "on call" to donate WB in the event of an emergency[7]. These donors are among those deployed and consent to be registered as prospective donors prior to deployment[8].

In addition to its essential role in increasing the survival rate of hemorrhagic patients, WB also offers biological advantages by providing all the blood components in a single transfusion to counteract the lethal triad observed in hemorrhage patients[9,10]. Essential blood components are often in short supply on the battlefield, especially platelets. Due to their short shelf life - between 5 and 7 days depending on the country - platelets are usually unavailable. This is why the use of WB, which contains platelets, can be essential for the treatment of certain hemorrhagic patients in extreme environments. Whole blood transfusion seems to be the only accessible solution in logistically challenging situations. This solution would address the need for platelets and logistical issues[5]. Any disadvantages that may arise seems far outweighed by the benefits of such a transfusion[11]. While risks will always exist, we can control and mitigate them. The literature shows that if the donor is pre-screened and a clear protocol is followed[11], WB transfusion from a WBB is safe and effective. WBB implementation currently appears to rely on several different protocol-driven techniques[11]. There is no existing interoperable protocol for the use of WBB even within the NATO coalition based on different national regulations.

The aim of this review is to identify situations where the benefits exceed the risks of resorting to a military WBB by focusing on these two questions: 1/ What

military context leads to the activation of a WBB (when/where)? and 2/ What measures can be taken to minimize the inherent risk of such an implementation on the battlefield?

2. MATERIALS AND METHODS

This systematic review was conducted according to Preferred Reporting Items in Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

2.1. Literature search and screening criteria

PubMed and Scopus databases were searched using the following keywords: “walking blood bank”; “walking AND blood AND bank”; “Emergency whole blood”; “Buddy transfusion”; “Blood far forward”; “walking donor”; “Emergency donor panel” and “warm fresh whole blood”. All articles published from 1985 (after HIV appearance in blood transfusion) until 25th of January 2023 were considered.

2.2. Selection of studies (exclusion and inclusion criteria)

First, the lead investigator identified relevant studies by reviewing the abstracts according to the inclusion and exclusion criteria. In addition, two authors independently assessed all the full texts, and then the full list of eligible studies was agreed by all the authors. The exclusion and inclusion criteria for study selection are described in table I. Studies were included if they described male military adults who were injured and required transfusion of blood collected in the field according to a WBB protocol. Our research focused specifically on adult male military patients, who make up over 95% of our deployable at-risk-population. Furthermore, studies in women tend to reflect transfusion in a perinatal setting, which is not representative of managing bleeding patients in the military. Moreover, studies reporting field-tested protocols and information on at least two of the three outcomes of interest (see table I) may be considered even if they did not include patients. A flowchart illustrating the selection procedure is presented in Figure 1.

Table I: Exclusion and inclusion criteria for military study selection

		Inclusion criteria	Exclusion criteria
Screening	**Language**	Papers written in English	Papers written in all other languages
	Study design	Prospective (including feasibility studies) or retrospective studies in international peer-reviewed journal	Unpublished material, communication, letter to the editor, reviews, and conference abstract
	Publication year	Papers published from 1986 onwards	Papers published up to 1985 to include the ITT related risk
	Participants	Military males adults if patients are involved	Females and children
Eligibility	**Outcomes**	At least 2 of 3: - Indication of resorting to a walking blood bank - Donor safety - Recipient/patient safety	Analysis of donor, patient, or use of the walking blood bank apart
	Setting	Military setting	Civilian setting

2.3. Data extraction and analysis

The data were extracted by the lead author and checked by a second author to ensure accuracy. Disagreements were discussed and decision was taken by a third author. The literature review was divided into two steps: activation indicators and risk mitigation measures.

To retrieve information from the selected studies, several tables were created. All relevant data regarding the activation factors of a WBB are compiled in Table II. The following contextual factors were determined: 1/ availability of a blood bank and type of product in stock, 2/ type of patient injury, and 3/ type of emergency situation (i.e., massive transfusion, mass casualty, remote, or combinations of the above). In addition, this table also included the activation criteria of the WBB as well as information on the type of WB used (i.e.: cold-stored WB or fresh warm WB).

All the mitigating and protective measures implemented in each study to minimize the risk associated with the use of a WBB were summarized in tables III to V. These countermeasures were grouped into two categories: donor-related and patient-related. The latter were likely to occur at two different times, before

deployment and on-site during blood collection. Information on donor-related activities is provided as follows: 1) donor screening before deployment and 2) donor screening at blood collection. This distinction was made because fully equipped laboratories and remotely accessible laboratories differ greatly in terms of resources, procedures, availability, as well as the sensitivity and specificity of the tests used. The blood grouping, the type of screening (i.e., infectious disease screening using questionnaire, nucleic acid testing, serology, or rapid test) and the virus tested were reported if mentioned. Donor screening included questionnaires and/or tests, and we considered both as one. The tests might differ depending on national requirements. Regarding the risk associated with the product, a distinction was made between the studies using only O WB and using type-specific blood or both depending on the situations. The tables also listed if the authors did consider the titer of hemolysins (low or not) in the product. All medical and related laboratory parameters helping to assess the patient's status were reported in the tables. Finally, the data concerning the patient's follow-up after transfusion were also included when available.

2.4. Assessment of the Quality of Evidence

The quality of evidence was assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach to rate the reliability of evidence from each included study. This was assessed by the lead author and independently verified by two others.

3. RESULTS

3.1. Search results

The literature search identified 352 records, of which 154 were assessed for eligibility after removing 198 duplicates. Based on title, abstract and article type, 115 studies were also excluded according to the inclusion and exclusion criteria defined in Table I. There was also one study exclusion on language grounds. The lead investigator identified 39 relevant studies through a review of abstracts against the exclusion criteria. Twelve papers agreed by all authors were included in the review[12–23]. A summary of the results of the literature search is shown in Figure 1.

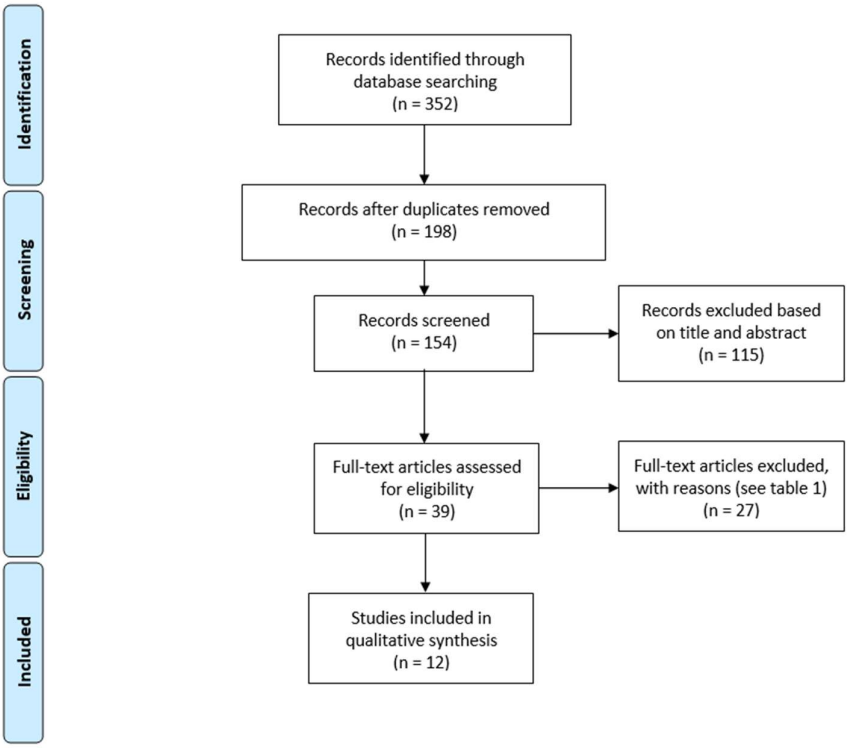

Figure 1: PRISMA flowchart illustrating the entire selection process, from the literature search to the selection of studies of interest based on the inclusion and exclusion criteria. The screening process allows the rejection of duplicates and papers that do not meet the inclusion criteria based on titles and abstracts (i.e., language, year of publication, study design and participants). The eligibility process involves full-text analysis of the remaining papers based on specific outcome criteria, namely the setting and reporting of at least 2 of the following 3 aspects: activation criteria, donor safety or/and patient safety.

3.2. Quality of evidence

9 of the 12 included articles were case reports and series[12,13,16–22]. Therefore, they were all graded "very low" according to the GRADE system. There were also three prospective observational studies[14,15,23]. They were all graded as "low" quality according to the GRADE system. Clustering by repeat authors did not appear to be an area of potential bias. These low-quality grading's were mainly due to the observational design of all studies, putting them at risk of bias, imprecision,

inconsistency, and indirectness. There was no disagreement between the reviewers with regard to the risk of bias and the GRADE rating.

3.3. Analysing results

a. Activation indicators of a WBB

Based on the situations considered (see Table II), the literature review identified four studies that only referred to a remote environment to support the use of a WBB[17,21–23]. Another reported having the WBB protocol ready to provide blood during an event combining remote situations, mass casualty and massive transfusion[18]. The remaining studies supported the activation of the WBB, either for massive transfusions[16–20], or for a combination of mass casualties and massive transfusions[12–15], or for a combination of massive transfusions and remote situations[19].

Accordingly, apart from the study by Strandenes and colleagues[23], all studies justified the use of a WBB as a response to shortages of blood products, and/or delays in evacuation (see table II)[12,13,22,14–21].

Shortages were either contextual or caused by the depletion of available supplies due to acute point-in-time demand[12,17–19,22]. Some of the authors also pointed out the shortage of a specific blood component: blood platelets[13,14,20]. As platelets were often scarce on the battlefield, they could only be obtained from WB. Whole blood has made the difference in the stabilisation and recovery of coagulopathic patients with certain types of injuries resulting in bleeding casualties[13,14,20].

All 9 retrospective studies described haemorrhagic patients with either uncontrollable bleeding or coagulopathy due to various traumatic injuries as the cause of injury leading to activation of a WBB (see table II)[12,13,16–22]. Among the remaining three prospective studies, two studies evaluated the feasibility of setting up a WBB and the supply potential generated by implementing the protocol[14,15], while the third one described the protocol they used to collect and bank WB from a pool of identified donors to anticipate potential needs on board[23]. It was also the only study to specify the use of cold-stored WB as a means of accessing and maintaining a “blood bank” without having home blood[23].

Table II: Summary of the indications of activation of a walking blood bank

Authors	Basic situation			Walking blood bank activation	
	Blood bank product available?	Type of Injuries	Situation	Activation indicator	WB used
Lewis et al., 2020	Yes (CSWB + Full CT)	Blast injury, haemorrhage	Mass casualties, massive transfusion	Depletion of CSWB/evacuation impossible or delayed	FWB
Miller et al., 2018	Yes (frozen pRBC + FFP)	No specific injury described: Helicopter crash	Mass casualties, massive transfusion	Platelets needed/severe coagulopathy	FWB
Bassett et al., 2016	Yes (Full CT)	Traumatic amputations, blast injury, shrapnel injury	Massive transfusion	Combat injured patients likely to require massive transfusion (benefits from early activation)	FWB
Strandenes et al., 2015	No (no blood bank available)	No specific injury described: Feasibility study for Norwegian frigate conducting antipiracy operations	Remote situation	Planning	CSWB for banking
Garcia Hejl et al., 2015	Yes (pRBC, FDP)	No specific injury described: Feasibility study	Mass casualties, massive transfusion	Platelets needed/severe coagulopathy	FWB
Hrezo and Clark, 2003	No	Rectal bleeding	Remote situation	Shortage of blood products	FWB
Gaspary et al., 2020	Yes (CSWB + Full CT)	No specific injury described: feasibility study	Mass casualties, massive transfusion	Shortage of blood products (CS LTOWB serve to start massive transfusion until FWB become available from the WBB)	FWB
Hakre et al., 2013	Not reported	IED Blast	Mass Casualties, massive transfusion + remote situation	Shortage of blood products	FWB
Malsby et al., 2005	Not reported	Gunshot wound	Massive transfusion + remote situation	Shortage of blood products	FWB
Liu et al., 2014	Yes (RBC, FFP, PLT)	No specific injury described: Hit by a ship cable	Massive transfusion	To correct coagulopathy when all other blood products failed	FWB
Gaddy et al., 2021	No (any products available at POI)	Gunshot wound	Remote situation	Absence of blood products (transfusion after extraction before evacuation, POI)	FWB
Song et al., 2021	Yes (CSWB)	Blast injury	Remote situation	No access to stored blood product at the POI: Delay for evacuation	FWB

Abbreviations: CSWB = Cold stored whole blood; pRBC = packed red blood cells; FDP = Freeze-dried plasma; CT = Components therapy; FFP = Fresh frozen plasma; RBC = Red blood cells; PLT = Platelets; POI = Point of injury; IED = Improvised explosive device; CS LTOWB = Cold stored low titer O whole blood; FWB = Fresh whole blood; WB = Whole blood.

b. Risk mitigation measures of a WBB

Linked to the donor

Two measures are reported to be used to limit donor-related risks, namely blood typing (table III) and donor screening (table IV). Both can be performed in early pre-deployment planning and/or on-site at the time of collection.

Across studies, blood typing prior to deployment and its confirmation at the time of collection were often combined with the aim of establishing a registry of potential donors and their blood groups that could be confirmed at the time of collection[13,16,17,21,23]. Three studies focusing on patient or donor screening failed to report pre-deployment or on-site blood group typing[18,20,22]. The study by Song and colleagues reported on the use of a donor registry, but did not provide any details on the potential risk reduction measures that were taken either prior to deployment or at the time of collection[22]. Nevertheless, it seemed to be a relatively important measure as most authors reported it, even though the protocols were quite different, and the lack of reporting did not mean that it was not done. The use of WB from only O donor, rather than type-specific or compatible blood, was reported in only 3 studies[15,19,22]. Furthermore, two studies did not even address this issue and did not specify the product used[16,20].

For donor screening risk mitigation measures (i.e., tests or questionnaires), all details provided by authors are shown in Table IV. Eight studies reported pre-deployment screening as part of the donor registry planning in the preparedness phase[12,13,15–19,23] and seven at the time of collection[13–18,23]. Six studies performed pre-deployment and on-site screening[13,15–18,23]. Two studies did not report on-site testing but did report pre-deployment testing[12,13,16–19], and one reported on-site testing but did not report pre-deployment testing[14]. Despite this, only two studies reported no screening at least once during the process[20,21]. In their study, Song and colleagues did not report any screening before or at the time of collection, but specified that the protocol was to 'call' donors from a registry[22].

Table III: Summary of the "typing" risk mitigation measure.

Authors	Type of WB	Pre-deployment	At collection
Lewis et al. 2020	Type sp. & LTOWB	Not detailed	Not reported
Miller et al. 2018	Type sp.	Only a 10% sample of on board personal	Confirmation
Bassett et al. 2016	Not reported	Refer to CPG	Refer to CPG
Strandenes et al. 2015	LTOWB + AWB	National standard procedure for regular donor in civilian health care: Grouping + titer	Confirmation (rapid test)
Garcia Hejl et al. 2015	Type sp.	No reported	Type
Hrezo et Clark 2003	Type sp.	Only a 10% sample of population	Type + Crossmatch
Gaspary et al. 2020	LTOWB	Not reported	Samples collected on site and send back to homeland for titer analysis
Hakre et al. 2013	OWB + AWB	Not reported	Not reported
Malsby et al, 2005	OWB	Not detailed	Not reported
Liu et al., 2014	Not reported	Not reported	Not reported
Gaddy et al., 2021	Type sp. LTOWB prehospital	Yes: blood ID card	Confirmation by rapid test required but not executed due to tactical limitations - use of blood ID card
Song et al. 2021	LTOWB	Not reported	Not reported

Abbreviations: WB = whole blood; Type sp. = ABO type specific; LTOWB = Low titer O whole blood; OWB = O whole blood; AWB = A whole blood; LTOWB = Low titer O whole blood; CPG = Clinical practice guidelines; ID = Identification.

Table IV: *Summary of the "screening" risk mitigation measure*

Authors	Pre-deployment	At collection
Lewis et al. 2020	Not detailed	Not reported
Miller et al. 2018	Only a 10% sample of on board personal HBV - HCV - Syphilis - malaria	Rapid tests
Bassett et al. 2016	Refer to CPG	Refer to CPG
Strandenes et al. 2015	National standard procedure for regular donor in civilian health care	Combined rapid test
Garcia Hejl et al. 2015	No reported	Questionnaire Rapid tests HIV, HCV + complete HBV vaccination
Hrezo et Clark 2003	Only a 10% sample of population. Questionnaire Serologic tests: HIV, HCV, HBV, HTLV	Rapid testing
Gaspary et al. 2020	Recommanded JTS CPG but not executed	Rapid testing
Hakre et al. 2013	Questionnaire Screening (90days): HIV, HCV, HBV, Syphilis, HTLV, West Nile virus (sample back to the US) Complete HBV vaccination	Rapid tests: HIV, HCV, HBV
Malsby et al, 2005	Not detailed	Not reported
Liu et al., 2014	Not reported	Not reported
Gaddy et al., 2021	Not reported	Not reported
Song et al. 2021	Not reported	Not reported

Abbreviations: HBV = Hepatitis B Virus; HCV = Hepatitis C virus; CPG = Clinical practice guidelines; HIV = Human immunodeficiency virus; HTLV = Human T-lymphotropic virus; JTS = Joint Trauma system; TTD = Transfusion transmitted diseases.

Linked to the patients.

It was not possible to identify only one or even a few important parameters for patient follow-up, as all authors used different parameters (see Table V), except for the prospective study by Strandenes et al, which used no parameters for follow-up[23]. From a transfusion perspective, the parameters reported in these studies can be divided into two main types: 1/ the medical parameters, where the most commonly reported were blood pressure, heart rate, survival rate, surgery, transfusion reactions and laboratory parameters reflecting the status of the patient (e.g.: haemoglobin or pH, lactate)[12,13,16,17,19–22] and 2/ adverse events related to TTDs

or screening on sample return to the home country[14–18]. Patient follow-up for potential TTDs was reported in five studies[14–18]. Hakre and colleagues focused their analysis on one patient's seroconversion following an on-site walking blood transfusion[18].

Table V: Summary of the patients' follow-up parameters

Authors	Patient follow-up/Measured indicators
Lewis et al., 2020	TACO - Surgery - recovery
Miller et al., 2018	HR - blood pressure - pH - Lactate - Hb - Plt count
Bassett et al., 2016	pH - BE - Hb - 30days follow-up: survival + Transfusion reaction/blood borne pathogens transfer - OR Time - time to transfer
Strandenes et al., 2015	Not reported
Garcia Hejl et al., 2015	Sample for immunoassays infectious agents: HTLV, HIV, HBV, Syphilis + Nucleic Acid Testing: HIV, HCV, HBV
Hrezo et Clark 2003	Blood count - PT/PTT - Hb - HR - BP - sO2 - Sample for future serologic testing - 48h follow-up - surgery
Gaspary et al., 2020	Sample back for pre-screening to add donor to register
Hakre et al., 2013	Transfusion associated adverse events TTD's: HTLV - WBC - temperature
Malsby et al., 2005	Pulse - BP - surgery - Follow-up 4weeks
Liu et al., 2014	Temperature - HR - respiratory rate - BP - Hb - PT- INR - PTT - Plt count - Calcium level - surgery - Acute lung injury - respiratory distresses
Gaddy et al., 2021	sO_2 - BP - HR - respiration - pulse - Glasgow score - surgery
Song et al., 2021	Survival - surgery

Abbreviations: TACO = Transfusion-associated circulatory overload; HR = Heart rate; Hb = Haemoglobin; Plt = Platelets; BE = Base excess; OR = Operating room; HTLV = Human T lymphotropic virus; HIV = Human immunodeficiency virus; HBV = Hepatitis B virus; PT/PTT = Prothrombin time/Partial thromboplastin time; BP = Blood pressure; sO_2 = Oxygen saturation; TTD = Transfusion transmitted disease; WBC = White blood cells; INR = International normalized ratio.

4. DISCUSSION

This review aimed to identify activation criteria for military WBBs as well as the risk mitigation measures associated with their use.

Our first research question investigated the rationale for its application. Two main trends have been identified in the literature to justify the use of WBB protocols: 1/ access to blood products in case of shortage (i.e., logistical indication

of activation)[12,15–23] and 2/ access to blood products for the treatment of a haemorrhagic patient when a required specific component is not available (i.e., clinical indication of activation)[13,14,20].

All but two of the studies[21,23] reported on the use of fresh WB to overcome the shortage of blood products[12–22]. Gaddy et al reported collecting blood for a casualty during a combat assault and withdrawing it at the site of injury. There was no shortage of blood, but blood was not immediately available on site[21]. Strandenes and colleagues, however, chose a different strategy, collecting blood to build up an emergency bank[23]. These two different strategies are equally acceptable and can be chosen according to the initial situation: collecting to meet a specific need based on a shortage or creating a bank based on an absence. Yet, both strategies are named differently: one is called a "walking blood bank" (WBB) while the other is called an "emergency donor panel" (EDP). The NATO Blood Panel recently discussed this difference[24]. It was decided that the WBB refers to WB collected for banking. In contrast, the emergency donor panel refers to a pool of pre-screened donors who are ready to give blood for immediate use without banking[24]. One may notice that this distinction is not yet clear in the literature. Therefore, to ensure that all studies were included, we decided to extend our search to the most used terms in the literature. Furthermore, all authors reported using this protocol to avoid overwhelming their designated transfusion system for highly demanding patients presenting with uncontrolled bleeding leading to massive transfusion or haemorrhagic shock. As previously reported in the literature, WB is an essential resource for DCR, e.g., at sea, it offers operational flexibility as the use of component therapy, the ratio "1:1 RBC": FFP" is not always and everywhere sustainable[23]. Our analysis led us to the same conclusion. The use of FWB collected on site could become, in exceptional situations, the only solution to access blood and save lives. While this review focuses only on the military setting, it was also used in isolated and large geographical areas presenting blood supply challenges comparable to military theatres (e.g.,[25–28]). The Norwegian Preparedness Plan is the more developed and published model for using WBB/EDP in the civil world when geography or supply is difficult to secure[27]. Finally, some authors reported choosing to use FWB in order to obtain a clinical advantage[20], as FWB offers a better survival rate in haemorrhagic shock[29]. However, it is still a highly controversial topic as the purported benefits of FWB are still not clearly evidence based[30,31].

Concerning our second research question, while the awareness of risk is common to all articles, the protocols differ in their implementation regarding the use of risk mitigation measures, both in terms of the type of test and the timing of its implementation. Our review showed that risks related to both donors and products need to be considered. It is well established in the literature that FWBs should come from pre-screened donors to reduce the higher risk of TTD[29]. However, in our review, even if both TTD screening and blood typing are considered to reduce the risk, the techniques used, and the timing of the interventions varied widely and did not allow standardization of practice. There are two main explanations for this. The first one would be the national regulation, which is quite specific to each country. Therefore, because all requirements and protocols are different (Germany, USA, UK, Canada)[32], interoperability in the use of WBB cannot be adopted by all NATO members. As it also depends on the prevalence in the home country, there are no standards for TTD screening[29]. The second one relates to the bias inherent to the design. Most of the reviewed studies were case studies. This implies that the data used are those that are available a posteriori and some of the data may be missing without necessarily indicating that the procedure such as testing was not carried out. Furthermore, it is also possible that some information is missing because the authors choose to omit reporting some data and not because the full test was not carried out. Not reporting did not mean that it did not happen. Regarding the product used, it would be more convenient in terms of the risk of transfusion reactions to use only O donors. However, our results do not reflect this. Most authors reported using ABO type specific WB, but unfortunately did not rationalise their choice[12–14,17,18,21,23]. From our perspective, availability. Indeed, O donors represent approximately 45% of the Caucasian population, whereas A donors represent approximately 45% of the same population. By limiting the sample to O donors, an important part of the donor pool is excluded. This may be important for obtaining sufficient resources. Nonetheless, this presupposes that the typing has been determined pre-deployment or at the time of donation. In addition, some authors report also considering the haemolysin titer in O WB[12,15,21–23]. However, there is no consensus on titer determination, either from a technical point of view or from a cut-off point of view. Therefore, not every nation would consider a donor as a low titre donor using the same levels. This is part of the limitation of the use of low titer in an international setting[33]. This would lead to complications in communication, monitoring and interoperability decisions. Finally,

patient outcomes were also considered in the studies reviewed, but there was no evidence of a consensus on these and their reporting was inconsistent. Nevertheless, all efforts should be made to assess patients' stability according to the resources available.

5. CONCLUSIONS

A blood collection protocol, whether a WBB or an emergency donor panel, must be part of the transfusion support concept because it provides access to resources that are otherwise inaccessible. Obviously, this will only be implemented in exceptional situations due to the risk associated. Most stakeholders are aware of these risks, which, if mitigated, are outweighed by benefits. Therefore, measures are taken to prevent, monitor and minimize the risks entailed by such protocol. To ensure a comprehensive selection of donors for the registry, it is essential to include this comprehensive protocol in the medical support planning process of operation. The key to success is the donor, their education and regular follow-up. Based on this review, there is a clear need for such a protocol in the military operational setting, but it can also be applied in the civilian world, particularly in remote locations. However, it must be part of the country's preparedness plan to ensure the best possible care for patients.

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CHAPTER 2: MINIMAL TACTICAL IMPACT AND MAXIMAL DONOR SAFETY AFTER A BUDDY TRANSFUSION: A STUDY ON ELITE SOLDIER PERFORMANCES IN BOTH LABORATORY AND FIELD ENVIRONMENTS.

Published article:
Degueldre, J., Dessy, E., T'Sas, F., Deneys, V., & Pattyn, N. (2021). Minimal tactical impact and maximal donor safety after a buddy transfusion: A study on elite soldier performances in both laboratory and field environments. *Transfusion*, *61*, S32-S42. 10.1111/trf.16463

ABSTRACT

BACKGROUND - The major cause of death of combat casualties in austere environments are related to hemorrhage and occur early after injury. The implementation of a walking blood bank may overcome the logistical issues raised using blood component therapy. Nonetheless, it is important to ensure that this buddy transfusion is not going to compromise the mission success by altering the donor's performance. The results available so far cannot ruled out this issue with certainty. Therefore, this study aimed at investigating the immediate effect of a 450-ml blood donation on the performances of elite soldiers in laboratory and field environments.

STUDY DESIGN AND METHODS - This double-blind, randomized controlled study included two experiments. For both experiments, subjects were randomly assigned either to a control group ($n_1 = n_2=7$) or a 450-ml-blood-bag donation group ($n_1=7$ and $n_2=8$) (. All participants underwent before and after a potential blood donation a multifactorial assessment including adapted physical tasks, haematological variables, vigilance parameters as well as subjective assessments.

RESULTS - No significant results were evidenced in this study. There was no impact of the blood donation on the participants' performances in both the hospital and the combat-like environments.

CONCLUSION - From a donor point of view, a 450-ml blood donation has no impact on the required abilities of our elite soldiers to fulfill a demanding tactical mission. Thus, the results of this study support the fact that buddy transfusions could be part of the operational clinical armamentarium in austere environments for elite soldiers when no blood components are available.

KEYWORDS: Walking blood bank, performance, Whole blood, donor safety, buddy transfusion

1. INTRODUCTION

Hemorrhage is the leading "preventable" cause of death from combat injuries[1,2]. Survival rates in the hemorrhaging patient depend on rapid and adequate management of the patients[3] as well as early initiation of balanced resuscitation[4]. Unfortunately, most of them die before reaching a Military Treatment Facility[5]. Therefore, the immediate availability of blood in pre-hospital conditions can save life and improve prognosis. However, blood supply and storage in austere environments remains an important logistical challenge. To compensate the unavailability of blood components in exceptional operational circumstances, on-site collection of whole blood (WB) from a "buddy" deployed on the same site[6], are successfully used in operational settings[7]. This method is known as a walking blood bank (WBB).

Because of the well-known biological advantages of WB and the logistical issues raised using components, most NATO countries are developing their own WBB procedures in accordance with their national requirements. This is also the case in Belgium; we are currently developing WBB guidelines to aid our medical staff deciding when to trigger a buddy transfusion whilst having minimal tactical impact and ensuring maximal donor safety. Therefore, we study the Belgian Special Forces (SF) operators, our most exposed and at-risk population, to evaluate the effect of a blood donation on their vigilance and physical performances. This study aims at guaranteeing that our SF operators will still be able to fulfill their mission and return to a safe place.

To fulfill this objective, we focus on the immediate effect of a 450-ml blood donation (i.e., a standard blood donation) on physical performance, vigilance, haematological parameters, and psychological aspects (e.g., stress, fatigue, well-being) of SF operators. Over the last decades, a few studies have arisen in the literature investigating some of these parameters among a military population[8–10]. With regard to vigilance, no effect of the blood donation were reported[11]. Yet, several studies reported a detrimental effect of standard blood donation on physical performance in a laboratory environment[9,11–13]. These studies reported that standard blood donation reduced haemoglobin level, maximal oxygen uptake (VO_2max) and maximal exercise capacity in a laboratory environment[9,11,12]. Unfortunately, only a few studies investigated the effect of a standard blood donation on performance in field scenarios[9,14]. These studies showed that the

combat abilities of the participants are preserved immediately after a 450-ml blood donation[10] . However, a recent meta-analysis highlighted important limitations in these studies, precluding the possibility of establishing a clear effect of blood donation on performance[12]. Indeed, all these studies used different populations, procedures, designs or/and variables[12]. Thus, the results were not readily transposable to the operational world for such a specific population. Indeed, the increased circulating blood volume of our highly trained SF operators [15] could decrease the visible impact of the donation on performance[9].

We aim to evaluate precisely the potential immediate impact of the donation on the SF operators' performances in two different set-ups. First, we conduct a study in a laboratory and secure environment in order to verify the results of the literature. Then, we study our target population in an ecologically valid environment (i.e., a field exercise in a desert environment) to ensure the transferability of our results to real-life operational settings. In each setting, we evaluate in a double-blind randomized controlled experiment, the effect of a single 450-ml blood donation through a multifactorial assessment including haematological parameters, vigilance, and physical performance measures. Moreover, even if psychological parameters such as the expected effect of the blood donation were ignored in previous researches, we consider these factors in both set-ups as they could significantly impact the participant's performance[16] through placebo effects.

2. MATERIALS AND METHODS

This double-blind, randomized controlled study consisted of two distinct experiments, first in a laboratory setting and then in the field. The study was approved by the Medical Ethics Committee of the University Hospital (UZ-Brussel) and the Vrije Universiteit Brussel (VUB) (B.U.N.: 143201835663). All participants provided written informed consent. The manuscript was written and edited according to the Consolidated Standards of Reporting Trials statement. The study was made in accordance with the guidelines for good clinical practice and the Declaration of Helsinki. No changes to trial design and methods were made following trial commencement.

2.1. Study design

There were 14 Belgian male SF operators included in the laboratory experiment and 15 in the field experiment. Participants received an oral and written explanation concerning the study and, if they expressed a wish to participate in the study, they signed an informed consent. Inclusion and exclusion criteria as well as criteria for discontinuation of the study are provided in Table I.

Table I. Inclusion, exclusion, and discontinuation criteria.

Inclusion criteria	- Physically active operator of the Belgian Special Forces who volunteer to participate in the study. - Military operational (i.e., Med Ops cat A) according to their yearly medical examination at the military hospital. - Medically fit to donate blood (based on their medical questionnaire and measurement of vital signs). - Right after a strenuous training period for both experiments
Exclusion criteria	- Does not meet blood donation criteria (according to Belgian law for blood donation) - Donated blood within the last 3 months - Takes antihypertensive therapy - Suffers any physical injuries before the start of the study
Discontinuation criteria	- Any medical condition or physiological reaction that arises during testing deemed unfit by the supervising medical doctor. - Decision of the participant to interrupt his participation

For both experiments, subjects were randomly assigned either to a control group or a donation group under the supervision of the study coordinator. To avoid compensatory mechanisms and the potential effect of blood loss awareness, participants wore blacked-out goggles and earmuffs during the blood donation (see figure 1). Moreover, the field researchers who collected the data were not aware of the group distribution, as the donation procedure was conducted by a different team. All the material and procedures were identical among both groups to guarantee a double-blind design. Moreover, all the equipment's were calibrated, controlled, and used according to the manufacturer's requirements.

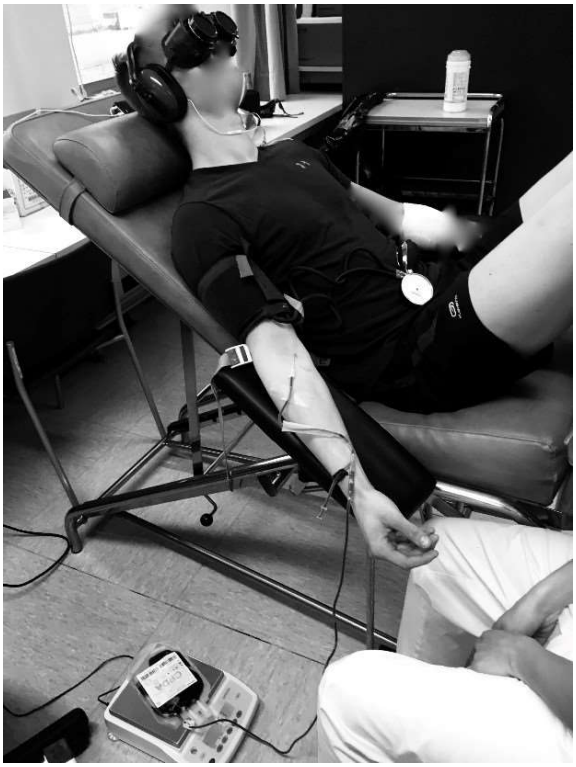

Figure 1: During the blood donation, the participant was lying down with his legs raised and his arms alongside the body. He wore blacked-out goggles and noise-cancelling headphones while listening to his favorite playlist for approximatively 10min. This specific set-up ensured that he remained blind about his group attribution. The most suitable arm was chosen to set-up the sterile single collection bag and start the blood donation. Control group participants donated a small blood sample (5-10 ml) while donation group participants gave a 450-ml-blood-bag. The collection bag was placed on a scale to guarantee that the same amount of blood was collected from each participant (approximately 450mL).

The laboratory experiment took place in the military hospital Queen Astrid, Neder-Over-Heembeek, Belgium. The field experiments took place during an exercise abroad under similar conditions to those experienced in operations (e.g., gear, climate, …). All participants underwent a multifactorial assessment including measures of haematological parameters, vigilance, and physical performances as well as subjective assessments. Only physical performance measurements differed between both experimentations. During the laboratory experiment, the potential effect of blood donation on performance was determined by comparing the results

of the baseline with the results of the post-donation measures (see figure 2). During the field experiment, the population performed one strenuous ecologically valid physical task (i.e., the warrior competition) after the blood donation (see figure 3).

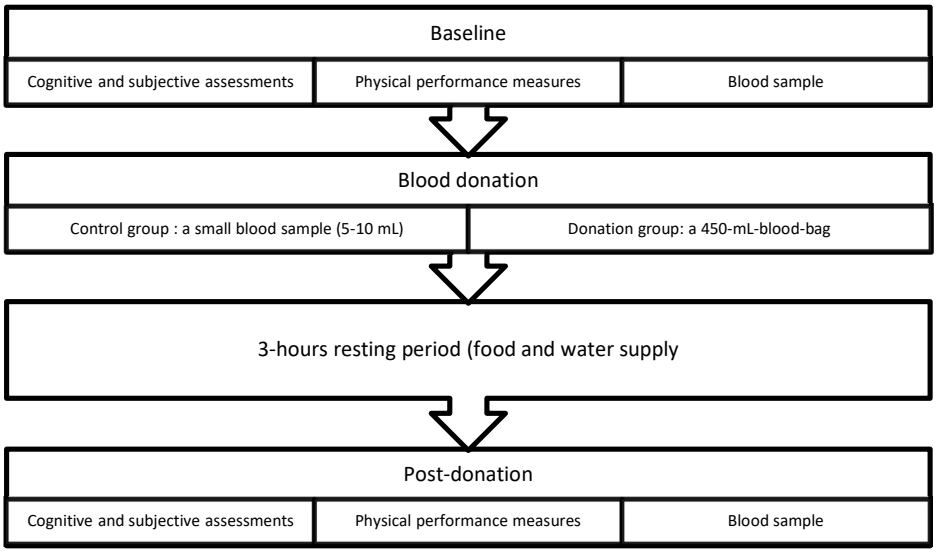

Figure 2: Summary of the testing sequence followed by each participant of the laboratory experiment in the Military Hospital. At their arrival, participant received detailed information about the experimental set-up and the planning of the day, and they filled in the first subjective questionnaire. Then, they were interviewed by the doctor responsible for the blood collection to attest that they were fit to be a blood donor. Once the medical interview was finished, blood samples were collected. Then, participants performed the baseline psychomotor vigilance task (PVT) as well as the first incremental graded exercise tests until exhaustion. At the end of the first exercise test and before the blood donation, participants filled in a second subjective questionnaire and performed a second PVT. For the blood donation, control group participants donated a small blood sample (5-10 ml) while donation group participants gave a 450-ml-blood-bag (see figure 1). A 3-hours resting period followed the blood donation. During this period, participants must drink at least 500ml water and performed a standardized intellectual assessment with a trained psychologist in the framework of a unit's internal project, completely independent from our study. After the resting period, they performed their second exercise test followed by the last PVT. They finished the assessment by filling in a third subjective questionnaire and a last blood sample was drawn.

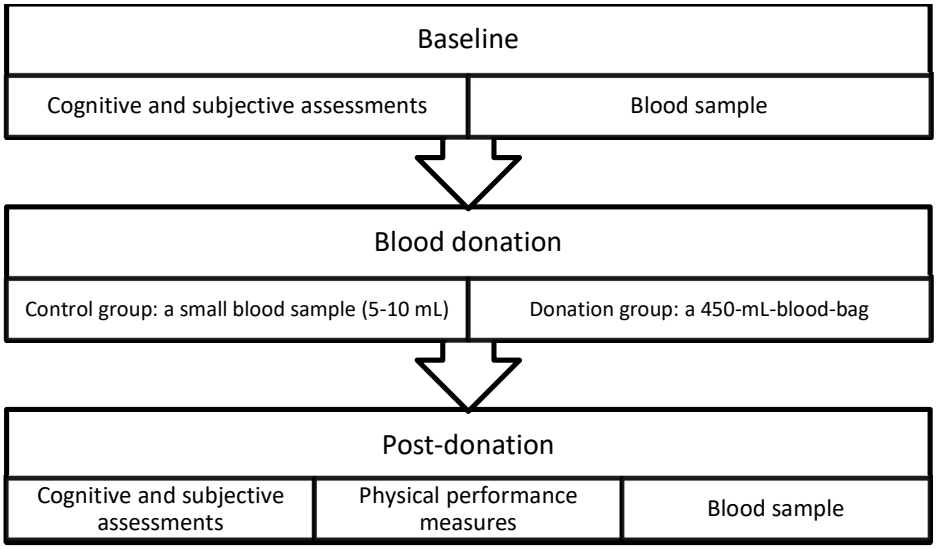

Figure 3: Summary of the testing sequence followed by each participant of the battlefield experiment. Upon their arrival, participant received detailed briefing about the study and explanation about the tasks of the day. Then, they were interviewed by the doctor responsible for the blood collection to attest that they were fit to be a blood donor. Once the medical interview was finished, blood samples were collected, and participants filled in their first subjective questionnaire. Before starting the blood donation, participants performed the baseline psychomotor vigilance task (PVT). For the blood donation, control group participants donated a small blood sample (5-10 ml) while donation group participants gave a 450-ml-blood-bag in a double-blind set-up (see figure 1). Then, participants started directly after blood donation the strenuous military circuit (i.e., the warrior competition) which was followed by the second PVT as well as a subjective questionnaire. Finally, a last blood sample was drawn.

2.2. Outcomes

As the main study objective was to assess the impact of a 450ml-blood donation on tactical capacity, and thus vigilance and physical performance right after a strenuous training period, these criteria were defined as the primary outcomes while subjective assessments and haematological variables were considered secondary outcomes.

a. Blood parameters

The 5-ml-venocapured blood samples were collected at the start and at the end of the testing day in both experiments. Unfortunately, due to logistical problem these samples were not analyzed for the laboratory experiment. For the field

experiment, blood parameters were immediately measured on a i-STAT handle analyzer with the chemistry (Chem8+) and the blood gas (CG4+) cartridges (ABBOTT, Chicago Illinois, US).

b. Blood donation

Blood donation procedures were the same in both experiments. Upon arrival, the doctor responsible for the blood collection evaluated all the participants. He performed a medical interview through a standardized blood donor questionnaire to verify the participant's eligibility to be a donor as required by Belgian law. Moreover, the doctor also assessed their vital parameters (including body mass and height), to attest that they were fit to donate blood and could be included in the study. Then, participants were randomly assigned to either a control group or a donation group. Control group participants donated a small blood sample (5-10 ml) while the others gave a 450-ml-blood-bag. Every participant was connected to a sterile single collection bag with anticoagulant solution adequate for whole blood (i.e., Citrate phosphate- dextrose-adenine) (TERUMO BCT Inc).

c. Vigilance and subjective assessments

The 10min-computerized Psychomotor Vigilance Test (PVT) recorded reaction times (RT) to visual stimuli that occurred at random inter-stimulus intervals [17]. Participants were instructed to monitor a screen and click as fast as possible once a millisecond counter appeared in the box and starts incrementing (from 0 to 1000 milliseconds). Reaction speed, lapses (reaction time over 500ms) and misses (missed stimuli) were recorded. This vigilance test was performed three times, before and after each physical task.

At the beginning of each experiment, participant rated their subjective levels of stress, mood, alertness, mental and physical fatigue as well as the quality of the previous night on a 10-cm visual analogue scale (VAS). After each physical evaluation, participants rated again on a VAS their subjective levels of physical and mental well-being, muscle pain, training intensity, stress, mood, alertness, mental and physical fatigue. Scores ranged from 0 to 100, as physically measured on the VAS. A higher score indicated a greater intensity of the subjective feeling being measured[18–20]. At the end of both experiments, participants were asked about the eventual impact of the blood donation on their vigilance and physical performances, the perceived side-effects as well as if their evaluation regarding whether they were in the donation or the control group.

d. Physical assessment

Laboratory experiment

Two incremental graded exercise tests until exhaustion[21] were performed, with 3h of rest in between, in the Sports lab of the Military Hospital under medical supervision. One hour before each test, the participants received a standardized meal. The exercise test was performed on a treadmill associated with an exercise testing system (Ergocard Clinical, Medisoft, Belgium) according to a protocol specially designed and currently used at the exercise lab for the SF population. The protocol started at 5.4 km/h and consisted of 3-minute stages at increasing running speeds, with an increase of 1.8 km/h, and with a total maximum speed of 23 km/h. Treadmill inclination was kept constant at 0% for all the candidates. At the completion of each stage, blood was drawn from the right fingertip to evaluate blood lactate concentration; moreover, the maximal heart rate (HR) was determined using a HR monitor linked to the computer (Polar Sporttester, kempele, Finland). The exercise was stopped when participants reached complete exhaustion. Gas exchange data with the candidate's oxygen intake and carbon dioxide output were measured using an automated breath-by-breath system (Ergocard Clinical, Software Medisoft, Belgium). After the test, the relative maximal oxygen consumption (VO_2max) was transcribed from the report of the device[21].

Field experiment

Physical performance was evaluated, after the blood donation, by a strenuous military circuit (i.e., the warrior competition) whilst carrying approximately 27kg of personal equipment and weaponry. Participants were involved in a competition throughout all the circuit. They had to performed as fast and as accurately as possible all the following tasks: a basic obstacle run, a 25m-shooting range, a 100m-shooting range, an 8-storey climb of a commando tower, a rappelling descent, a close quarters battle house run as well as an urban climbing parkour. In our study, we considered the score computed by the instructors based on the individual results on each task, the time to perform the obstacle run as well as the score obtained for each shooting task. Moreover, the HR was determined at rest and within a 3-minutes interval after finishing the circuit using a fingertip pulse oximeter (Onyx II, Nonin, Minneapolis, Minnesota, USA).

2.3. Statistical analysis

All statistical analyses were performed using IBMS Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 25.0 for Windows. For all statistical tests, statistical significance was accepted at the $p \leq .05$ level. Partial eta-squared (η^2) and Cohen's d (d') were used to measure the effect size.

Regarding the laboratory experiment, a 3 (Time [start, post-effort test 1 and post- donation]) x 2 (Group [control and donation]) mixed ANOVA, with Time as within-subjects factor and Group as between subjects' factor, was used to test the results of the repeated parameters (see figure 4). To investigate the difference between the measures of the other repeated subjective parameters of the two groups (i.e., non-donor vs. donor), a 2 (Time [post-effort test 1 and post- donation]) x 2 (Group [control and donation]) mixed ANOVA with time as within-subjects factor and Group as between subjects' factor when the assumptions of normality and homogeneity were met (see figure 4). Greenhouse-Geisser Epsilon corrections were used when sphericity was violated. The assumptions of normality (Kolmogorov-Smirnov with a Lilliefors Significance Correction) and homogeneity (Levene's test) were tested before performing these statistical analyses.

Laboratory experiment	Between-subject factor	Groups	Donation
			Control
	repeated parameters	physical parameters	Relative VO2max*
			Lactate*
			Heart rate*
		Vigilance parameters	Reaction time
			Lapses
			Misses
		Subjective parameters	Physical fatigue
			Mental fatigue
			Sleepiness
			Stress
			Physical well-being*
			Mental well-being*
			Training intensity*
			Muscle pain*
	Within subject factor	Time	Start (not for *)
			Post-effort test 1
			Post-donation

Figure 4: Overview of all laboratory experiment variables included within the statistical analyses and the results section.

Regarding the field experiment, a parametric independent-samples *t*-test (two-tailed) was used to investigate the difference between the measures of the physical parameters as well as the non-repeated subjective parameters of the two groups

(see figure 5). Moreover, the repeated parameters were tested using a 2 (Time [pre, post]) x 2 (Group [control and donation]) mixed ANOVA (see figure 5). Time was used as within-subjects factor and Group as between subject's factors. Greenhouse-Geisser Epsilon corrections were used when sphericity was violated. These statistical analyses were performed as the assumptions of normality and homogeneity were met. When the assumptions of normality and homogeneity were not met, scores on pre- and post-test were compared for each group separately using a non-parametric Wilcoxon signed-rank test.

Field experiment	Between subject factor	Groups	Donation
			Control
	Repeated parameters	Haematological and physical parameters	Haematocrit
			Haemoglobin
			Heart rate
		Vigilance parameters	Reaction time
			Laspes
			Misses
		Subjective parameters	Physical fatigue
			Mental fatigue
			Sleepiness
			Stress
	Non repeated parameters	Physical parameters	Obstacle run*
			25m shooting range*
			100m shooting range*
			Overall score*
		Subjective parameters	Physical well-being*
			Mental well-being*
			Training intensity*
			Muscle pain*
	Within subject factor	Time	Start (not for *)
			End

Figure 5: Overview of all field experiment variables included within the statistical analyses and the results section.

3. RESULTS

3.1. Laboratory experiment

One participant of the donor group was injured and was not able to perform the physical and vigilance assessments, therefore, he was excluded from all the analysis. The ANOVA analysis of physical parameters during the laboratory experiment revealed only a significant decrease over time for the variable relative $V0_2max$ [$F(1, 12) = 7.455$, $p = 0.018$, $\eta 2 = 0.383$]. No significant interaction effect between time and group was evidenced for any of the physical parameters (see table II).

Table II. Means and standard deviations for the physical, vigilance and subjective parameters during the laboratory experiment.

		Start		Post-effort test 1		Post-donation	
Parameters	**Measures**	**Control (*n*= 7)**	**Donation (*n*= 7)**	**Control (*n*= 7)**	**Donation (*n*= 7)**	**Control (*n*= 7)**	**Donation (*n*= 7)**
Physical parameters	**Relative VO_2-max (ml/kg/min)**	-	-	54.38 (4.79)	53.96 (7.66)	53.30 (3.79)	49.81 (6.63) *
	Maximal Heart rate (beats/min)	-	-	196.86 (3.29)	198.14 (13.21)	197.43 (4.83)	197 (11.27)
	Lactate (mmol/L)	-	-	9.52 (1.89)	9.13 (3.14)	9.41 (1.16)	7.63 (1.92)
Subjective parameters	**Physical fatigue**	40.31 (24.83)	55.82 (19.69)	35.74 (19.15)	52.65 (20.08)	57.74 (13.02)	49.20 (22.42)
	Mental fatigue	36.90 (24.95)	27.59 (20.45)	23.24 (21.19)	35.76 (19.84)	33.25 (25.13)	38.58 (27.50)
	Sleepiness	35.40 (10.57)	33.77 (20.94)	32.14 (26.7)	39.15 (22.89)	37.40 (24.33)	32.06 (28.40)
	Stress	13.37 (12.34)	15.63 (8.94)	12.43 (17.79)	19.61 (5.82)	11.28 (14.34)	14.02 (10.47)
	Physical well-being	-	-	65.86 (17.01)	63.93 (18.07)	67.13 (18.44)	68.91 (27.89)
	Mental well-being	-	-	84.99 (16.51)	81.52 (7.99)	85.43 (14.74)	80.02 (8.76)
	Training intensity	-	-	75.98 (10.36)	61.10 (21.29)	69.46 (12.92)	63.74 (20.08)
	Muscle Pain	-	-	29.75 (25.86)	3.99 (25.21)	27.81 (27.81)	46.18 (27.89)
Cognitive parameters	**Reaction Time (ms)**	298.95 (18.51)	298.06 (18.01)	296.43 (21.77)	297.60 (24.65)	304.23 (23.81)	311.88 (34.07) *
	Lapses	1.50 (1.52)	0.33 (0.52)	2.17 (1.94)	0.67 (0.82)	1.33 (1.97)	1 (1.09)
	Misses	0.33 (0.52)	0 (0)	0.17 (0.41)	0 (0)	0 (0)	0 (0)

Values are mean (standard deviation).

The symbol * indicates that there was a significant difference in the statistical analysis.

The analysis of the four subjective measures (i.e., physical fatigue, mental fatigue, sleepiness, and stress) with independent samples t-tests showed no significant differences between both groups at the start of the experiment. The

Regarding the vigilance assessment, data recording on the PVT was problematic for two participants, they were excluded from the analysis ($n_{non\text{-}donor}$=6 and n_{donor}=6). The ANOVA analysis of vigilance during the laboratory experiment revealed only a significant main effect of time for the variable reaction time [F (2, 20) = 4.101, p = 0.0327, η2 = 0.291] (see table II). However, no other significant effects were evidenced.

ANOVA analysis of these four repeated subjective parameters during the laboratory experiment revealed no significant main effect of time or interaction effect between time and group. The analysis of the four subjective measures at the end of each effort test (i.e., physical well-being, mental well-being, training intensity and muscle pain) showed no significant main effect of time or interaction effect between time and group (see table II). Moreover, only two out of the seven non donors correctly indicated being part of the donor group (see table III). These two candidates were the only one to report a minor effect (average of 12,44%) of the blood donation on their physical performance.

Table III. Crosstab representing the real group distributions versus the participant's expectations in laboratory experiment.

		Participant expectations		
		Non donor	**Donor**	**Total**
Real group distributions	**Non donor**	4	3	7
	Donor	5	3	8
Total		9	6	**15**

Values are number of subjects (n)

3.2. Field experiment

Four independent-samples t-test were conducted to evaluate the impact of the 450ml blood donation on the physical performance during the field experiment. No significant differences were evidenced between the non-donor and the donor groups. However, the non-donors obtained a better performance than the donors in both the obstacle run and the 25m shooting range while the donors performed better than the non-donors for both the overall score and the 100m shooting range (see table IV).

Table IV. Physical performance assessment during the field experiment

	Control (n= 7)	Donation (n= 8)	p. t-test	Effect size
Obstacle run (s)	158 (18.01)	175 (19.05)	0.100	0.915
Shooting range – 25m	101.43 (13.96)	96.00 (11.02)	0.415	0.436
Shooting range – 100m	74.29 (7.34)	78.50 (11.73)	0.428	0.423
Overall Score	49.43 (14.89)	63.88 (16.29)	0.098	0.923

Values are mean (standard deviation).

The ANOVA analysis of vigilance parameters during the field experiment revealed no main effect of time and no significant interaction effect between time and group.

Haematological parameters were not normally distributed; therefore, two non-parametric Wilcoxon Signed-Ranks Tests were performed. These tests indicated that post-test ranks for the donor group were significantly lower than the pre-test ranks for haematocrit (Z= -2,456, p=.014) and for haemoglobin (Z=-2,388, p=.017) while no differences were evidenced for the non-donor group.

The analysis of the four subjective measures at the start of the experiment with independent samples t-tests showed significant differences between both groups for the mental fatigue level (t(13)=-3.286, *p*=0.006, d'= 1.701), the physical fatigue level (t(13)=-2.875, *p*=0.013, d'= 1.488), and the stress level (t(12)=-2.875, *p*=0.014, d'=1.528). The donors reported to be significantly more stressed and mentally and physically tired than the non-donor group at the start of the experiment (see table V). The ANOVA analysis of the four repeated subjective parameters during the field experiment revealed only a significant main effect of time for the physical fatigue [F (1, 12) = 8.016, *p* = 0.01, η^2= 0.40] and the sleepiness [F (1, 12) = 7.454, *p* = 0.018, η^2= 0.383]. However, no significant interaction effect between time and group has been evidenced. The analysis of the four subjective measures at the end of the testing day showed no significant differences between both groups.

Table V. Means and standard deviations for the haematological, vigilance and subjective parameters during the field experiment.

		Start		End	
Parameters	**Measures**	**Control (*n*= 7)**	**Donation (*n*= 8)**	**Control (*n*= 7)**	**Donation (*n*= 8)**
Haematological and physical parameters	**Haematocrit (%PCV)**	41.14 (2.19)	42.63 (2.77)	42.00 (2.00)	40.50 (2.726)
	Haemoglobin (g/dL)	13.99 (0.75)	14.49 (0.92)	14.27 (0.69)	13.77 (0.93)
	Heart rate (beats/min)	68.86 (12.24)	68.63 (10.51)	146.43 (31.59)	160 (13.51)
Subjective parameters	**Physical fatigue**	18.57 (15.80)	46.25 (20.71) *	43.00 (21.82)	54.75 (21.87) *
	Mental fatigue	19.00 (14.34)	43.88 (14.86) *	24.57 (16.79)	39.63 (18.98)
	Sleepiness	14.71 (14.20)	31.00 (18.45)	11.86 (13.73)	16.38 (11.38) *
	Stress	11.29 (12.16)	32.29 (15.16) *	8.71 (8.54)	28.25 (26.75)
	Physical well-being	-	-	84.14 (15.04)	66.00 (17.65)
	Mental well-being	-	-	90.57 (11.46)	78.13 (9.75)
	Training intensity	-	-	66.57 (26.18)	73.75 (15.39)
	Muscle Pain	-	-	19.43 (14.57)	41.25 (28.09)
Vigilance parameters	**Reaction Time (ms)**	303.62 (13.95)	314.27 (19.59)	298.68 (20.29)	304.38 (21.49)
	Lapses	0.43 (0.53)	1.87 (1.64)	1.57 (2.07)	1.75 (1.49)
	Misses	0.14 (0.38)	0.12 (0.35)	0 (0)	0 (0)

Values are mean (standard deviation).

The symbol * indicates that there was a significant difference in the statistical analysis.

Moreover, only three out of the eight donors correctly indicated to be part of the donor group (see table VI). These 3 candidates were the only one to report a minor effect (average of 19,33%) of the blood donation on their physical performance.

Table VI. Crosstab representing the real group distributions versus the participants' expectations in the field experiment.

		Participant expectations		
		Non-donor	**Donor**	**Total**
Real group distributions	**Non-Donor**	5	2	7
	Donor	5	3	8
Total		10	5	**15**

Values are number of subjects (n)

4. DISCUSSION

Our double-blind randomized controlled study examined the immediate impact of a 450-ml blood donation on SF donor performances, in two distinct experiments: a laboratory experiment and a field experiment. In each trial, participants were randomly assigned to either a control or a donation group. Then, they were submitted to a multifactorial assessment including haematological measures, vigilance, and physical performance measures as well as subjective assessments. This study aimed at evaluating precisely the potential immediate impact of the donation on the SF operators' performances. First, we analyzed the results of the laboratory experiment to confirm the assumptions of the literature and to guarantee the safety of the participants for a blood donation in an operational-like environment. Indeed, to the best of our knowledge, no double-blinded randomized controlled study so far has examined the effects of a blood donation after a strenuous battle-like task in this context. This is why we adapted the settings for the field experiment.

Regarding the haemoglobin and haematocrit, even if technical reasons prevented us from analyzing the blood samples in the laboratory setting, the analysis of the field data evidenced only significant immediate effect of the 450ml-donation in the donor group. Our results were consistent with the literature[13,22] even if other studies focused only on the effect 24 hours after donation[13,22].

With regard to the impact of blood donation on performance, we did not find any significant effect of the blood donation on the physical performance in both set-ups which corroborates the results obtained by Nadler and colleagues[8]. Moreover, our results evidenced no significant effect of the blood donation on the vigilance level which is consistent with the litterature[11,23].

This absence of significant differences in performance between both groups at the end of the testing days was evidenced regardless of individual differences (e.g.,

level of fatigue, stress or expected effect of blood donation). Indeed, we could have expected an impact on performances due to the significant higher fatigue and stress levels of the donors in the beginning of the field experiment. Furthermore, their expectations regarding their group distribution could have impact their physical performance (i.e., "placebo" effect) or at least the SF operators could have allowed themselves to have a diminished physical performance (i.e., motivational effect).

Despite our attempts to counteract the limitations reported in the literature, certain limitations are inherent to our target population and must be accepted. The major weakness of our study is obviously the rather small sample size which may lead to the impression of an under powered study. Nevertheless, even if our sample size seems to be limited, it is still representative of our target population. Indeed, our research focused on an elite military unit composed only by a really restricted number of highly trained male individuals. Therefore, by agreeing to compromise on the sample size rather than on the ecological validity of our field setting, we ensure that the guidelines are tailored to the specificities of our target population. Moreover, it also offered the actual future "client" to this exceptional procedure the opportunity to safely experience the potential effect of a blood donation on their performances.

5. CONCLUSIONS

A 450ml-blood donation has no significant impact on the SF operator performances even for a strenuous exercise in an ecologically valid field environment. Thus, a 450-ml blood donation has no immediate effect on their capacities to fulfill their demanding mission in tactical circumstances. Therefore, from a donor point of view, we are in favor of allowing under strict medical supervision the use of a buddy transfusion in exceptional operation life-threatening situations when no blood components are available.

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CHAPTER 3:
WHEN DO BENEFITS TURN TO RISKS? IMPACT OF A 900ML WHOLE BLOOD DONATION ON SPECIAL FORCES PERFORMANCE.

Published article:
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Transfusion, *64*(4), 656-664.
10.1111/trf.17757

ABSTRACT

BACKGROUND - Special Forces (SF) teams operate in remote environments with limited medical support. As a result, they may need to rely on buddy transfusions to treat bleeding teammates. Considering that 450ml has no direct impact on their combat performances, it might be tempting to take more blood from a compatible donor to save a haemorrhaging teammate. This study investigates the effect of a 900mL blood donation on SF operator performance and recovery time following this donation.

STUDY DESIGN AND METHODS - Participants underwent a multifactorial assessment including measures of physiological parameters, vigilance, and physical performance. Results from the day of blood donation were compared with baseline values obtained one week earlier (i.e., immediate effect), as well as repeated testing at 7, 14 and approximately 30 days after blood donation (i.e., recovery period).

RESULTS - Haemoglobin levels and heart rate were affected by giving blood. The participants also experienced a significant decrease in physical performance of more than 50% immediately after blood donation. Recovery was slow over the following weeks, remaining significantly different from baseline until full recovery around day 30. However, participants were still able to respond to a simple stimulus and adjust their response, if necessary, even immediately after donating blood.

DISCUSSION - A 900 mL blood donation greatly affects the physical fitness of SF operators. A donation may be worthwhile if it is the only life-saving procedure available and does not endanger the donor's life. The donor would then become a patient and unable to complete the mission.

KEYWORDS: Walking blood bank, performance, elite soldier, donor safety, buddy transfusion

1. INTRODUCTION

Special Forces (SF) operate with limited logistical and personal footprints in remote and dangerous areas. In such austere conditions, they might be required to take care of a teammate with a life-threatening hemorrhage while awaiting evacuation[1–4]. Compensating for large blood loss may require considering a buddy transfusion[5,6].The immediate collection of whole blood from a compatible donor may be the only way to obtain blood, as there are no components available in such circumstances[7]. To ensure the safety of our SF operators who need to donate blood to optimise patient survival, we must identify and quantify the potential impact of whole blood donation.

Previous studies showed no significant effect of a 450mL blood transfusion neither on the vigilance of the operators nor on the physical performances[8,9]. This means that a blood donation of 450mL has no impact on the ability of the donor to carry out the mission and should not have any tactical consequences[8,10]. Therefore, in an emergency situation where there are few compatible donors available, it may be tempting to increase the amount of blood collected from a matched donor, especially considering the above findings[8–10].

The maximum volume of whole blood that can be collected is strictly limited so as not to exceed the proportion of the circulating volume that might affect a regular donor[11]. Consequently, research on the effects of large blood donations is relatively sparse and dated. Reports of performance studies after donations of more than 450mL are very limited[12,13]. One of these studies examined physical performance immediately after donating 800mL and showed an immediate significant 13% reduction in haemoglobin (Hb) and 30% reduction in time run at maximal capacity[12]. The authors also looked at the long-term effects of such a large blood donation and found a progressive recovery that began four days after donation and continued for four weeks, with no return to baseline values. However, Hb level returned to the baseline level after 14 days[12]. Another study found that a blood loss of one litre leads to a decrease in Hb level, which affects maximal oxygen uptake and therefore working time[13].

This raises the question of how the SF operator feels after this 900mL exceptional but potentially strategic collection. According to Convertino and colleagues, total blood volume increases by 100mL per 10 kg of body weight in athletes[14]. Since the SF operators are comparable to the elite athletes, it is expected

that they will also have a higher circulating blood volume[15]. In this specific population, this means that a double donation would remain below the legal percentage threshold[11]. However, the tactical implications of such a medical decision must be assessed in terms of performance and duration of effect. This assessment could determine whether the donor should be considered as an additional patient or whether, from a tactical point of view, he would still be able to carry out the mission. According to the literature, physical performance is likely to be affected immediately and significantly[12,13]. In addition, the return to normal may take several weeks and is likely to depend on the haemoglobin level[12,13].

Our study aims to investigate: 1) the immediate effect of 900mL blood donation on physical performance, vigilance, and executive functions, and 2) the recovery time of potentially altered capacities.

2. MATERIALS AND METHODS

This Prospective observational study was approved by the Medical Ethics Committee of the University Hospital (UZ-Brussel) and the Vrije Universiteit Brussel (VUB) (B.U.N.: 143201941913). The manuscript was written and edited according to the Consolidated Standards of Reporting Trials statement. The study was conducted in accordance with the guidelines for good clinical practice and the Declaration of Helsinki. There were no changes to the study design or methods since the start of the study.

The study was explained to the Belgian SF operators both verbally and in writing, and the participants signed an informed consent form if they agreed to take part. Inclusion, exclusion, and withdrawal criteria are shown in Table I. To ensure the donor safety, operational restrictions were added to the usual criteria of the Belgian Blood Transfusion Service, in particular no diving or skydiving for 15 days after blood donation. Seven Belgian male SF operators, who fulfilled all the inclusion criteria, were included in the experiment.

Table I. Inclusion, exclusion, and withdrawal criteria of the study.

Inclusion criteria	- Being a physically active operator of the Belgian Special Forces - Being military operational (i.e., Medical Operational category A) according to the yearly medical examination at the military hospital. - Meeting the Belgian Blood Transfusion Service criteria. - Being medically fit to donate blood (based on medical questionnaire and vital sign measurements). - Sufficient circulatory volume calculated using Nadler's formula $[(0{,}3669 * (Height\ in\ m)^3) + (0{,}03219 * (Weight\ in\ Kg)) + 0{,}6041]$ which should ensure that collected volume remains below 20% of circulatory volume.
Exclusion criteria	- Failed to meet blood donation criteria. - Have donated blood in the last 3 months. - Antihypertensive therapy intake. - Any physical injury before starting to study. - Heart disease history. - Diabetic. - Haemoglobin level lower than 14g / dL.
Withdrawal criteria	- Any medical condition or physiological reaction that arises during testing deemed unfit by the supervising physician. - Decision of the participant to interrupt his participation. - Operational constraints.

2.1. Study design

14 days before donating blood, participants came to the Military Hospital Queen Astrid (MHQA) to perform an incremental graded exercise test to exhaustion (i.e., VO_2max test) and to become acquainted with the testing protocol (i.e., familiarisation). The participant completed a baseline test seven days after the familiarisation. The study design is shown in Figure 1.

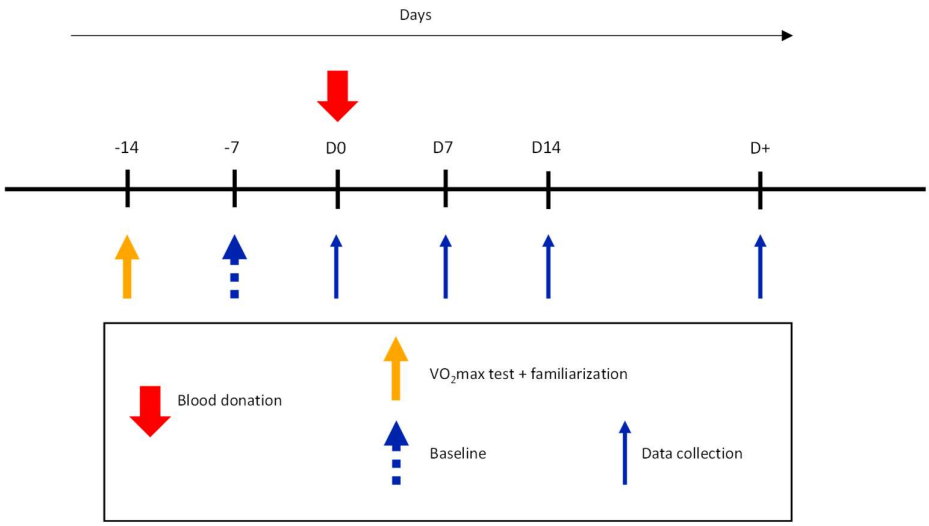

Figure 1: Study design and testing plan. VO_2max testing was performed 14 days before blood donation, followed by baseline testing seven days later. On day 0, the test sequence performed at baseline was repeated after donation to measure the immediate effect of the 900mL blood donation. It was repeated on days 7, 14 and + to assess potential recovery over time. D+ is an average of 30 days after donation, depending on the availability of the donor.

On day 0, before the start of the test, participants completed a medical questionnaire and were interviewed by a doctor who assessed whether the participant was medically fit to be a blood donor. The blood donation occured only on D0. The first blood unit was collected using a sterile single collection bag with a 16-gauge needle and filled with CPDA (Citrate-phosphate-dextrose-adenine) (TERUMO BCT). After a 30-min resting period, a second bag was filled. The blood donation was followed by another 30-min resting time before beginning the testing sequence. The collected blood was not stored and was destroyed immediately after the donation. After the blood donation, a physician assessed their wellbeing and cleared them for testing. The whole procedure was supervised by an anaesthesiologist. To reduce as much as possible potential dizziness due to dehydration, the participant had to drink at least 900mL of water and eat a standardized salted snack during the whole blood donation. The participant received a hot meal for lunch from the hospital canteen and had to eat it at the end of the testing phase before leaving.

The testing phase (Figure 2) was repeated 7, 14 and approximatively 30 days (28 or 35, depending on the availability of the participants) after blood donation. All participants underwent this multifactorial assessment including measures of physiological parameters, vigilance, and physical performances as well as subjective assessments.

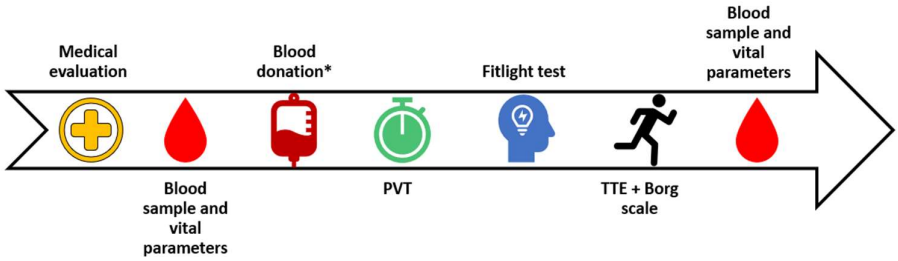

*Figure 2: Sequence of a testing day from the baseline to D+. The test sequence is standardized and repeated in the same order each time the participant completes it. It consists of a medical interview with blood sampling, the blood donation (*only performed on D0), a vigilance test (i.e., psychomotor vigilance test), a fitlight test, a physical performance test (i.e., the maximal run measured by a time to exhaustion and the Borg Scale) and, finally, another blood sample is taken at the end of the sequence.*

2.2. Outcomes

As the main study objective was to assess the impact of a 900mL blood donation on tactical capacity, the study design included physiological parameters, vigilance, and physical performance measurements.

a. Physiological parameters

Participants had vital signs measurements (e.g., heart rate) at the beginning and end of each day of the trial. On day 0, the parameters were also measured before each test to ensure participants remained healthy. The anaesthetist released the participant from test to test based on these parameters.

Blood samples (EDTA tube 3 mL) were taken at the beginning and end of the testing sequence on days 0, 7, 14 and +. A complete blood count was performed on a Sysmex XP-300 automated haematology analyser. This study only reported haemoglobin level. On the day of donation, 5 hours were left between the collection of the first sample and the collection of the end-of-test sample.

b. Vigilance assessment

The 10min-computerized Psychomotor Vigilance Test (PVT) recorded reaction times (RT) to visual stimuli that occurred at random inter-stimulus intervals. Participants were instructed to monitor a screen and click as fast as possible once a millisecond counter appeared in the box and starts incrementing (from 0 to 1000 milliseconds).

c. Fitlight test

The visuomotor task requiring the Fitlight-hardware and software (http://www.fitlighttraining.com/) developed by Van Cutsem was used[16]. This task measured simple and complex reactive time to visual stimuli. Seven lights were placed against a wall and lit in different colours for two seconds, one behind another in a random order. If a light turned red, green, or yellow (i.e., simple stimuli), participants had to put out the light as fast as possible by passing before the light with the left or right hand within a range of 5cm. However, if a light turned blue (i.e., complex stimuli), participants were instructed not to respond to the stimulus on the wall. Instead, they had to turn around and put out another light lying behind them on the floor (1m50). After each stimulus, participants had to return to the indicated starting position and focus again on the fixation cross. Each colour was presented 16 times, yielding a total of 64 stimuli. The inter-stimulus time varied between 3 to 6 seconds and each inter-stimulus time was randomly used 16 times. The total test lasts approximately 6 min 30 s. Reaction times (RT) was measured for both simple and complex stimuli. Furthermore, we calculated the interference effect by measuring the difference between the RTs to simple and complex stimuli. This measures the ability to inhibit an automatic response (i.e., the simple task) to provide another, more appropriate response to a complex task.

d. Physical assessment

To assess the effect of the 900mL blood donation on physical performance, the participants performed a maximal running test to exhaustion after the blood donation[12,17]. The "running test to exhaustion" (TTE) was performed on a treadmill (Ergocard Clinical, Medisoft, Belgium) at a speed determined by the individual VO_2max results from the preliminary incremental maximal exercise test (VO_2max protocol)[18]. The TTE started after a five-minute warm-up on the treadmill. Participants run until volitional exhaustion at a pace corresponding to 90% of the speed associated with their VO_2max (ranging from 13.8 to 15.4km/h). Time to

exhaustion is measured with a manual chronometer and expressed in seconds. The participants were not informed on their recorded times until completion of the whole study and no watches were viewable during the maximal run. Blood lactate concentration was measured at the beginning and at the end of the TTE. Immediately after the end of the TTE, the subjects completed a perception of exertion questionnaire, the Borg scale. This is a self-rated scale ranging from 6 to 20. 6 corresponds to no exertion and 20 to maximum perceived exertion[19].

e. Logbook

Participants were required to complete a daily questionnaire in addition to the test sequence. The questionnaires were designed to assess how people felt day-to-day and over time in terms of exercise tolerance, fatigue, and experienced symptoms. This allowed us to better interpret the effects of the donation and to record unmeasured but perceived information.

2.3. Statistical analysis

All statistical analyses were performed using IBMS Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 28.0 for Windows. A parametric paired sample t-test was used to test the results of the repeated parameters compared with the baseline at every step of moment of the measurement. Except for the physiological parameters, which are compared to those measured when D0 started. The assumptions of normality (Kolmogorov-Smirnov with a Lilliefors Significance Correction) and homogeneity (Levene's test) were tested before performing these statistical analyses. For all statistical tests, statistical significance was accepted at the $p \leq .05$ level. Cohen's d (d') were used to measure the effect size.

3. RESULTS

3.1. Physiological parameters

This section analysed two key metrics. The first was the change in haemoglobin and heart rate on the day of the donation (Table II). This was based on samples and measurements taken at the start and end of the D0 test (5 hours delayed). The second was the haemoglobin level variation on each test day to measure recovery over weeks (Table III and Figure 3). The haemoglobin level of the participants was significantly lower 5 hours after 900mL blood donation than before donation. The

heart rate of the donors remains significantly higher 5 hours after the 900mL blood donation than right before (Table II).

Table II. Mean values and standard deviations of the haemoglobin level and of the heart rate at the beginning and at the end of the D0.

Measures	Pre-Donation (*n*= 7)	Post-Donation (+5hours) (*n*= 7)	p. t-test	Effect size
Haemoglobin (g/dL)	15.41 (1.21)	14.33* (1.14)	.005	1.653
Heart rate (bpm)	69.29 (7.91)	94.14* (8.13)	.003	-1.812

p. t-test = p-value of the student's t-test.
Values are mean (standard deviation).
The symbol * indicates that there was a significant difference in the statistical analysis (i.e., $p < .05$).

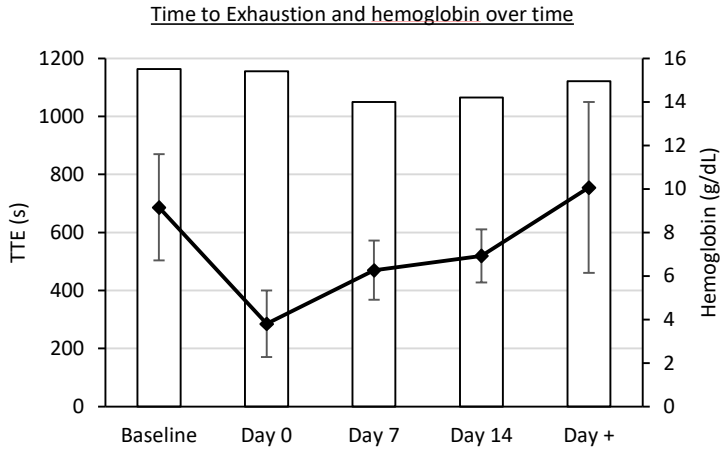

Figure 3: Graphical comparison between TTE and haemoglobin changes over time. The mean values of haemoglobin are represented by the bar graph and are related to the right axis. The mean values are plotted on the left axis with their associated standard deviation.

Statistical analysis of haemoglobin levels showed that haemoglobin levels were significantly lower 7 and 14 days after blood donation, while returning to normal at 28 days (Table III).

Table III. Means and standard deviations for the hemoglobin level at D0, D7, D14, D+.

Measures	D0 *(n = 7)*	D7 *(n = 7)*	D14 *(n = 7)*	D+ *(n = 5)*
Haemoglobin (g/dL)	15.41 (1.21)	14.00* (0.75)	14.20* (1.07)	14.96 (0.74)
p. t-test	-	.003	.030	.511
Effect size	-	1.860	1.069	0.322

D+= testing day 28 or 30 depending on the availability of the participant; p. t-test = p-value of the student's t-test. Values are mean (standard deviation).
The symbol * indicates that there was a significant difference in the statistical analysis (i.e., $p < .05$).

3.2. Performance parameters

Exhaustion was reached significantly faster after the blood donation compared to baseline. Moreover, the 900mL blood donation still had a significantly negative effect on the physical performance of the donors 7 days and 14 days after the donation (table IV and Figure 3). After approximatively 30 days, their performance had normalized. Regarding the vigilance and fitlight testing, donating 900mL of blood had no significant negative effect on participants' reaction time (Table IV and Figure 3).

Table IV. Means and standard deviations for the performance's parameters: TTE, PVT and fitlight test at D0, D7, D14, D+.

	Measures	B *(n = 7)*	D0 *(n = 7)*	D7 *(n = 7)*	D14 *(n = 7)*	D+ *(n = 5)*
Physical performance	**TTE (s)**	686.71 (182.93)	285.71* (115.00)	470.29* (101.64)	519.57* (91.19)	755.20 (294.27)
	p. t-test	-	< .001	.004	.007	.990
	Effect size	-	2.932	1.720	1.510	-.006
	Borg scale Score	17.57 (0.787)	18 (1.15)	17.43 (1.40)	17.57 (1.51)	17.40 (1.52)
	p. t-test	-	.200	.689	1.000	0.477
	Effect size	-	-.545	.159	.000	.351
	Lactate (mM/L)	11.98 (2.27)	10.63 (2.72)	12.95 (1.78)	12.51 (0.87)	11.64 (0.41)
	p. t-test	-	.418	.388	.606	.378.328
	Effect size	-	.328	-.352	-.206	.443
PVT	**RT (ms)**	293.18 (20.24)	288.58 (24.77)	283.70 (15.41)	288.97 (23.74)	283.43* (19.98)
	p. t-test	-	.549	.068	.352	.030
	Effect size	-	.240	.841	.381	1.477
Fitlight test	**RT blue (ms)**	1317.96 (82.09)	1332.41 (112.56)	1305.57 (93.79)	1350.20 (104.68)	1342.78 (146.65)
	p. t-test	-	.546	.649	.277	.276
	Effect size	-	-.242	.181	-.452	-.563
	RT color (ms)	1066.05 (117.77)	1044.93 (142.91)	1019.10* (106.97)	1034.15 (122.69)	1014.03 (153.61)
	p. t-test	-	.131	.003	.099	.224
	Effect size	-	.661	1.825	.739	.643
	Interference effect (ms)	251.91 (69.03)	287.48 (85.83)	286.47 (105.16)	316.05* (103.29)	328.74* (71.19)
	p. t-test	-	.096	.113	.017	.008
	Effect size	-	-.744	-.701	-1.236	-2.223

PVT= Psychomotor vigilance test; TTE= Time to exhaustion; RT= Reaction time; D+= testing day 28 or 35 depending on the availability of the participant; p. t-test = p-value of the student's t-test.

Values are mean (standard deviation).

The symbol * indicates that there was a significant difference in the statistical analysis (i.e., $p < .05$).

3.3. Logbook

Logbook analysis showed that 5 participants reported significant shortness of breath on days 1 to 4, 2 on days 7 and one on days 14. Orthostatic disturbances occurred in 3 participants on day 1 or by day 4 and 7. Finally, increased exercise

intolerance was reported by two participants up to day 7 and by two more up to day 14.

4. DISCUSSION

This observational study examined the immediate effects of a 900mL blood donation on the performance of 7 Belgian SF operators and the time required for recovery.

The results showed that a 900mL blood donation had a significant and immediate effect on participants' physiological parameters. This was demonstrated by comparing heart rate and haemoglobin data at the beginning and end of the day. After the 900mL blood donation, haemoglobin levels were lower and resting heart rate was higher. Several studies have already reported decreased haemoglobin levels following standard donation[10,20–23]. However, an increase in resting heart rate after donating 450mL has only been reported twice[20,24]. In addition, the effect of such an important blood donation on the heart rate was to be expected on the basis of the ATLS classification of hypovolemic shock[25]. The fact that our study reported both effects can be explained by the large volume of blood collected. Removing an average of 15% of circulating volume means removing 15% of available red blood cells, resulting in a significant reduction in haemoglobin. As cardiovascular function is known to be strongly influenced by circulating volume, cardiac output must be increased to maintain oxygen delivery and compensate for the significant volume loss[14,26]. The volume loss was apparently not fully compensated for by the fluid intake. This volume was replenished at a later stage by the application of the Frank-Starling forces[10,14]. However, as we observed a significant decrease in the haemoglobin levels at the end of D0, it can be assumed that the volume was at least partially recovered, allowing for dilution[10,14]. The orthostatic intolerance experienced by our participants in the days following donation may be explained by these same significant variations in circulating volume.

In terms of physical performance, the immediate effect of the 900mL blood donation was rather severe. All participants experienced at least a 50% reduction in exercise capacity as measured by the time to exhaustion test. Recovery was slow over the following weeks. It remained significantly different from baseline values until complete recovery at D+. The findings can be compared to Ekblom and colleagues in 1972[12]. They observed a loss of 30% in the maximum running time immediately after an 800mL blood donation. They also observed a gradual recovery

in the weeks following the blood donation. However, they did not observe a complete recovery after donation, whereas the haemoglobin levels were reported to be comparable to baseline values 14 days after the donation[12]. The results also showed that the lactate inflexion point, measured at the end of the maximal effort, was far exceeded. In addition, the participants completed the Borg Scale after the physical task to assess perceived fatigue. There was no difference in perceived exhaustion during recovery, and we can confirm that participants stopped at the point of physical exhaustion and not because of a motivational bias. This potential motivational bias was already minimised by our experimental design[12,17]. In our setting, the relative bias was limited by the fact that the participants had to perform a maximal run of about 5 minutes at 90% of their maximal speed. The motivational aspect would have become more relevant in a submaximal exercise of longer duration and could have influenced the interpretation of the results. Moreover, the maximal exercise mimics a rapid response to outrun the enemy or launch an attack. This may be the least well-described in the literature, yet the one most likely to be influenced by donating blood, and thus have the greatest tactical impact on mission success or personal safety[27].

In terms of vigilance, no effect of a 900mL blood donation could be demonstrated, either immediately or in the long term. These results are in line with our previous findings after a standard double-blind blood donation[9]. Overall, reaction times improved over time. There was also no significant effect on complex responses involving executive functions. Thus, participants remained able to respond to a simple stimulus and adapt their response pattern to a more appropriate response when needed.

Although the design was robust, given the repeated measurements and the homogeneity of the sample, this study presents some limitations that must be addressed. The sample size is the most obvious limitation. This limitation is inherent to the population studied. We are dealing with a small elite unit with significant operational constraints. The number of missions, exercises and constraints associated with maintaining their professional skills made significant and sustained participation more difficult. Nevertheless, despite the small sample size resulting from these constraints, it has been possible to present a very consistent, convergent, and overall strong set of results. We would like to emphasise that these results can certainly not be generalised to other members of the military or to the average civilian population. The population studied has already demonstrated

tolerance to donating 450mL of blood and was able to perform combat tasks in the same way[8,9]. In addition, this population has been shown to possess a higher circulating volume, comparable to highly trained athletes[15]. Working with this specific population allowed us to ensure that the volume withdrawn remained below the 20% total blood volume limit. For this reason, our results are only intended as a recommendation to ensure the safety of the SF operators in exceptional operational circumstances. Regrettably, study protocol did not mimic the operational environment. The study was carried out in a standardised and controlled environment, without being exposed to real life, to anticipate the consequences of a hypovolemic response. Therefore, it was impossible to take fully into account the realities of this population, which include strenuous exercise under extreme conditions, stress, dehydration, fatigue, and the enormous weight of the material to be carried. As a result, our conclusion cannot include any specific recommendations for the "usual" battlefield environment.

5. CONCLUSION

In conclusion, a 900mL donation could be considered for Belgian SF operators in exceptional life-saving circumstances in remote environments with scarce resources and delayed evacuation. This would require a reassessment of the mission. The donor would become a patient and would no longer be able to carry out the mission as his physical capabilities would be severely compromised. A donation of 900mL of blood should only be considered as a last resort in an absolute emergency and under strict medical guidelines.

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CHAPTER 4:
WHAT IS THE MOST EFFECTIVE METHOD OF PRESERVING THE REMAINING WHOLE BLOOD COLLECTED UNDER AN EMERGENCY PROTOCOL TO SAVE MORE LIVES?

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ABSTRACT

BACKGROUND: Should an urgent need for blood products arise, the use of emergency collection procedures may be the sole means of accessing blood products. It is therefore crucial to ensure the preservation of blood bags collected but not used so that they can be utilised for another patient. Whole blood can be stored at either cold or room temperature, depending on the case studies reported in the literature. The question then arises as to which storage temperature will result in the best overall preservation of whole blood (WB) properties. Our study investigated this question for storage periods of up to 48 hours.

METHODS: A prospective randomised interventional study was conducted on 30 WB bags collected from military personnel eligible for donation. The blood bags were randomly kept either at 22°C or at 4°C. Samples of each bag were taken immediately following blood collection to perform baseline measurements. The bags were then stored for 48 hours before undergoing a second analysis. The measurements included general product quality assessments, such as metabolic and haematological parameters. Furthermore, coagulation factors, thrombin generation potential, and platelet function were evaluated. The latter included platelet activity, clot formation capacity, and aggregometry.

RESULTS: The results showed that after 48 hours of storage, both groups presented sufficient overall quality as assessed by metabolic parameters. Haemoglobin levels remained stable in both groups after 48 hours, whereas platelet count decreased if WB was stored at 4°C. Aggregometry results were significantly affected after just 48 hours, in both groups. For viscoelastometry measurements, WB stored at RT showed better preserved clot stiffness. While the platelet-related component was unaffected in RT-stored WB, it was reduced in 4°C-stored WB. RT-stored WB also demonstrated an increased coagulation time. Regardless of storage conditions, thrombin-generating potential was maintained.

DISCUSSION: Both temperatures offer products of sufficient quality for managing haemorrhagic patients. However, based on platelet count and clot stiffness measurements, the cold storage temperature appears slightly less favourable. The choice of either temperature should depend on the frequency of the patient's treatment.

KEYWORDS: whole blood, Emergency donor panel, haemostatic potential, cold stored whole blood, warm whole blood

1. INTRODUCTION

Standards of care evolved from the use of whole blood (WB) in the first half of the century to component therapy in the second half. However, traditional transfusion support cannot always be provided in the operational theatre, and logistics may mean that WB is the only option on site. The current resurgence in military use of WB is therefore a response to the realities of medical care in austere environments. Clinically, the properties of WB have been shown to improve survival in haemorrhagic casualties[1–4] compared to component therapy. This is particularly true for the treatment of coagulopathic patients[5]. This product alone allows the three blood components to be administered in physiological proportions, reducing donor exposure and dilution[6,7]. It also overcomes storage limitations imposed by individual component storage requirements[8]. Using WB in the field to treat bleeding casualties is the most appropriate clinical and/or logistical solution[9].

There are two ways of obtaining WB in the field. One way is through the supply chain (i.e., low titre O WB), WB is collected and prepared in the home country before being sent to the field stored cold[10]. This home-prepared WB is usually leukodepleted for safety. By reducing the number of white cells, one decreases the risk of transfusing immunomodulators and transmitting viruses carried by these cells[11]. Alternatively, warm WB can be collected directly on-site using a walking blood bank (WBB) or an emergency donor panel (EDP)[9]. In this case, time is usually critical and there is no time for leukodepletion. The collection of WB in the field is an easy way to obtain and prepare WB in a challenging environment[12]. Occasionally, though, a few bags of blood remain. This may occur because the patient has been stabilised, evacuated, or has passed away. It is important not to waste this precious and limited resource and to consider storage options.

There are two options for the storage of WB. Some recommend storing blood bags at 4°C as this gives a longer shelf life[13]. Others suggest storage of blood at room temperature to keep it warm and ready for use. However, due to bacterial risks, storage at room temperature reduces shelf life. Therefore, both shelf life and product characteristics are affected by the choice of storage temperature. In fact, many studies have already been carried out to analyse the properties of cold-stored WB since it was reintroduced into the military supply chain. These have included analyses of the effect of leukoreduction on platelet function, haemostasis, and general product quality[11,14], general quality during storage for the non-leukoreduced variant[13,15,16] and the effect of pathogen reduction on haemostasis[17].

Non-leukoreduced WB showed a decrease in agonist-stimulated aggregation which was attenuated in whole blood stored cold in comparison with measurement in whole blood stored at 22°C[17]. Other studies showed a gradual decrease in the number of platelets and in platelet function when measured by aggregometry[15,16]. All studies showed stable coagulation values over time when measured by viscoelastometry[13,15,16]. Warm WB, although not extensively studied, was found to have only moderate loss of platelet function and clotting factors after storage at 25 °C for 72 hours[18]. Haematocrit, platelets, and coagulation factors were stable and viscoelastometric measurements showed no change in coagulability over time[18].

To our knowledge, no one has directly compared the quality of non-leukoreduced cold-stored WB (CSWB) and non-leukoreduced warm WB (WWB). Therefore, the goal of this study was to compare two storage temperatures to determine the best way to store excess blood collected in the field to preserve its clinical benefits for bleeding patients.

2. MATERIALS AND METHODS

2.1. Donor population

Active male military personnel were informed of the study by e-mail. Those who wished to participate in the study contacted the laboratory to make an appointment for testing. Participants were medically fit to donate and did not meet any of the following exclusion criteria: blood donation within the last 2 months, three blood donations in the current year, use of medication affecting haemostasis such as anti-inflammatories, antiaggregants or anticoagulants in the 10 days prior to participation. Thirty participants were enrolled in the experiment. They received a written and oral explanation of the protocol and signed an informed consent form.

2.2. Study design and protocol

This prospective randomised interventional study was conducted at the Queen Astrid Military Hospital. Before giving blood, the donor underwent a medical interview to make sure he was fit to donate. A 450 ml blood bag was then collected by a nurse. The blood bags were stored at room temperature for two hours after collection. They were then randomised into two storage temperature groups. The blood bags that were stored at a controlled room temperature of 22 °C were

included in the warm WB (WWB) group (n=16), and those that were stored at 4 °C in the cold WB (CSWB) group (n=14).

Blood was collected directly for measurements at D0 to establish baseline values and after 48 hours to compare the overall product quality of both groups over time. Blood quality was evaluated by measuring parameters related to 1/ metabolic function, 2/ haematological factors such as the presence of platelets and RBCs, 3/ coagulation factors, and 4/ haemostatic potential to confirm adequate platelet function.

2.3. Whole blood collection and sampling

The volume collected from each volunteer was standardised and scaled during donation. An empty blood bag contained 63 mL of CPDA-1 (citrate phosphate dextrose adenine) (Terumo BCT Europe). A 16-gauge needle was attached to this bag for collection. During collection, the bag was homogenised every 30 seconds to ensure quality and preserve the collected product. On the day of donation and after 48 hours, samples were taken for measurement. Blood bags were gently homogenised 10 minutes by placing them on a shaker before sampling. For the collection of the samples the bags were connected to a TSCD connector (Terumo BCT Europe, Brussels, Belgium) under sterile conditions. The sampling bag, which was placed on a balance, was filled by gravity to obtain 80g. Tubes for each analysis were filled from these bags. Sampling tubes without additives (Avantor-VWR, Leuven, Belgium) were used because blood was already anticoagulated by the CPDA-1 present in the collection bag.

2.4. Outcomes

a. Metabolic parameters

Lactate, pH, glucose, sodium, and potassium levels were measured on an i-STAT handle analyser (ABBOTT, Chicago, IL) using chemistry (Chem8+) and blood gas cartridges (CG4+).

b. Haematological parameters

Haemoglobin (Hb), white blood cell count and platelet count were measured using a Sysmex XP-300 automated haematological analyser (SYSMEX Europe, Hoeilaart, Belgium).

c. Coagulation factors

Whole blood samples were centrifuged at 2500g for 2*10 minutes to obtain platelet-poor plasma. Plasma was stored at -80°C for analysis on all samples at the end of the study.

Fibrinogen, and factor VIII (Fact VIII), were analysed on an ACL-TOP 750 (WERFEN, Barcelona, Spain). Fibrinogen was determined by HemosIL QFA thrombin (WERFEN). FVIII was determined by a one-stage assay using HemosIL SynthasIL, HemosIL FVIII deficient plasma and Calibration plasma (WERFEN).

d. Haemostatic function

Quantitative in vitro determination of platelet function was also studied by flow cytometry on a FacsCanto II (BD Biosciences, New Jersey, US). Pre-analytical processing was previously described[19]. Blood samples were kept at room temperature for one hour before adding a mix of fluorochrome coupled antibodies and HEPES buffer (WB dilution of 1/12). For platelet activation experiments, agonists 2-MeSADP (1 µM final concentration) and TRAP-6 (3.3 µM final concentration) were added to the mix. Samples were incubated for 20 minutes at room temperature. Incubation was stopped by adding 1 mL of HEPES-PFA 1% buffer and analysed within 48 hours. The following platelet surface markers were included: CD41a phycoerythrin (PE, clone: HIP8, BD), PAC-1 fluorescein isothiocyanate (FITC, binds active conformation of αIIbβ3, BD) and CD62P APC (anti-P-selectin, alpha-granule release, Miltenyi, Bergisch Gladbach, Germany).

The platelet aggregation potential was determined by Multiplate (Roche diagnostics GmbH v 5.0, version du logiciel: 2.0, 04/2021; Basel, Switserland) using different agonists: ADP, TRAP, and arachidonic acid (ASPI).

Viscoelastic haemostatic properties were studied using the Quantra haemostasis analyser, based on a fully automated Sonic Estimation of Elasticity via Resonance (SEER) Sonorheometry proposed by HemoSonics (Stago BioCare, Asnieres-sur-Seine, France). The QStart cartridges were used to determine clot time (CT), clot stiffness (CS), fibrinogen contribution to stiffness (FCS) and platelets contribution to stiffness (PCS).

e. Thrombin generation

Thrombin generation was evaluated on a thrombinoscope (Stago, Asnières sur Seine, France) to generate peak of thrombin generation (peak), lag time (LT),

thrombin generation velocity index (VelInd), and endogenous thrombin potential (ETP). Whole blood samples were centrifuged at 2500g for 2*10 minutes to obtain platelet-poor plasma. All samples were stored at -80°C for analysis at the end of the study.

2.5. Ethics

This prospective randomised interventional study was approved by the ethical committee of Saint-Luc University Hospitals (2022/11OCT/372) and conducted at the Military Hospital Queen Astrid. The manuscript was written and edited in accordance with the Consolidated Standards of Reporting Trials statement. The study was conducted according to the Good Clinical Practice Guidelines and the Declaration of Helsinki.

2.6. Statistical analysis

All statistical analyses were performed using IBMS Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 28.0 for Windows. The assumptions of normality (Kolmogorov and Smirnov with Lilliefors significance adjustment) and homogeneity (Levene test) were tested prior to statistical analysis. If normality was not met at D0, outliers were removed from all subsequent analyses for comparison to a normal population.

A 2[time (D0, 48H)] × 2[groups (CSWB, WWB)] mixed ANOVA with time as the within-subjects factor and groups as the between-subjects factor was used to compare all the measured outcomes. Greenhouse-Geisser Epsilon corrections were used when sphericity was violated. The p-value significance level was set at $p < 0.05$ for all statistical tests. Effect size was determined using partial eta-squared.

3. RESULTS

All results are presented in Table I.

Table I: Overview of the measured data of metabolic, haematological and VET parameters as well as thrombin generation and coagulation factors compared at D0 and 48H for CSWB and WWB.

Parameters	Measures	WWB			CSWB			*Statistics (time*group)*	
		N	D0	48H	N	D0	48H	*p*	η^2
Metabolic parameters	Na (mmol/L)	16	143.94 (1.29)	143.13 (0.81)	14	143.07 (1.14)	140.29 (1.20)	<0.001	0.405
	K (mmol/L)	16	3.30 (0.41)	4.80 (0.50)	14	3.31 (0.39)	6.26 (1.05)	<0.001	0.43
	Glu (mg/dL)	16	294.88 (65.58)	323.06 (13.62)	14	266.64 (79.02)	400.36 (16.45)	0.001	0.315
	Lac (mmol/L)	16	0.93 (0.42)	12.65 (0.95)	14	1.15 (0.55)	3.71 (0.55)	<0.001	0.981
	pH	16	7.18 (0.06)	6.72 (0.03)	14	7.19 (0.08)	6.94 (0.03)	<0.001	0.672
Haematological parameter	Hb (g/dL)	15	13.85 (1.00)	13.47 (1.58)	14	13.89 (0.91)	14.36 (2.64)	0.212	0.057
	Plt (10^3/µL)	15	198.60 (37.84)	177.80 (37.18)	14	223.43 (47.26)	165.07 (55.09)	<0.001	0.367
	WBC (103/µL)	15	5.65 (0.85)	5.57 (0.84)	14	5.46 (1.00)	5.47 (0.95)	0.566	0.012
Coagulation factors	Fib (mg/dL)	16	289.44 (58.07)	256.31 (45.26)	14	244.86 (56.14)	209.36 (36.86)	0.817	0.002
	Fact VIII (%)	16	129.61 (19.41)	71.34 (15.81)	14	143.15 (42.63)	64.63 (15.02)	0.036	0.148
Haemostatic potential	Activation platelet markers: FACS								
	PAC-1 ADP (RFU)	14	97.53 (1.78)	86.77 (7.95)	14	97.70 (1.19)	85.62 (6.57)	0.599	0.011
	PAC-1 TRAP (RFU)	15	98.50 (0.93)	68.22 (24.91)	11	98.51 (0.57)	72.61 (12.04)	0.587	0.013
	PAC-1 Unstimulated (RFU)	14	11.98 (9.60)	1.51 (1.04)	14	6.11 (5.71)	23.44 (10.85)	<0.001	0.696
	P-Selectin ADP (RFU)	14	53.49 (10.86)	44.80 (17.89)	14	60.81 (10.15)	63.46 (14.58)	0.062	0.128
	P-Selectin TRAP (RFU)	15	85.70 (7.09)	61.09 (24.23)	13	90.28 (5.68)	58.65 (19.68)	0.051	0.144
	P-Selectin Unstimulated (RFU)	13	0.92 (0.53)	9.87 (6.65)	14	1.42 (0.96)	22.14 (10.91)	0.002	0.325
	Aggregometry: Multiplate								
	ADP ([AU*min])	15	482.53 (133.54)	21.87 (23.49)	14	430.79 (171.86)	111.79 (69.35)	0.014	0.204
	TRAP ([AU*min])	15	955.33 (234.89)	242 (115.62)	14	888.64 (278.03)	379.14 (151.96)	0.023	0.178
	ASPI ([AU*min])	15	571.80 (253.77)	17.60 (32.53)	14	543.07 (238.34)	168.71 (120.31)	0.048	0.137
	Viscoelastometry test: Quantra								
	CT (sec)	15	165.60 (15.23)	203.93 (16.17)	14	167.71 (28.39)	181.36 (21.33)	0.010	0.240
	CS ([hPa])	15	15.48 (3.20)	14.53 (3.93)	13	15.73 (2.75)	11.21 (4.18)	0.009	0.237
	PCS ([hPa])	15	14.05 (2.93)	13.21 (3.66)	13	14.41 (2.37)	10.20 (3.88)	0.013	0.214
	FCS ([hPa])	15	1.43 (0.42)	1.32 (0.45)	14	1.29 (0.46)	0.97 (0.46)	0.600	0.125
Thrombin generation	ETP (nM)	14	1294.65 (206.36)	1321.33 (204.09)	13	1328.10 (292.28)	1353.24 (163.27)	0.987	<0.001
	LT (min)	14	5.19 (1.19)	5.25 (1.16)	13	4.43 (1.01)	5.41 (1.05)	0.001	0.377
	Peak (nM)	14	135.76 (26.17)	113.90 (25.06)	13	155.86 (71.87)	142.88 (54.00)	0.719	0.005
	VI (nM/min)	14	26.24 (7.90)	14.53 (5.20)	13	35.29 (30.69)	23.07 (15.98)	0.958	<0.001

Values are mean (standard deviation). The p-value significance level was set at $p < 0.05$ for all statistical tests.

Abbreviations: ADP= Adenosine diphosphate, ASPI= Arachidonic acid, CT= Clotting time, CS= Clot stiffness, D0= measures realized at day 0, ETP= Endogenous thrombin potential, Fact VIII= Factor 8, FCS= Fibrinogen part of the clot stiffness, Fib= Fibrinogen, Glu= Glucose, Hb= Haemoglobin, K= kalium, Lac= Lactate; LT= Lagtime, N= sample size, Na= Natrium, p= p-value, PAC1= Plt= Platelets, PCS= Platelet part of the clot stiffness, TRAP= Thrombin receptor activator, VET= viscoelastometric testing, VI= Velocity index, WBC= White blood cells, 48H= measures realized at 48hours of conservation, η^2= effect size.

3.1. Metabolic parameters

All measured parameters differed significantly between groups over time. CSWB had a greater increase of potassium and glucose and a greater decrease of sodium than WWB, whereas WWB had a greater increase of lactate associated with a decrease of pH.

3.2. Haematological parameters

Among the haematology parameters, only the platelet count was significantly different between the CSWB and WWB groups over time. Both groups showed a decreasing trend, which was more pronounced in CSWB than in WWB (Figure 1). Hb and WBC remained stable in both groups over time.

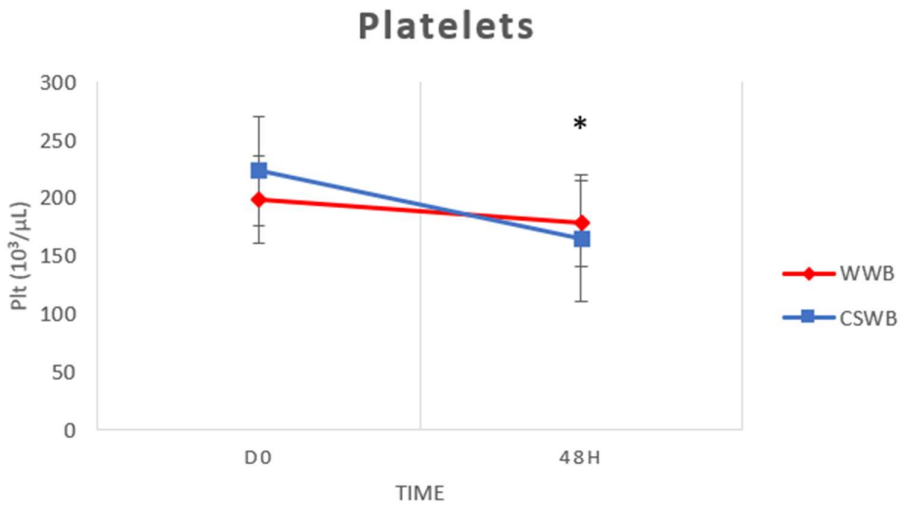

Figure 1: Graphical comparison of the impact of storage over time (D0 and 48h) on platelets (Plt). The data pertains to cold-stored whole blood (CSWB) and warm whole blood (WWB). The mean values are plotted with their associated standard deviations. Statistical significance (p<.05) is indicated on the graph by an asterisk (*).

3.3. Coagulation factors

There was a trend towards a decrease in all coagulation factors over time in both groups. However, a statistically significant difference between the groups over time was only seen for FVIII.

3.4. Haemostatic function

Platelet activatability studied by flow cytometry, representing alpha granule release (membrane bound P-selectin) and activated $\alpha_{IIb}\beta_3$ (PAC-1), showed a similar decline over time in both groups in response to either ADP or TRAP activation (Figure 2). In the unstimulated condition, we demonstrated a statistically significant increase in membrane bound P-selectin over time, especially in CSWB. Activation of $\alpha_{IIb}\beta_3$ (PAC-1) declined in WWB but increased in CSWB at 48h.

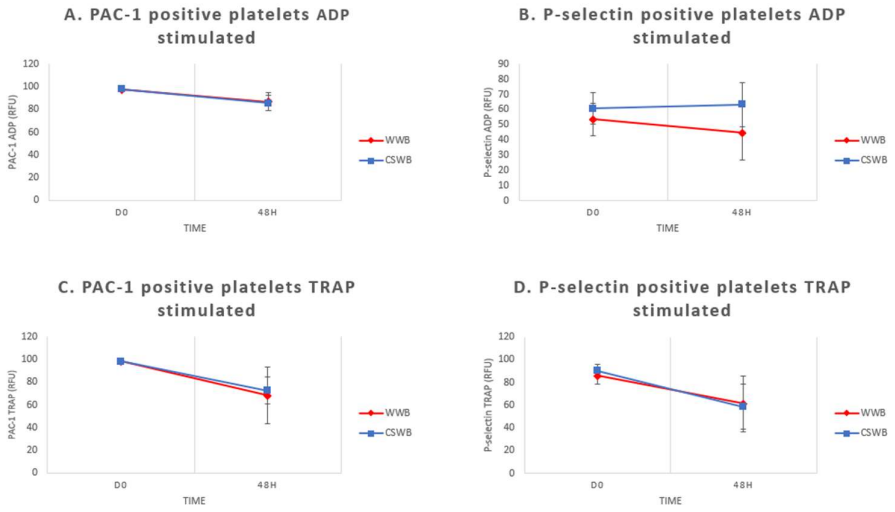

Figure 2: Graphical comparisons of the impact of storage over time (D0 and 48h) on FACS parameters are presented here. The data pertains to cold-stored whole blood (CSWB) and warm whole blood (WWB). The graphs A and B depict the platelet activatability through the expression of PAC-1 marker in response to either ADP or TRAP stimulation. The graphs C and D depict the platelet activatability through the expression of P-selectin marker in response to either ADP or TRAP stimulation. The mean values are plotted with their associated standard deviations.
Abbreviations: FACS= fluorescence activated cell sorting, TRAP= thrombin receptor activator, ADP= adenine diphosphate.

For the Quantra parameters, all values, except the fibrinogen part of the clot stiffness (FCS), were significantly different between groups over time. The clotting time (CT) shows increased values in the WWB compared to CSWB while the clot stiffness (CS), and the platelet part of the clot stiffness (PCS) showed increased values in CSWB compared to WWB (Figure 3). There was no statistically significant interaction group*time for the FCS.

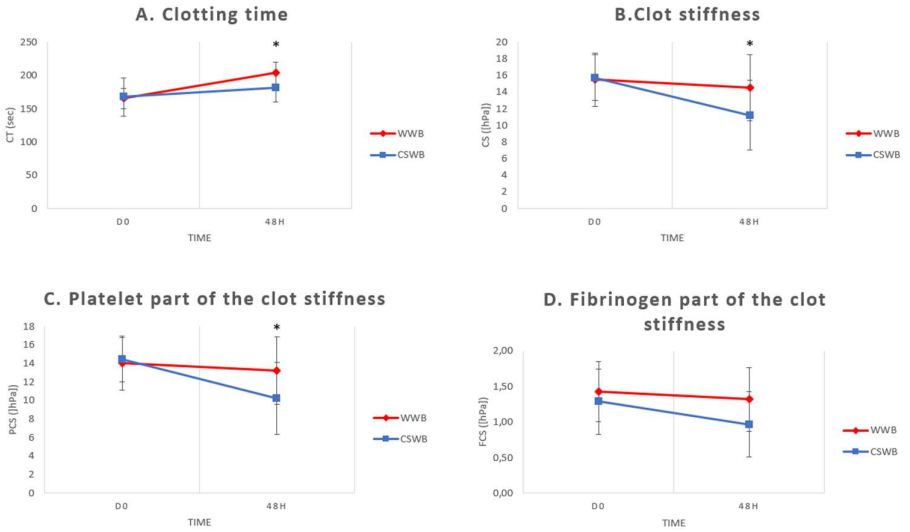

Figure 3: Graphical comparisons of the impact of storage over time (D0 and 48h) on the = viscoelastometric testing (VET) parameters are presented here. The data pertains to cold-stored whole blood (CSWB) and warm whole blood (WWB). Graph A depicts the increased clotting time (CT) over time in the WBB compared to the CSWB. Graph B shows the decreased clot stiffness (CS) over time in the CSWB compared to the WBB. The graph in C shows the decrease in clot strength associated with the platelet component (PCS), while the graph in D shows the portion of clot strength associated with the fibrinogen component (FCS). The mean values are plotted with their associated standard deviations. Statistical significance (p<.05) is indicated on the graph by an asterisk (*).

3.5. Thrombin generation

Thrombin generation parameters showed no significant differences between the two groups over time except for lag time (LT) (Figure 4).

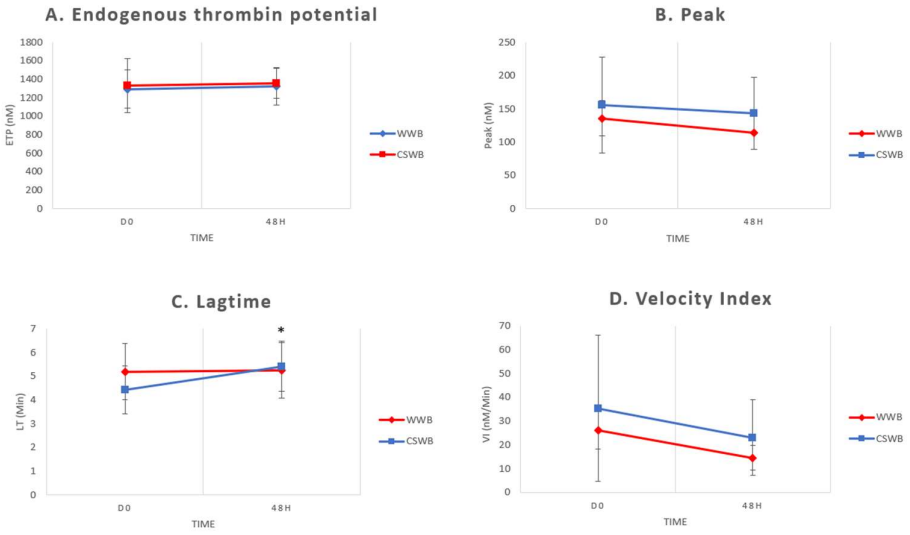

Figure 4: Graphical comparisons of the impact of storage over time (D0 and 48h) on the thrombin generation factors are presented here. The data pertains to cold-stored whole blood (CSWB) and warm whole blood (WWB). Graph A shows that there is no significant difference at any storage temperature in the potential for the generation of endogenous thrombin (ETP), which is important for clot formation in secondary haemostasis. The same applies to the generation peak shown in Graph B, which evolves similarly between the two storage types. Thrombin production velocity index (VI), shown in Figure D, is comparable at all storage temperatures. Delay to start thrombin production (i.e., lagtime, LT), shown in Figure C, is increased when whole blood is stored at 4 °C compared to storage at RT. The mean values are plotted with their associated standard deviations. Statistical significance (p<.05) is indicated on the graph by an asterisk (*).

4. DISCUSSION

This study compared the overall quality and haemostatic potential of non-leukoreduced WB collected by emergency protocol (i.e., WBB/EDP) and stored at 4°C (CSWB) or 22°C (WWB) for 48 hours.

The European Directorate for the Quality of Medicines and Healthcare[20] published the guidelines used in Europe, and specifically in Belgium to determine and guarantee the quality of the products supplied. This guide defines the parameters to be monitored in each blood component to ensure their quality over time. In contrast, there is no consensus in the literature regarding the parameters that attest to the general quality and sufficient haemostatic potential over time for whole blood. No cut-offs or standard methods are available in other references

books or guides. Consequently, the results are discussed in the absence of any thresholds.

The metabolic parameters exhibited temporal evolution in line with cellular metabolic processes. These alterations are frequently described as storage lesions[21]. In the current study, some parameters demonstrated a temporal trajectory in alignment with expectations. Specifically, potassium concentration increased, and sodium level decreased over time[13]. This effect was more pronounced in the CSWB group for both parameters. As anticipated, a decline in pH was accompanied by an elevation in lactate concentration over time. Storage at room temperature[13,18] appeared to result in a more acidic environment in comparison with cold storage. As the pH remained above 6.4[20], the environment was considered suitable for the maintenance of platelet viability. However, the glucose profile demonstrated a distinct trajectory in comparison with previous observations[13,18]. An increase in the concentration of glucose was observed over time. However, in accordance with existing literature, it is the presence of this substance that is of greatest consequence, as it functions as a substrate for cellular metabolism[22]. Moreover, its absence has been demonstrated to have an impact on platelet function[23].

Regarding haematological parameters, haemoglobin (Hb) and white blood cell (WBC) levels remained stable and did not differ significantly between the groups over time. Consequently, after 48 hours, non-leukoreduced WB retained the same capacity to deliver RBCs to the patient as when first collected, regardless of storage temperature. This is the critical factor in delivering oxygen to the patient. One of the three principal objectives of whole blood administration has been attained. With regard to the platelets, there was a decrease in their count over time. This decrease was even more pronounced in CSWB than in WWB. The decline in platelet count was observed in CSWB at an earlier stage than that reported by Jobes and colleagues in cold-stored whole blood on day 4[13]. However, in WWB, our results were comparable to those reported in the study published by Hughes and colleagues. The researchers observed no decrease in platelet count over time when WB was stored at room temperature.

In contrast to classical light transmission aggregometry, whole blood platelet aggregation measured by multiplate, is known to be strongly dependent on platelet count[24]. However, in the present study, aggregometry appeared strongly affected over time in both groups, with greater reductions in WWB than CSWB. Hughes and

colleagues also reported a significant and rapid reduction of aggregation in WWB[18]. Similarly, Meledeo justifies the reduced response to aggregation observed by the formation of platelet microaggregates. They are thought to be induced by the presence of fibrinogen in whole blood. Their hypothesis is that fibrinogen is responsible for the premature activation of platelets, which predisposes them to aggregation[25]. Interpreting of this static test remains difficult, as it does not integrate the dynamic flow parameters of haemostasis and the endothelial contribution.

In line with these results, the activatability of platelets in response to high concentrations of agonists, as measured by flow cytometry, was found to decrease over time in both groups. The proportion of PAC-1 and P-selectin positive platelets was found to be lower in both the WWB and CSWB groups at 48h compared to baseline levels following stimulation with TRAP and ADP agonists. The aforementioned markers of platelet activation are indicative of platelet aggregation ability (activated integrin α2bβ3) and the release of α-granules, respectively. No significant differences were observed between the groups. Nevertheless, in the unstimulated condition, P-selectin expression was upregulated after 48 hours in both groups. However, the increase in expression was significantly higher in CSWB than in WWB. This could be due to the presence of more storage lesions[26]. P-selectin is one of the factors identified by the AABB (American association of blood banks) as being associated with storage lesions. Regarding α2bβ3 expression in WWB, a reduction in signal intensity was observed in contrast to what was demonstrated in CSWB. A more activated phenotype of cold-stored platelets was reported in the absence of ADP or TRAP stimulation. These findings are consistent with those previously reported by Tohidi-Esfahani and colleagues[27].

Although WB platelets appeared to lose some platelet activatability, we hypothesize that their function is sufficient to seal a vascular occlusion in a bleeding patient. This preserved in-vitro haemostatic potential was reflected in the Quantra VET. There was a significant difference between the two storage conditions over time, with better stability reported at 48h for CS and PCS for WWB. However, the CT was significantly higher in WWB than in CSWB, which means that the clotting time was better in CSWB, but the clot formed was less stiff. FCS did not differ significantly between groups over time. This is reassuring given that fibrinogen is one of the first factors to be affected in haemorrhage[8]. This is also consistent with our findings. The fibrinogen remained stable over time in both groups which is

equivalent to what has already been shown in the literature[13,18]. FVIII, on the other hand, was reduced to a greater extent in CSWB than in WWB. Nevertheless, because, this factor is sufficiently preserved if 30% remains in the product, we can assume from our results that it is sufficiently preserved throughout the 48H period of blood storage, independently of storage temperature[11]. In particular, the reduction has been described as being particularly pronounced in the initial 24 hours before subsequently decreasing at a more gradual pace[28].

In relation to thrombin generation, the parameters measured at 48 hours did not exhibit any discernible differences between the two study groups in terms of ETP, VI and peak. Nevertheless, there was an observable increase in LT in CSWB compared to WWB. This could be due to a more important decrease in coagulation factors in CSWB. However, the endogenous thrombin potential was preserved at 48h. A similar conclusion was reached by Assen and colleagues[16].

In addition to the aforementioned considerations pertaining to leukoreduced WB, another crucial parameter that can impact the coagulation cascade is the WBC. These cells are known to participate in haemostasis at various stages[29]. WBCs are a potential contributory factor in the formation of clots. Leukocytes are known to interact with platelets in the process of aggregation, rolling onto the endothelial surface and fibrin production, which is mediated by P-selectin from activated platelets[30]. No specific tests were conducted in the present study to evaluate the involvement of WBC in the haemostasis process. However, this could be a fruitful area for further investigation. The results demonstrated that, following a 48-hour incubation period, both coagulation factors and platelets were capable of forming and maintaining a blood clot in both temperature conditions.

Non-leukoreduced WB should be stored at room temperature when collected in the field and potentially used within the next 48 hours. However, the circumstances of the field situation would also be a determining factor in this decision, as both products appear to be acceptable. It may be advisable to store the non-leukoreduced WB at 4 °C if the number of patients arriving is low and the risk of having to discard the product after storage at 22 °C is high. If the product is immediately stored at 4°C, this will increase its shelf life to the 35 days accepted by manufacturers for a CPDA-1 storage medium. This choice may be recommended since it allows the product to be kept in acceptable conditions if the number of patients does not guarantee that it will be used within 48 hours. Further investigation is required to assess the biochemical properties and haemostatic

potential of the non-leukocyte WB over time during extended storage, in the case of a WBB strategy.

5. CONCLUSION

It can be concluded that WB collected as part of a WBB or EDP can be used within 48 hours, whether stored at room temperature or at 4°C. Both products are acceptable, although WWB's overall rating is better. In cases where there is a large influx of casualties and where the probability of use is relatively high, warm storage would be preferable. However, given the bacterial risk associated with this type of storage at high temperatures, if the frequency with which patients are treated would be low, cold storage might be considered in order to extend the storage time.

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GENERAL DISCUSSION

"He that will not sail till all dangers are over must never put to sea."

Thomas Fuller

The objective of this thesis is to develop an adapted emergency blood collection protocol for the Belgian Defence. To achieve this goal, the following research questions were considered:

1) What circumstances warrant the implementation of an emergency blood collection protocol in operation?

2) What risks are associated with implementing an emergency blood collection protocol and how are these risks mitigated?

3) What are the immediate effects of a 450mL donation on the physical performance and alertness of elite soldiers?

4) What are the immediate effects of a 900 mL donation on the physical performance, alertness, and decision-making of elite soldiers, and what is the recovery time for these functions to revert to their original state?

5) What is the optimal method for storing surplus blood collected in an emergency blood collection protocol within the first 48 hours that preserves all its therapeutic benefits for bleeding patients? Is there a difference in quality, and therefore efficacy in haemostasis, between cold- or warm-stored blood?

1. REFLECTION TOWARDS THE RESEARCH QUESTIONS

To address the two first research questions, a systematic review of the pertinent literature was conducted. Regarding the first research question, namely, " **What circumstances warrant the implementation of an emergency blood collection protocol in operation?** ", the analysis identified the circumstances under which the activation and use of this emergency blood collection protocol would be appropriate considered within the context of a military setting. This investigation has revealed that Belgium is not facing a unique set of circumstances but rather is facing the same limitations of the transfusion support system in operation as other nations[1]. The rationale for activating the protocol may be classified into two principal categories. The first category comprises logistical considerations, which arise when the existing logistics system is no longer fit for purpose[2–10]. The second category concerns clinical issues, which are primarily relevant to the management of haemorrhagic casualties with coagulopathies[11–13]. This on-site emergency collection protocol is the only means of obtaining platelets, a vital resource that most operational blood banks lack. However, the availability of this component could represent the critical difference in a patient's vital prognosis. The scientific

community emphasizes the necessity of integrating this protocol into the range of capabilities available as part of medical support to operations, from the planning phase onwards[14]. The process of establishing a pool of pre-screened donors is indeed time-consuming and resource-intensive, particularly once deployed. Additionally, the personnel responsible for implementing this protocol require training. As the emergency protocol is not employed on a routine basis, staff must be trained in its use. The necessity for such training is heightened when non-medical personnel are tasked with carrying it out. It is crucial to differentiate between two concepts that are related to the previously described protocol by the NATO Blood Panel[15]. This protocol is typically initiated when the conventional transfusion support system is exceeded. If this protocol is enacted in a system where the panel of potential donors is called upon for an immediate blood donation intended for transfusion to a casualty being treated, it is referred to as an "Emergency Donor Panel" (EDP). In contrast, the term "walking blood bank" (WBB) is employed when the operational context does not permit sufficient replenishment, resulting in the absence of a conventional blood bank. In such instances, blood is collected in the theatre from pre-screened donors in anticipation of the arrival of a casualty, with the objective of establishing this renowned stock (i.e., the blood bank). This strategy may be considered an act of forecasting. However, our literature search revealed that this strategy is only described once—in the case of a ship[4]. The two strategies can be considered complementary, with the selection of one over the other depending on the specific circumstances of the transfusion support plan.

A consensus emerges from the reviewed articles that the use of the protocol carries a risk. Therefore, as a second step, the literature review was extended to include this risk analysis question, allowing us to answer our second research question: **"What risks are associated with implementing an emergency blood collection protocol and how are these risks mitigated?"**. Two categories of risk can be identified: those linked to the blood compatibility and those related to the TTD. To counterbalance these risks, the protocols include the use of rapid tests, which are employed for both blood group confirmation and detection of the most significant TTDs, namely HIV, HBV, and HCV. It is important to note that these tests are preventive measures designed to reduce the risk to a minimum, dependent on the available resources. It is typically the case that the donor and patient's blood group is confirmed at the collection stage using a rapid test, thereby reducing the

risk of major reactions[3–5,9,11,12]. Furthermore, the decision to collect only LTOWB donors[6,10] represents another effective method of reducing the risks associated with transfusion reactions to a minimum. The compatibility risk may therefore be considered minor rather than major. This risk needs to be considered preventively prior to any operation by titrating haemolysins in type O donors, as this test cannot be performed in the operating theatre. Consequently, if this risk management measure is to be successfully implemented, it must be planned and the relevant LTOWB donors must be identified. Moreover, there exists a risk of the transmission of infectious agents, which is increased due to the lack of systematic leukodepletion, the absence of pathogen reduction treatment, and the inability of the polymerase chain reaction (PCR) viral genome detection test to be performed during the donation procedure, as mandated by Belgian law. These treatments are time-consuming and/or require non-deployed logistics. To mitigate this risk, several strategies have been proposed. In some article, a preliminary screening is conducted to identify suitable donors, resulting in a pre-defined pool of pre-screened candidates[2–8,11]. Alternatively, a rapid serological test is employed at the time of donation to detect infections[3–7,11,12]. These approaches are even sometimes combined[3–7,11]. However, when time is of the essence for the recipient or limited manpower available, some may not have reported implementing them. Regardless, whether they were planned or not is no longer a concern, given the limited time available for the patient[9]. It is crucial to acknowledge that these rapid tests typically take between 30 minutes and one hour to yield results. Furthermore, although these tests have been validated for use in screening patients, they have not necessarily been validated for use in screening blood donors. Sensitivity and specificity are distinct for these diagnostic or screening tests. Finally, the associated preventative measures were classified based on the timing of their implementation. It is evident that the quality and reliability of the results will differ depending on the conditions under which the test is conducted; for instance, when performed on the national territory in accordance with all legal recommendations and using the appropriate equipment, personnel, and procedures, or when conducted by less experienced personnel using alternative testing methods in an operational setting. Another effective method frequently highlighted is the utilisation of a pre-donation medical interview to identify individuals who engage in risk-taking behaviours and to exclude those deemed to be ineligible donors. This strategy serves to minimise the risk of exposure[3–5,7,12]. The cornerstone of safety in

such circumstances is donor selection. Therefore, it is crucial to ensure that donors are aware of the significance of their role, and to involve them in the process, initially by providing a comprehensive explanation of the associated risks and then by monitoring them on a regular basis.

Finally, the objective of the literature review was also to identify standard parameters used for patient monitoring. However, no consistent measures were identified across the reviewed studies. There is considerable variability in the parameters monitored due to several reasons, including the retrospective nature of the studies which resulted in a lack of control over the variables under investigation and the use of material that can be retrieved a posteriori. Additionally, the different nations have different Point of Care Testing (PoCT) equipment for monitoring patients with severe haemorrhage, leading to differences in monitoring parameters. A limited number of studies have evaluated the quality of monitoring, the results provided, and their effectiveness in an austere environment[16,17]. Similarly, the parameters to be monitored to justify the activation of the WBB/EDP have not been standardised, with each protocol including measures that are specific to the nation concerned. In a groundbreaking study, Strandenes and colleagues demonstrated that ROTEM (a device for rapidly and globally studying coagulation based on rotary thromboelastometry) was reliable at sea[4]. They could prove useful with regards to Patient Blood Management (PBM) and blood savings when blood product resources are limited. Nevertheless, it cannot be assumed to be a viable option in all cases due to its considerable logistical footprint. In light of this innovative concept, Belgium has acquired Quantra devices. These devices facilitate a functional measurement of whole blood clotting potential and provide information on any imbalances in certain factors (platelets, fibrinogen). The information is then used to guide and assist in the management of transfusions for patients, thereby enhancing patient care. The deployment of these devices will be contingent upon the enhancement of logistics and the augmentation of medical personnel in a sufficient and appropriate manner. This will be achieved mainly through the utilisation of a role 2 configuration (i.e., equivalent to a field hospital). This device was selected on the grounds of its robustness, its measurement technique, which is independent of the vibrations frequently encountered in the field, and most importantly, its ease of interpretation when considered alongside other equivalent devices on the market. The device facilitates a rapid and efficacious response, even among personnel less experienced in its utilization.

The safety of donors during deployment in operations should be a primary concern in developing clear recommendations, which is addressed by the third research question: "**What are the immediate effects does of a 450mL donation on the physical performance and alertness of elite soldiers?**". The findings of a double-blind controlled longitudinal study performed in a laboratory setting were consistent with those of previous research in demonstrating no impairment in physical performance[18] or alertness[19,20] during maximal treadmill running. The testing was subsequently relocated to an ecologically valid environment, namely a training facility in a hot and humid climate, in addition to being carried out in a state of fatigue and dehydration. It was representative of the tasks performed on a mission. Furthermore, the test was conducted with a full set of mission-specific gear, weighing in at approximately 30 kg (Figure 1). This was done to replicate the conditions encountered during a mission.

Figure 1: Special forces operator wearing basic military equipment weighing 30 kg (SFGP©).

Nevertheless, in this setting, it became evident that the double-blind controlled testing could no longer be conducted longitudinally, due to the lengthy and challenging nature of the physical task. The findings demonstrated that there was

no significant difference or impact on alertness or physical right after a standard donation of 450 mL of whole blood, whereas there was a notable difference in haemoglobin levels between the donor group and the control group. This is consistent with previous findings in the literature[21,22]. However, in both settings, a crucial insight emerged: the potential psychological effect of donation. Individuals who believed they were part of the donor group predicted that donation would result in a negative effect on their performance. Consequently, the operator may be negatively impacted if he were to become aware that he had donated. Notwithstanding, it is not to be presumed that all candidates will react in such a manner. However, this risk should be considered as part of the protocol implementation recommendations.

Another limitation of this analysis is that the results are applicable to a relatively small population. The population selected for this study consisted of special forces operators, considered to be elite soldiers. These individuals were chosen because they are the most at risk of falling back on the protocol, due to their work in austere conditions and small isolated groups with a small logistical footprint. Furthermore, the risk of donors is heightened due to the demand on this group during operational missions, which is further compounded by the fact that they are already over-trained. Consequently, they are unable to be compared to other soldiers. Indeed, should a staff member donate blood, they may resume work at the office or engage in less physically demanding, less isolated tasks within the enemy's territory. In such a case, there is a reduced risk of impaired performance. Consequently, blood donations may be conducted within the legally permitted donation frequencies without any adverse physical or tactical consequences. As demonstrated in Chapter 2, there is no discernible impact on the studied population; therefore, tactical commanders will not have to consider this component in their planning. It is important to consider that donation is not an option for specific populations or individuals who do not meet specific qualifications, either in Belgium or on operations. For instance, if the donor candidate is required to perform tasks such as flying an aircraft, machine operation, or parachute jumping and diving, donation is not possible[23].

Special Forces operators are highly trained soldiers who consistently seek to push the boundaries of physical and mental endurance[24]. However, when it comes to blood donation, this drive to go beyond limits can lead to unwise risks being taken without sufficient consideration of potential consequences. Furthermore,

when faced with a shortage of resources and compatible donors, these individuals may wish to make multiple donations, thereby increasing their colleagues' survival prospects in the event of haemorrhagic injury. This is the reason why further research was conducted on the impact of donating blood on this specific population. Additionally, the military has expressed significant interest in this area. This study addresses the concern expressed by the fourth research question: "**What are the immediate effects of a 900 mL donation on the physical performance, alertness, and decision-making of elite soldiers, and what is the recovery time for these functions to revert to their original state?**". The study employed a 35-day longitudinal design to assess the recovery process following a double blood donation. Due to the demanding nature of our participants' schedules and the study design, we were unable to achieve a high participation rate. Despite this, we observed a significant and conspicuous effect after the completion of maximal exercise, with a relatively similar recovery profile observed among all participants. For the sake of the participants' safety and comfort, this exercise could not be performed in double-blind conditions; this decision was further reinforced by the knowledge that donation may have a psychological effect. There was no control group; the donor's performance was compared with his own. This was an effective approach as the participants' profiles were relatively varied. In consideration of the population, an effort of extended duration, and a relatively low intensity, such as an endurance run on a treadmill at 70% maximum speed, was deemed to be susceptible to motivation. Conversely, a brief but intense maximal effort was considered less likely to be affected. Moreover, this maximal effort more accurately represents the effort required in a real-life situation, such as an escape from a dangerous situation. Notwithstanding the body size of participants, whether small and thin or large and robust, the effect observed was consistent: nearly a 50% loss of physical performance immediately post-donation of 900mL as previously reported[25]. There was no discernible loss of alertness or decision-making abilities. The full recovery of the participants' basal physical performances was achieved within a period of approximately four weeks. In addition to these performance losses, we observed orthostatic phenomena in several participants during the few days following the donation.

In the event of a double donation, the donors are to be considered as patients by tactical command. They must be evacuated from the battlefield in the same way as any other patient would be since they will no longer be able to complete their

mission as safely as their colleagues who donated a pocket or did not donate at all. Furthermore, they are to be granted a period of recuperation before being rehired for an equivalent mission. This is an essential aspect that tactical command must consider in its planning processes. Furthermore, as the circulatory volumes of Special Forces operators are elevated, they represent a distinct category from other military personnel. Thus, the recommendations in question are not applicable to other military personnel. Consequently, it is not possible to collect a double donation from the general military population under conditions of extreme isolation and in the presence of a significant threat to life. Finally, the collection of an equivalent of two blood bags is not envisaged in circumstances where there are sufficient donors or resources, which is the case in most operational military deployments.

The objective of the study presented in this thesis' final chapter is to identify an appropriate conservative method for whole blood bags collected via the emergency protocol and not transfused. Given the scarce and precious nature of this product, it is crucial to ensure its safe preservation in the most optimal manner, tailored to the specific situation at hand, to ensure its reuse for another patient while maintaining the requisite quality standards. Considering this, the following question arises: "**What is the optimal method for the storage of surplus blood collected in an emergency blood collection protocol within the first 48 hours to preserve its therapeutic benefits for bleeding patients? Is there a difference in quality, and therefore efficacy in haemostasis, between cold- or warm-stored blood**?". Whole blood is used in a variety of clinical contexts, with the general qualities and haemostatic potential representing one of the most important criteria for its application. Therefore, it was essential that these parameters were monitored throughout the study. This investigation was thus designed to assess the impact of storage conditions during the initial 48 hours. The two temperature settings evaluated – ambient temperature and 4°C – are those typically used in the literature, yet a comparison of the maintenance of haemostatic properties at these temperatures had not yet been performed[26,27]. The whole blood samples collected for the study were stored in CPDA-1. The anticoagulant solution was selected for its capacity to extend the storage period by several days through the addition of adenine to the initial citrate-phosphate-dextrose mixture. According to the manufacturer, the product has a shelf life of 35 days from the date of sampling[28].

The biochemical and metabolic parameters revealed that degradation was more pronounced during hot storage compared to cold storage. However, the levels remained within the acceptable ranges for both conditions as demonstrated by the pH levels, in accordance with the European Directorate for the Quality of Medicines & HealthCare[29]. Regarding haematological characteristics, a significant reduction in the number of platelets was observed after 48 hours of cold storage. These platelets appear to have reduced activity, activation, and activability after 48 hours in both groups. Conversely, coagulation factors demonstrated stability over time. A more functional in vitro test was employed to assess the ability to generate clots. The Quantra instrument was selected because of its rapid interpretation and ease of use. Another factor that motivated our choice was that this viscoelastic test has been acquired by Belgium for placement in its most robust and reliable medical support structures, with the objective of guiding the clinical choices of transfusion management to facilitate the optimal process of blood preservation (PBM) and to ensure the most efficient blood savings possible. The test revealed a deterioration in the overall stiffness of the clot when stored at 4°C. This was observed to be due to a significant decrease in the platelet-related part, with no corresponding decrease in the fibrinogen-related part. This may be interpreted in the light of the observed decrease in platelet numbers and the stability of fibrinogen levels. However, clot formation time was found to be significantly longer during hot storage than during cold storage. The potential for thrombin generation was found to be preserved in both types of storage. This factor plays a crucial role in the process of secondary haemostasis, which is vital for the consolidation of the blood clot. It acts as a catalyst for the transformation of fibrinogen into fibrin. However, there is only a slight difference in the time taken for thrombin to be generated in cold-stored blood. This may result in a slight delay in the production and consolidation of the clot.

Both storage temperatures yielded satisfactory results in terms of overall quality and haemostatic potential. However, in terms of the stiffness of the clot formed in vitro, whole blood stored at a warm temperature demonstrated superior results. Despite an increase in clotting time, there was no corresponding increase in clot stabilisation time, as the time required for thrombin generation was not prolonged compared to measurements taken with whole blood stored at 4°C. In addition, it is vital to ensure the hygiene of the donation process to reduce the risk of bacterial contamination. In the event that the risk of contamination persists or

cannot be mitigated, it is recommended that the blood be discarded or, alternatively, stored cold. Consequently, the optimal condition for the blood is 4°C. This temperature increases the blood's shelf life. This is precisely the choice that other nations have already made; they propose storage for up to 24 hours at room temperature or 8 hours at room temperature before placing it at 4°C. Once cold, the blood is no longer considered fresh but rather stored[30].

2. THE BELGIAN PROTOCOL: A PRACTICAL PROPOSAL

Based on the aforementioned information and in accordance with the Belgian haemovigilance recommendations, a new protocol is put forth, as detailed. The protocol enables the implementation of a patient blood management policy, as advocated by bodies such as BeQuint, in a military context. It should be noted, however, that this protocol has been developed and validated by military clinicians but has not yet undergone a crash test. Consequently, there is still scope for further modification in the future.

First, the protocol establishes parameters for assessing the likelihood of shock developing in a patient. These were developed in collaboration with medical professionals and designed to be measured using the PoCT equipment made available to medical teams. The PoCT for Belgium utilises the Istat®, which was proposed by Abbott. The device can be used to measure several biological parameters, including blood gases, haemoglobin, electrolytes, and lactate, as well as PT/INR. For the purposes of patient monitoring, the biological parameters haemoglobin, pH and lactate are deemed to be the most appropriate. In addition, pulse and blood pressure are included in the decision-making process as they are relevant and relatively simple to measure. Finally, blood loss is identified as a parameter that would enable quick and easy decision-making by deployed personnel.

With respect to the rationale for implementing the emergency collection procedure, whether for a WBB or an EDP, the two primary categories of triggers for activation (i.e., logistical, or clinical considerations) pertain to Belgium. Due to the logistical constraints associated with the storage of platelets and their short shelf life, Belgium is unable to supply blood banks deployed in the field. In the event that a patient requires such a transfusion, this constitutes the sole means through which Belgium can provide medical personnel with an adapted protocol for accessing it. This observation applies equally to forward medical capabilities (role 1) and to field

hospitals with more robust logistics (role 2). It therefore constitutes a crucial factor in the justification of the provision of this emergency protocol, without which Belgium is unable to offer its personnel appropriate and adequate care. Furthermore, the logistics of making blood products available in the blood banks deployed are cumbersome. Another illustrative example of the potential for the logistics chain to be overwhelmed is the deployment of small, isolated teams such as special forces teams. These personnel lack the requisite logistics to store and transport blood products. Consequently, if they require it, this protocol is the only viable option for obtaining the necessary supplies. In practical terms, this justifies the availability of this emergency protocol, which allows the Belgian medical component to fulfil its role of providing medical support to operations, if the conventional logistics chain is exceeded. Without this protocol, the Belgian medical component would be unable to fulfil its role.

Furthermore, this protocol could be employed to alleviate the burden on military medical logistics and the pressure on Blood Transfusion Centres (BTC) to deliver the blood products required by the military. The scenarios encountered by the Navy are particularly conducive to this example. The probability of utilising blood products is low, yet the risk of not doing so is high. This frequently leads medical logistics to make blood products available, yet a significant proportion of them are ultimately wasted. Building on the insights gained by Strandenes and colleagues, the Navy could leverage the opportunity to proactively withdraw products, setting up a modest blood bank or simply maintaining a pool of donors, ready to provide when necessary[4]. This would empower Defence to participate in more effective blood product management at the national level, while ensuring that patients receive the optimal product. A decision-making algorithm has been developed and is proposed to those involved in the field (Figure 2).

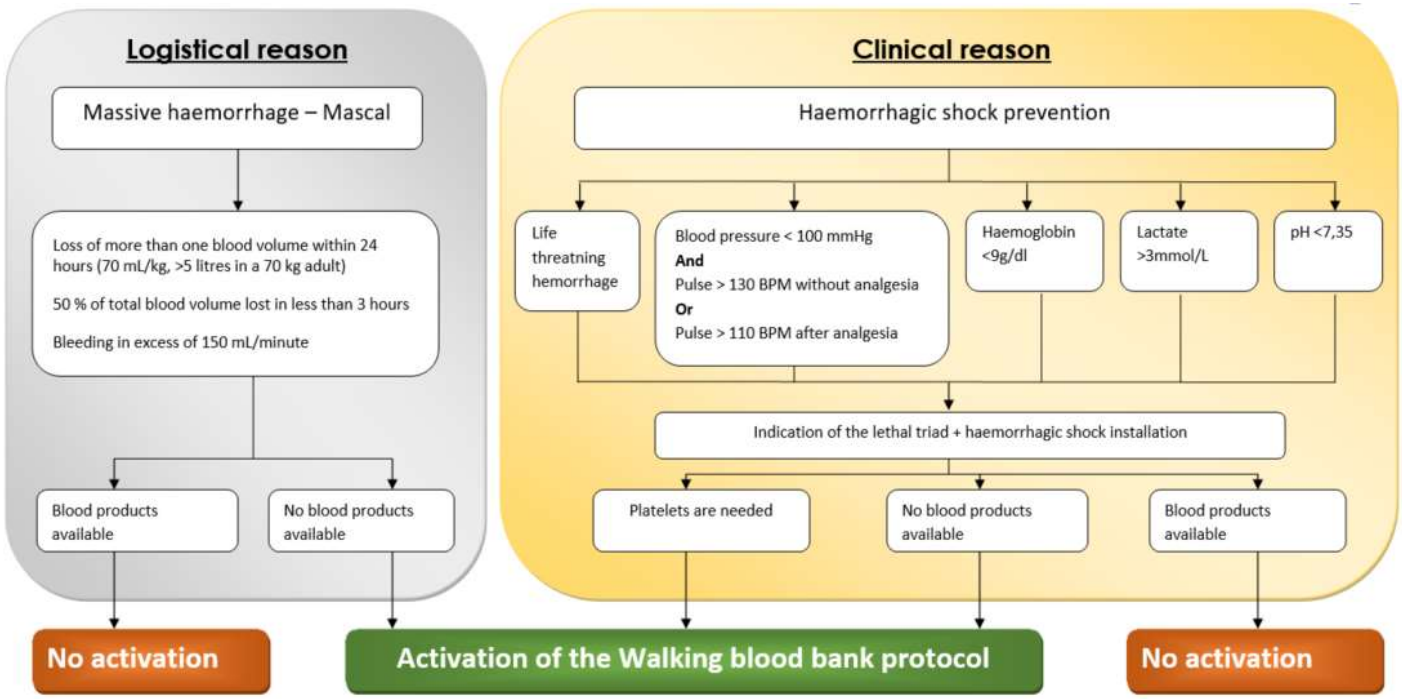

Figure 2: A decision-making algorithm based on the findings of a comprehensive review of the relevant medical literature and developed in collaboration with medical professionals in the field, including anaesthetists and emergency physicians, has been devised by Belgian authorities. The protocol was adapted to accommodate the point-of-care test (PoCT) available to deployed units.

The risk mitigations implemented to ensure the safety of the donor and recipient during the activation of the Belgian protocol are comparable to those implemented in other countries, as evidenced in the chapter 1[1]. It is of the utmost importance that the protocol be included in the planning phase, as this allows for the implementation of all necessary preventive measures (Figure 5). These include the selection of potential donors and appropriate education to make them aware of the risky behaviours. A complete pre-screening is also carried out before deployment. Such procedures must be equivalent to those implemented by a BTC. Furthermore, the relevant legislation is duly adhered to, up until the point of deployment. These measures serve to guarantee the safety of both the donor and theonateent and to control any associated risk. Additionally, the analysis of haemolysin titration in type O donors' forms part of the donor screening process, with the aim to establishing a LTOWB donor database. Once the data has been collected, the information is organised and stored in a donor register. This register is used when the emergency protocol is activated.

In addition to this meticulous preparation, measures are taken to prevent residual risk in theatre at the time of donation. Firstly, particular importance is attached to the donor's questionnaire, which must be completed to verify the eligibility of the donor, while ensuring that their health remains uncompromised and that they have not engaged in any risky behaviour that would be detrimental to the recipient. To provide a working tool for staff deployed to select the safest and most suitable donor in a challenging operational context, a British team has developed a Questionnaire Triage Tool (QTT)[23]. The questionnaire has been adapted from the version used by a BTC in the country where the deployment is taking place. This adaptation is intended to reflect the specifics of the area of deployment[23]. Moreover, it is used to cover the period between pre-deployment pre-screening and the time of the donation. It should be noted that this questionnaire will in no way supplant a medical assessment conducted by an appropriately trained medical professional or the experience of an appropriately qualified collection doctor. Rather, it is intended to serve as a prompt for excluding those donors who present a risk that is deemed to be unacceptable. This is particularly important as the questionnaire can be completed with the donor by non-medical staff or staff with qualifications that are less rigorous than those expected of a doctor[23]. The primary triage stage serves the function of ensuring that the donation is made with the full knowledge and consent of the donor and

identifies regular donors who may be less likely to pose an unacceptable risk to the recipient. The secondary triage stage is employed to identify risks that are deemed too high for both the recipient and the donor, based on the donor's general condition. Finally, the tertiary triage stage is used to eliminate residual risks based on any high-risk contacts. The responses to the questionnaire are used to assign a score that is proportional to the risk, which enables donors to be classified according to their eligibility and reliability. The questionnaire published and approved by the NATO Blood Panel is the one proposed for the Belgian protocol (see Appendix B).

The necessary materials for the collection and transfusion of blood will be provided in a kit format (Figure 3), consisting of a donor kit and recipient kit (see Appendix C). Additionally, these kits contain all of the requisite components to minimise the risks outlined above, including rapid diagnostic tools and blood group identification cards (i.e., Eldon card). Rapid detection tests have the capacity to identify two of the three main TTDs (HIV and HCV) with high accuracy. It should be noted that tests for syphilis have not been included in the protocol, as this infection can be treated with antibiotics at a later point in time if it develops in the recipient. Similarly, it would not be advisable to include rapid malaria tests, as the donor is already screened before departure and thus is not a malaria carrier. Regarding the donor's potential exposure to endemic pathogens during his mission, it can be assumed that he is at an equivalent risk to the recipient. Should the donor have exhibited symptoms suggestive of contamination, he would have been excluded from donation based on the results of the pre-donation questionnaire. As donors are selected prior to deployment, it is possible to exclude those who have not responded positively to the hepatitis B vaccination programme, which is mandatory for Belgian military personnel. This allows for the elimination of donors who would otherwise require additional time for the donation test to be conducted in the field. Given the low prevalence of these cases[31], the number of donors who would be excluded is relatively small. However, the time saved in carrying out the tests is considerable.

Figure 3: *Donor and patient kits designed by Belgium to facilitate the implementation of the protocol. They contain all the equipment needed to manage the donor or patient.*

Prior to deployment, both the donor and recipient will have their blood group established, and this information will be corroborated by confirming the blood group through the Eldon Card (Figure 4). Once again, among the range of tests available and used by our partners, this is the one that involves the least logistics while providing control of the test and a rapid result. It is of the utmost importance to confirm both the donor and recipient groups to prevent any potential for a significant transfusion reaction. The determination of whether to collect only LTOWB or compatible blood should be based on a case-by-case assessment, considering donor availability and the potential risks associated with double donation. In larger medical facilities, where there are more potential donors a greater number of patients, it may be preferable to collect only LTOWB to speed up the availability of blood and avoid an identification problem. However, in smaller facilities, it is advisable to prioritise transfusion of compatible blood to minimise the number of potential donors and, in turn, reduce the risk of double onateon.

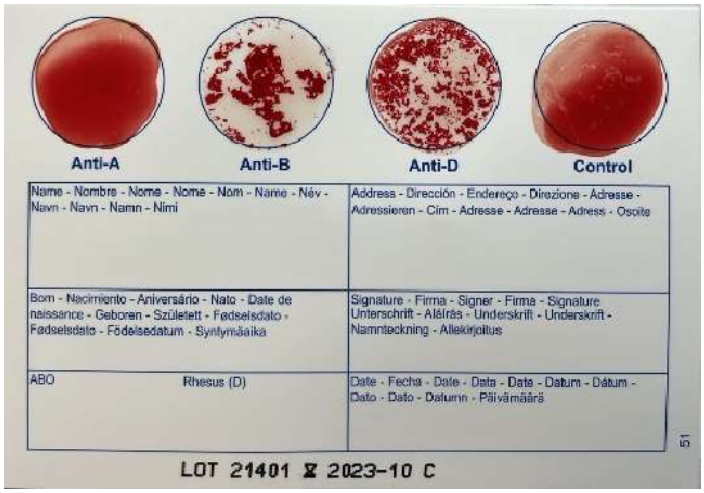

Figure 4: The representation depicts the results of a test conducted using an Eldon card. This card represents a straightforward and efficacious method of confirming the blood group through the utilisation of water and a whole blood sample. A noteworthy feature of the Eldon card is its inclusion of an internal control mechanism, which allows for the verification of test results. The kit contains all the components necessary for the test to be performed, apart from water. The instructions for use, which include interpretation rules, ensure that individuals without medical training can read the result.

The developed protocol addresses the safety of donors and recipients, while providing guidance for staff who are required to implement it (Figure 5). It should be noted that the recommendations presented in this work are primarily oriented toward the perspective of blood bankers, which often conflicts with the views of front-line doctors who prioritize the availability of products over rapid testing, given that they perceive the risk of mortality to be higher than the probability of seroconversion. The objective is to devise the optimum protocol by incorporating all pertinent transfusion rules. It is the responsibility of the medical professional applying the protocol to determine the level of urgency and, where appropriate, to deviate from certain stages. The necessity for robust donor identification and follow-up in planning is therefore paramount.

The protocol in question, along with its subsequent implementation, must be considered alongside the necessary training and education programs[32–35]. It is of the utmost importance that the training encompasses both theoretical and practical elements. Military personnel must be taught to implement the protocol, use the tools provided and conduct the various tests included within the kits. Moreover, it is crucial that those involved in these activities are fully aware of the

crucial role they are to play and the significance of their responsibilities. In addition to medical personnel, training must also be provided for non-medical personnel who will be required to assume temporary responsibility for a patient and implement the protocol as closely as possible to the point of injury, sometimes without any medical support. Such personnel may include ambulance drivers, combat medics, or even personnel available at the most critical sites. It is therefore the duty of these staff members to guarantee the safety of not only the donor but also the patient and the product itself. This is the reason why the protocol contains checklists to be followed if the procedure is initiated. Furthermore, the overall procedure is set out in a standard operating procedure (SOP). Training will therefore be developed on this basis, with the objective of enabling all those identified as potential effectors to perform all the tasks associated with this protocol, regardless of their initial background. Unfortunately, the training for the Belgian protocol is not yet operational due to the lack of legal authorisation for its implementation. The protocol has been designed to meet the requirements of the military, but some exceptions are pending and require approval from the Ministry of Health. It is only once these approvals have been obtained that we can proceed to develop theoretical and practical training. This training will obviously be developed in different stages and according to the needs identified in the target groups. It will be oriented towards qualifying the learner, with tests designed to ensure that the individual has acquired the requisite technical and theoretical knowledge. It is also anticipated that regular refresher courses will be provided as part of the training for this protocol, given the potential rarity of its implementation. Regular training will better enable military personnel to react in an appropriate manner when the occasion demands.

Firstly, it is necessary to emphasise the exceptional circumstances that could lead to the activation of this protocol. Indeed, it is not being proposed as a panacea for all issues; rather, it is being put forward as one of several potential tools that could be employed by the armed forces with a view to enhancing care for those who have sustained injuries. In addition, while whole blood is currently a popular topic of discussion in military literature and is frequently promoted as a highly valuable commodity in the management of haemorrhagic casualties, its suitability in all cases is open to question. Indeed, some patients will still require transfusion, with the components balanced in a manner that reflects the characteristics of their condition. It must also be noted that whole blood has a limited shelf life in

comparison to red blood cells (RBCs). Furthermore, it is important to acknowledge the logistical constraints and wastage associated with making blood units available. In light of these factors, it is crucial to maintain a critical mindset that considers all the constraints and that is oriented towards the optimal care of each individual patient. It is evident that, contingent on the patient's condition and the available management resources, the most suitable products may vary[36–38].

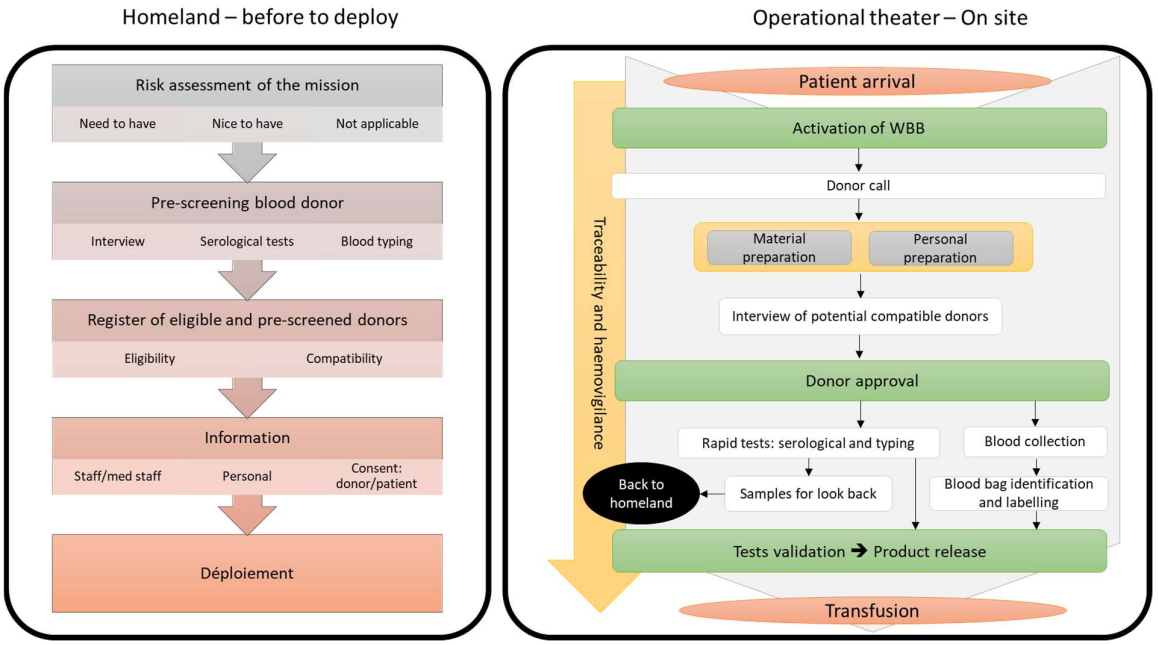

Figure 5: The figures present an overview of the actions to be undertaken in accordance with the prescribed sequence for the preparation and execution of the emergency protocol for whole blood collection. The stages to be completed prior to deployment in the mission preparation planning are illustrated in the left-hand frame, progressing from the initial risk assessment stage to the deployment stage. Each intermediate stage is a requisite precursor to the subsequent stage, which must be carried out in layers. The right-hand chart depicts the sequence of actions required for the enactment of the emergency protocol. This begins with its activation and subsequent call for donations, followed by the interviewing of prospective donors. Once selected, the donors are scheduled for blood collection. Rapid tests are performed on samples collected simultaneously with the blood unit. Biological qualification (confirmation of donor's blood group and negative test results) allows the product release and the transfusion. Throughout this process, it is imperative that both haemovigilance and traceability measures are maintained to guarantee the security and wellbeing of all parties involved.

3. CRITICAL EXAMINATION OF THE RELIABILITY OF THE PROTOCOL DEVELOPED.

A critical analysis of the Belgian protocol can be conducted along four primary axes (Figure 6). First, the protocol's strengths, advantages, and parameters that contribute to its resilience must be identified and preserved. Next, its weaknesses, parameters requiring attention to enhance its efficacy, and parameters where deterioration could occur must be evaluated. These weaknesses and parameters must be addressed as soon as possible to ensure the continued optimization or at minimum, prevent deterioration. Additionally, opportunities for further development or collaboration are analysed, with a view to committing resources to make them a reality. This would also serve to enhance the system's robustness and facilitate its wider exploitation. Finally, the potential threats that could impede the development or quality of the protocol are considered, with a view to reducing or preventing risks from external sources.

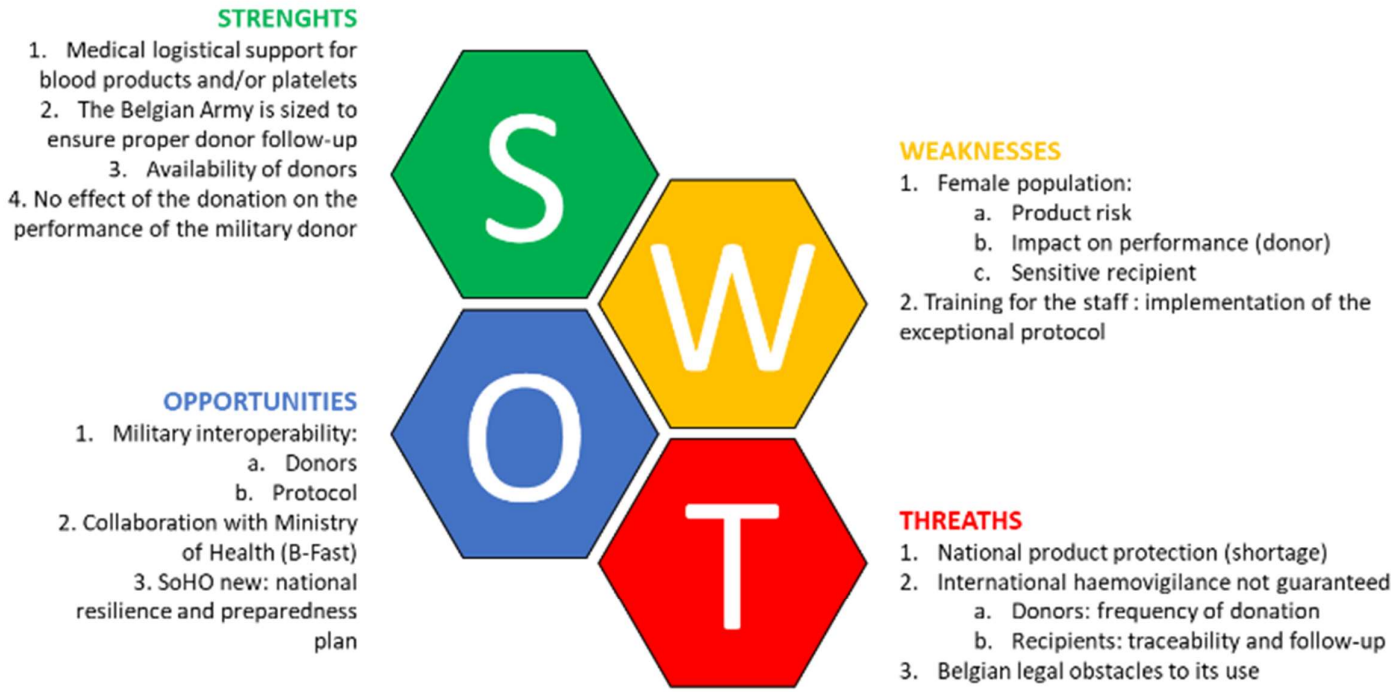

Figure 6: the illustration provides a summary of the swot analysis conducted at the conclusion of the research project. The diagram below provides a summary of the strengths, weaknesses, threats, and opportunities presented by the implementation of an emergency collection protocol.

3.1. Advantages, military prospects, and external opportunities

A significant advantage of this protocol is its ability to provide access to blood products and platelets, particularly when standard transfusions are unavailable in the field. This protocol enhances logistical support and the quality of care provided to combat casualties, thereby enabling the Belgian Defence to achieve its objectives. The development of this protocol, in conjunction with related research, ensures that donors can perform their duties without undue concern for their safety. This is an important aspect, as it expands the potential pool of donors, including those in special forces, while not compromising the quality of their mission. Our findings have confirmed existing results and contributed to data collection by exploring potential limitations associated with extreme donations. This thesis now offers recommendations to tactical commanders on how to manage donors in this case of a double donation.

The size of the Belgian army in comparison to other partner nations may present a limitation, but it can also be regarded as an asset, particularly in the context of personnel management. This enables a more cost-effective approach to donor management, as fewer simultaneous deployments or even smaller deployments are required, which in turn leads to a more frequent and affordable frequency and quantity of donor tests. These tests encompass all the pre-screening and regular donor follow-up tests that serve to minimise the risk of transmitting infections. They also include double blood grouping and haemolysin titration for type O donors. Nevertheless, it continues to represent a significant challenge to perfect and develop this routine use. In this context, it is beneficial to draw upon the work already carried out by countries with a more advanced approach to implementing WBBs[39–41].

Furthermore, the act of donating blood on behalf of a fellow soldier represents a situation whereby the donor is identified with the recipient, which is likely to stimulate donations and reduce the likelihood of a shortage of donors. However, there is a potential for this to lead to an additional risk, namely a desire to donate blood even if the donor has already incurred risks, in order to save the soldier's life. It is therefore of the utmost importance that the Questionnaire Triage Tool is carried out with the greatest care to be able to rule out any potential donors who would be willing to donate despite being ineligible.

Finally, the military advantages of the protocol itself are twofold. Firstly, it offers the potential for inter-operability, thereby facilitating international medical support. The capacity to deploy donors and medical personnel in an interoperable manner represents an asset in the context of emergency protocols, both in terms of availability and the capacity to implement the protocol itself. Conversely, a greater similarity between the equipment used by partner nations tends to place greater pressure on manufacturers, as they face a greater challenge in producing items that meet the needs of a wider range of users. This can result in delivery delays, but it can also facilitate joint stockpiling in the context of a potential global war.

3.2. Internal limits to the protocol

One of the limitations of the protocol is its failure to include female staff as either donors or recipients. Amongst other, women are unsuitable as donors because they often have a smaller circulating volume, less accessible veins, and an iron deficiency partially increased by menstruation[42,43]. Additionally, with successive pregnancies, the development of anti-HLA antibodies in the maternal plasma increases the risk of transfusion reactions and complications. Conversely, as recipients, despite current studies indicating the possibility of using O RhD pos for female staff[44–46], and despite the recommendations of blood bankers, RhD-negative RBCs are still planned for the management of women of childbearing age[47,48]. This choice is based on the hypothesis that it may prevent complications in future pregnancies should an anti-D antibody develop. It is important to note that this limitation is not absolute, but rather, relative. Currently, despite recruitment efforts, the military population is still predominantly male, with no more than 11% of personnel identified as women. This figure falls even further when considering the number of women deployed on operations. Therefore, this limitation is not absolute, but rather relative and could potentially be further mitigated by targeting only high-risk female personnel, defined here as those with a negative RhD blood type. This would represent a reduction to approximately 6% of women.

An additional challenge arises in that this kind of exceptional procedure, designed for use in austere environments with limited resources, may have to be carried out by non-medical staff. In practice, it is often the case that neither doctors nor nurses are present or are primarily involved in patient care. It is therefore essential that the procedures and documents associated with emergency blood

transfusions are readily available and accessible during the activation process. These work rules include medical triage to prevent the waste of blood on an injured individual who is beyond the point of saving, as well as an assessment of evacuation time, which informs the decision to commence transfusion[49,50]. Furthermore, the various stages, from the request of whole blood to transfusion, via the collection process and the tests required, are referenced, and detailed in order to provide the personnel involved with the correct, concise aide-memoire for their duties. These measures facilitate the enhancement of safety for both the donor and the patient and must be included in a standard operating procedure (SOP), which is accessible to the relevant personnel and serves as the basis for any training conducted[40]. It is of paramount importance that those involved receive training on the emergency blood collection protocol and how to use it, in addition to following the procedure to guarantee the necessary traceability. Consequently, it is of the utmost importance to integrate practical training within a controlled environment, during which the soldiers shall be overseen by specialists capable of providing requisite advice and information[32]. This is especially the case given that training of staff members in the implementation of emergency protocols represents a crucial limitation. This endeavour is challenging, particularly in the absence of regular practice. Without regular training, it is inevitable that the risk of error will be amplified. It is thus imperative that those responsible for implementing the protocol be adequately trained and informed. In addition, they must possess the capacity to respond effectively and swiftly in high-pressure situations. Training, including regular practice, represents a key component of a successful collection operation. Other nations have likewise highlighted the importance of training[47,51].

3.3. Risks associated with military interoperability

It is a common occurrence for countries to experience shortages of blood products. Given that blood is a scarce resource, each nation is at risk of favouring the use of its products for its own personnel, which could limit the scope of international partnerships.

Another issue that deserves consideration is that of traceability. This is an important consideration for both donors and recipients alike and can be planned for accordingly. It is recommended that transfused patients be tested at regular and consistent intervals during their stay at different facilities. Such regular testing enables potential seroconversions to be monitored, thus facilitating the

identification of any potential issues at the earliest opportunity. In the United States, transfusion recipients will be tested three, six, and 12 months after the transfusion. Furthermore, donors who return from deployment are also tested retroactively to ascertain the safety of the donation[52]. However, in cases where subsequent recipients are managed at facilities of different nationalities, it is possible that data may not be transmitted correctly or that malfunctions may occur as a result of different regulations. The implementation of haemovigilance is relatively straightforward when all the requisite procedures are conducted within a single facility. If treatment is to be transferred to an international context where the use of electronic monitoring cannot be guaranteed, it becomes a challenging endeavour to guarantee. Furthermore, traceability must be assured for donors. It is imperative to ensure that the frequency with which donations are made is respected. At the administrative level, therefore, it is crucial to cross-reference data and to include potential donations for other nations. It is, therefore, important to enquire as to the date of the subject's last donation as part of the primary triage questionnaire. This question is designed to provide further information that can be used in conjunction with the question of whether the donor has ever donated blood.

4. UP TO DATE STATUS OF THE PROJECT

One of the most significant current challenges to the protocol is the possibility that it could never be authorised under Belgian law. The Belgian legislation includes a range of articles that restrict the implementation of the protocol (Figure 7). The exceptions that must be included to permit their implementation influence different stages of the process. They extend from the context of collection, the data information management, via screening (specific viral genome detection tests must be performed on the donor), to product processing (leukoreduction and pathogen reduction treatment). However, the protocol does not allow for systematic compliance with the article describing product processing, information relating to the donor and the delegation of collection and transfusion acts in specific instances. The principal constraints are associated with the domains of traceability and haemovigilance in general. It would be an unrealistic assumption to expect the entire Quality Management System that is required and put in place in an establishment within a national territory to be readily operationalised within the context of an operational theatre. For example, it is not feasible to furnish proof of training for the personnel engaged in the sampling process. Furthermore, tests for

the analysis of viral genomes, which require the use of sophisticated automated instrumentation and delicate techniques, are similarly unsuitable for execution in austere conditions. The preparation of products to confirm their quality and to limit the transmission of pathogens or white blood cells responsible for potential adverse effects can be carried out effectively in austere environments. However, the deployable technique proposed for such procedures tends to be both time-consuming and detrimental to the final product quality. Consequently, such procedures are often deliberately excluded. Moreover, as qualified medical staff are a scarce resource in this type of setting, they will be involved with the patient directly, not with the donor. In some cases, staff who are adequately trained and experienced in advance may be assigned tasks such as preparation and collection, despite not being legally authorised to perform these duties. In such circumstances, the use of telemedicine may be extended to include remote physician direction of teams with limited resources, including a paucity of qualified staff, in order to provide care in highly isolated settings. In conclusion, further examination is required with regard to the management of medical secrecy. It is possible that the communication of blood groups could be conducted without restriction, which could prove challenging to conceal – even if the rationale were to be kept confidential. Such circumstances could give rise to discussions within the units. It is evident that these adaptations are vital for the survival of this small group, given the numerous deviations from the law, both subtle and substantial. Notwithstanding the proposed risk management measures, residual risks persist, necessitating a strategy of risk acceptance and reliance on the training and information of those involved to enhance their effectiveness and safety.

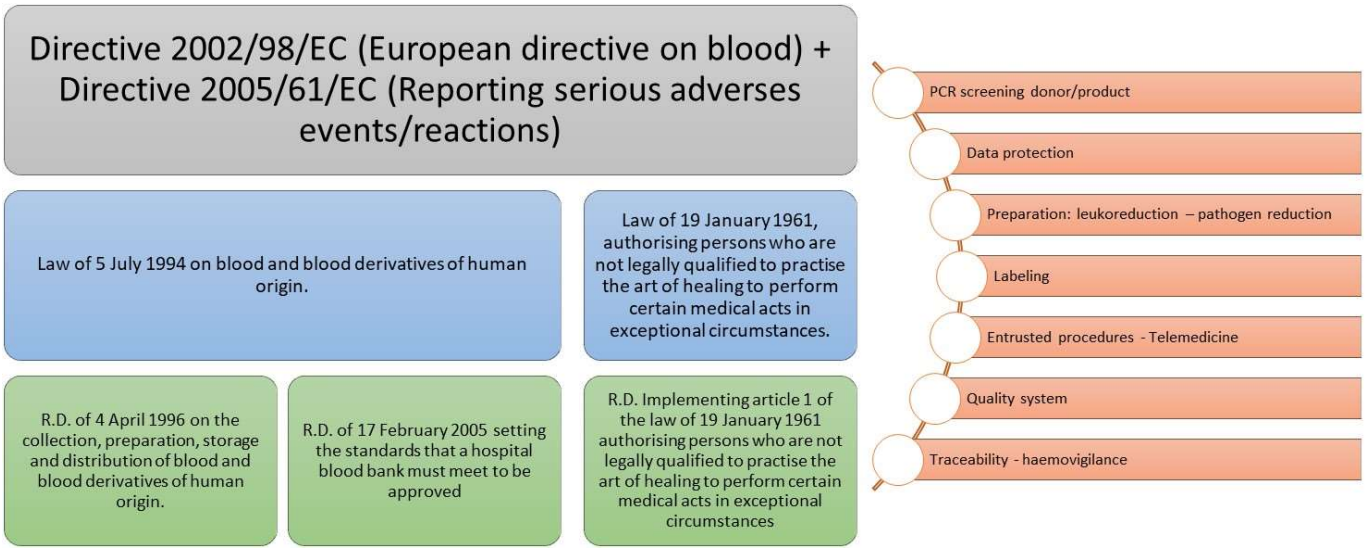

Figure 7: The figure depicts the regulatory framework for transfusion management in Belgium and the principal legal barriers to the implementation of the WBB/EDP. The left-hand side presents a hierarchical organisation of the principal legal texts, from the European directive through to the laws and royal decrees that implement it. On the right-hand side, the steps that cannot be fully respected or guaranteed when implementing the emergency whole blood collection protocol (WBB/EDP) are listed, from the European directive to the royal decrees, which provide further details of these steps.

In order for this emergency protocol to be allowed and authorised by the Ministry of Health, which oversees this law governing transfusion, it must be considered. The Ministry is the sole authority with the power to introduce a derogation article into the law, thereby authorising its use in the context of military operations or exceptional situations on national territory. This constitutes a framework comparable to the one that the French chose to adopt to permit the inclusion of this practice in exceptional situations[53]. This request for a derogation was submitted to and endorsed by the Minister for Defence to the Cabinet of the Minister for Health. In accordance with Article 4 of Law of 20 July 2006[54], relating to the establishment and operation of the Agence Fédérale des Médicaments et des Produits de Santé (*AFMPS*), the agency's mission is "to guarantee the quality, safety and efficacy of all operations involving blood, from its collection to its utilisation". This legislation pertains to areas within the remit of the agency and ensures the monitoring, application, and control of regulations pertaining to this remit, in addition to their implementing decrees. It is thus this agency that will determine the integration of these new regulations into the existing system.

5. PERSPECTIVES

The completion of this research does not signify the conclusion of the work. As previously stated, Belgian legislation prohibits the utilisation of whole blood prepared by a blood transfusion facility that adheres to established collection and preparation protocols. The product in question must first be reintegrated into the range of blood components made available for mainly military use. As previously stated, this product will not entirely supplant the existing range of products; however, it will serve as a valuable addition for patients experiencing haemorrhagic shock.

Moreover, modifications to the legislation authorising the utilisation of WBB/EDP in exceptional circumstances must be negotiated with the FPS Health in order to gain acceptance and comprehension of our constraints and objectives, and to integrate their requirements into the proposed protocol for improvement.

The aforementioned requests will be subjected to analysis by the Higher Health Council, which will evaluate both the advantages and the potential risks associated with them. Moreover, this well-founded and referenced scientific position will constitute a compelling argument for integrating this product and this protocol into Belgian medical practice. However, beyond this joint endeavour, the European

Union is taking a more definitive stance through the publication of a directive on substances of human origin[55]. This directive includes a national obligation to include and provide for preparedness and emergency plans that can also serve as resilience plans in the event that usual services are exceeded. It thus follows that emergency protocols must be put in place, which provides a justification for the provision of a WBB/EDP for transfusion.

With regard to whole blood, our intention is to extend the study on its conservation in order to contribute to the collection of information on its quality and the quality of the platelets and coagulation factors it contains. This will facilitate the optimal utilisation of the product in accordance with the circumstances that may arise. A deeper understanding of the product will facilitate its optimal utilisation and ensure the highest standard of patient care.

5.1. CIVILIAN PROSPECTS

The legislation and regulatory frameworks are primarily designed for civilian contexts, which are largely distinct from the constraints and exceptional circumstances encountered by medical staff in military settings. The public sector is not constrained by the same limitations as the military, and therefore may be less inclined to implement the proposed legal amendments. This is particularly relevant in view of the highly organised and efficient nature of the national blood transfusion services. In the case of other countries, such as Norway, the public sector is similarly unable to provide adequate transfusion support within its own territory. Consequently, it participates in discussions regarding the authorisation of this exceptional protocol and its incorporation into the national resilience and preparedness plan[33,47,56,57]. This provides an opportunity for the military to contribute their experience to the benefit of the national territory and the civilian world.

It is possible to collaborate on Belgian territory in a manner similar to that observed in Norway. The Ministry of Health has indicated that B-Fast is considering utilising the protocol for transfusion support for its deployed personnel. Furthermore, there is a regulatory imperative in the future to include a resilience protocol on national territory in conjunction with the publication of the revised European legislation regarding substances of human origin. Indeed, in practice, some civilian companies have already employed this type of protocol in the recent

past, as documented following attacks and natural disasters[5], the civilian medical community is preparing to utilize this protocol again, inspiring military advances[41].

An illustrative example is the national preparedness and resilience plan developed in Norway, which incorporates emergency collection protocols to compensate for the geographical isolation of certain regions of the country. It is therefore necessary to consider the delivery of pre-positioned stocks of blood products to remote areas of the country to limit the risk in the event of urgent treatment. It would take an excessively long time to transfer the patient to a blood facility with a well-stocked supply. This would render it impossible to adhere to the recommended treatment times for operations conducted on national territory. In this context, emergency collection protocols have been encouraged which involve the creation of pools of pre-screened co-located donors to avoid the unnecessary storage of blood products which are doomed to expire. This is a resilience protocol compared to the conventional blood bank system which is not guaranteed. These products are too often subject to shortages which would result in a high loss rate. The use of risk analysis has led to the military's experience being applied to the benefit of civilians.

Although the literature demonstrates the advantages of whole blood transfusions in certain cases, such as the massive transfusions in austere environments discussed in this work, it is notable that the civilian healthcare services in Europe have entirely neglected this practice. This is due to component therapy having demonstrated its logistical and safety advantages, which were preferable over the use of whole blood in the 1970s[58]. In contrast, in the USA, instances have been documented in which the civilian sector employs non-traditional military methods in the event of emergencies, disasters or catastrophes involving the destruction or unavailability of traditional blood products[8]. This is because the USA has to deal with a large number of casualties with war-like injuries, even on home territory. Consequently, trauma centres have been established, drawing inspiration from military medical advances, including the utilisation of whole blood. As previously stated, its utilisation in a civilian context has already been validated, with positive feedback[59]. Its deployment has been demonstrated to have a favourable impact on survival rates at 24 hours and 30 days, while reducing hospital stays[60]. Furthermore, additional studies have indicated a decline in 24-hour mortality[61] and a reduction in the necessity for transfusions following an emergency department (ER) visit[62]. Finally, the benefit to haemorrhagic patients has been

demonstrated in vivo, showing an increase in survival at 24 and 48 hours. It would be particularly advantageous if thromboelastography results were severely impaired (MCF less than 60mm)[63]. This recommendation has also been extended by Repine (2006) to include disaster and mass transfusions, along with cardiac surgery, liver transplantation, and obstetric haemorrhage[58].

6. CONCLUSION

It is of the utmost importance that patients presenting with haemorrhagic bleedings are treated promptly with blood products, even in the context of military operations. Therefore, it is essential to ensure that these products are made accessible as early as possible within the management of such casualties and as close to the point of injury (POI) as possible. In the absence of standard transfusion support in military operations, it is crucial to activate an emergency protocol that provides optimal care to our deployed soldiers. This is the purpose of this thesis. The development of a tailored protocol for our nation has been made possible through the careful consideration of potential risks and limitations. This approach ensures the safety of all involved, including donors, recipients, personnel carrying out the protocol, and those who may be responsible for activating it. The project serves to illustrate Belgian Defence's investment in the joint effort to constantly enhance the care provided to its war wounded, while maintaining a vision of the necessity to ensure interoperability. It is anticipated that the majority of the medical support for military operations will continue to be provided internationally in the foreseeable future. This is due to the significant burden in terms of personnel, equipment and infrastructure that would be incurred by individual countries. Consequently, procedures implemented by one country must become increasingly synergistic and interoperable in order to integrate efficiently and safely. However, to guarantee this security in practice, it is crucial not to lose sight of the need for effective traceability in the management of war casualties across international borders. This is yet another challenge for the future, and one that is already being addressed by the NATO Blood Panel.

It is evident that the Belgian protocol developed as part of this thesis will continue to evolve and improve over time. Nonetheless, it contains indications for the implementation, safety and traceability of the process from collection to administration, as well as recommendations regarding transfusion supervision, which are made following each stage of the project. Such matters will be the subject

of numerous discussions with the relevant public health authorities in order to allow flexibility for all parties involved while maintaining quality standards of care. To achieve this, it is understood that the military must ensure the implementation of effective planning and the monitoring of donors on a regular basis, as well as the training of personnel deployed in this field.

The legislation must be adapted to permit the utilisation of whole blood initially, and subsequently whole blood obtained through the application of emergency protocols. The use of whole blood is not yet expressly addressed by either the Belgian legal code or European directives. These legal limitations act as a barrier to the development of appropriate and effective protocols by European nations which would facilitate the treatment of war wounded, and thus represent a significant obstacle to progress in the field. Furthermore, these limitations impede compliance with the NATO guidelines requiring nations to provide comparable standards of care for deployed personnel[64]. Upon conclusion of the project, the Belgian Defence will propose a framework for discussion with public health authorities with a view to facilitating implementation.

In anticipation of the forthcoming revision of the European directives (SoHO)[55], there is a need for transformation, and it falls upon the public sector to consider our request and to provide assistance in proposing solutions and implementing them.

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APPENDIX A:
BELGIAN WBB PROTOCOL

WBB-WALKING BLOOD BANK MHKA-LABO-OPS@MIL.BE
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WORK

Walking Blood Bank

OPS

WBB

Validated by: Degueldre Julie

CONTENTS

1. KEY REFERENCES AND LEGAL INFORMATIONS

1.1. Superior

Belgian law, Belgian A.R

European directives:

- 2002/98/CE du 27 janvier 2003 établissant les normes de qualité et de sécurité pour la collecte, le contrôle, la transformation, la conservation et la distribution du sang humain et des composants sanguins et modifiant la directive 2001/83/CE
- 2005/61/CE du 30 septembre 2005 portant sur l'application de la directive 2002/98/CE du parlement européen et du conseil en ce qui concerne les exigences en matière de traçabilité et la notification des réactions et incidents indésirables graves
- 2004/33/CE du 22 mars 2004 portant sur l'application de la directive 2002/98/CE du parlement européen et du conseil concernant certaines exigences techniques relatives au sang et aux composants sanguins

NATO Standardization Agreements seeking the interoperability of NATO partner countries

Avis du conseil supérieur de la santé 8831 : Recommandations pour la prévention et la prise en charge des hémorragies massives.

1.2. Inferior

Pre-deployment screening and interview of the donor candidates

Activation of WBB: indications

Call for donors

Donor Interview

Donor selection on site

Blood group determination procedure

Serological rapid tests procedure

Blood collection

Transfusion

Follow-up results (a posteriori)

Medical records

1.3. Related

LABO-Proc 2. Immuno-hematologische analyses

MD muti card

MHQA Transfusion Handbook

2. ABBREVIATIONS

ABO: Blood group A, B, AB or O

BP: blood pressure

CMV : Cytomegalovirus

EDP: Emergency donor pool

Hb: Hemoglobin

HBV: Hepatitis B-virus

HBcAb: Hepatitis B core antibodies

HBsAg: Hepatitis B surface antigen

HCT: Hematocrit

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

HTLV: Human T-lymphotropic virus

IAT: Irregular agglutinins test

MTF: Military treatment facilities

NAT: Nucleic acid test

RHD: Blood group antigen-D of the RH blood group system

SORT: Special operation resuscitation team

SOST: Special operation surgical team

StO2: Oxygene saturation

WBB: Walking blood bank

3. GENERAL FRAMEWORK

What is Walking Blood Bank (WBB)? Basically, a blood bank is a storage facility in which blood is kept available for any patient in need of a blood product transfusion. In our current context, "walking" means a walking and living individual. Therefore, the location where we store the available blood is the donor him or herself. WBB means collection from pre-screened donors for banking, not for a specific patient or immediate use. Whereas EDP means an emergency donor pool, i.e. the same pre-screened donor pool but collected in case of immediate need without banking purposes.

Throughout the document, the term WBB is used for ease of reference because the principle of preparation and collection is the same. Especially as this allows banking to be covered if the blood collected is not immediately used (e.g. death of the patient).

Within an operational context, a walking blood bank involves a relatively small group of persons, who are screened before deployment and can be readily available "upon request" for donating blood in case of emergency.

Donors are healthy and voluntary military personnel who are able, fit and willing to donate blood.

These donors may be staff members, who are deployed in the field and who have expressed willingness to be included in the potential donor list before their departure.

In a remote area, a teammate might also be a suitable donor in an emergency situation. The most suitable donor will depend, among other things, on the mission, the level of risk, staff availability and the degree of urgency.

Therefore, potential donors agree to regular serological monitoring before and during deployment. Members of a donor group also receive guidance about the appropriate lifestyle to adopt.

4. THE PURPOSE OF WALKING BLOOD BANK

Main objectives of the walking blood bank include both logistical and clinical advantages:

- Logistical advantages for compensating shortages or delays in the resupply of blood components (used for managing massive hemorrhages) as well as delay of evacuation for the patient.

- Clinical advantages for the care of bleeding patients, since it is the only practical way to transfuse platelets in the field. Platelets are generally unavailable because of their limited validity and their storage difficulties (5 days at 25°C on a shake plate).

4.1. Logistical benefits

The blood products are shipped to the operational theatre, under conditions compliant with storage rules applicable to such products. On-site, the blood products have to be stored appropriately, their conservation temperature must be monitored continuously and their expiration date should be evaluated in order to plan for a timely resupply.

This necessitates the implementation of an organized system of administration and follow-up, both in theatre and in the homeland. Adequate logistical means, such as a transport container and a refrigerator, must be present as well. For the EDP, which uses fresh, warm, whole blood, the logistical impact is less elaborate: no fridge and no stock, because the blood products are prepared “in real time” and in precisely the

required quantity. It requires only a well-trained and well-educated medical personnel who have access to the medical report of the donor pool and the appropriate sampling equipment. In theory, such a donation can be done quickly (+ - 30 min).

In case of banking during a WBB, the use of the classical fridges provided for blood products delivered from Belgium will be adequate and sufficient

4.2. Clinical benefits: Remote damage control resuscitation

WBB offers a unique opportunity for providing the platelets, which are essential to the treatment of patients with massive hemorrhage, who cannot be saved by packed cells and plasma alone.

Damage control resuscitation (DCR) is the principle of resuscitating patients who are (going into) shock due to traumatic injuries, by concentrating on the treatment of those processes, which are an immediate and direct threat to the patient's survival.

An important concept in DCR is the "lethal triad", which consists of hypothermia, acidosis and coagulopathy. Hypovolemia, due to blood loss, will lead to poor tissue perfusion, tissular hypoxia, lactic acid production and this acidosis. Acidosis, in turn, will lead to fibrinolysis and a more global state of coagulopathy with platelet dysfunction. Blood loss will also mean the loss of clotting factors and platelets, which will make the bleeding worse. Finally, blood loss also causes hypothermia, which will exacerbate acidosis AND coagulopathy. All these phenomena will cause an increase in the existing hemorrhage. The utility of early administration of whole blood can also be understood in this context. One of the most pressing needs for such patients is the restauration of adequate and functional circulation volume. Only whole blood can provide this, without the risk of haemodilution.

The so-called golden hour is too late. Even is the bleeding is stopped before cardiac arrest occurs, without the restauration of the intravascular volume with a warm liquid capable of transporting oxygen to the tissues, the lethal triad will continue to exacerbate the patient's condition. Most fatalities occur within 30 to 60 minutes after the initial injury, regardless of the use of tourniquets and other means of haemorrhagic control.
For patients with no access to blood products, fresh, warm whole blood collected on-site would be the only possible way to obtain the necessary products at the right time.

5. EXCEPTIONAL SITUATIONS

The first step is to define a standard operational procedure for this exceptional situation. Blood donation and transfusion are, at a Belgian and European level, well defined by a legal framework, which also includes collection from and transfusions to Belgian soldiers in foreign operations.

Therefore, an emergency blood donation is not just about donating blood product in emergency situations. It also includes the circumstances in which the stock of blood products is insufficient or unsuitable. All actions from the selection, interview, ABO-

typing, pre-screening, blood donation, follow-up of the donor and ABO-typing to the follow-up of the patient or receiver are well described and framed.

Nevertheless, when blood products are not available or if the patient is in life-threatening danger and platelets are needed for adequate treatment, an on-site whole blood collection may be required. This is considered an exceptional situation in which the usual legal framework cannot be followed to the letter.

In this work document, we provide a risk analysis and means of action to undertake the transfusion of whole blood collected on-site and to minimize the risks involved. However, these situations force us to deviate from the legal framework; therefore, the final responsibility for the activation of this procedure will lie with BMed/MCC.

This procedure explains the different steps necessary to "follow the legislation", as well as to guarantee maximum protection of both the donor and the patient, if all the mandatory legal steps cannot be executed at the right time.

6. INDICATIONS FOR WALKING BLOOD BANK

As mentioned earlier in this document, the indications should be either logistical either therapeutic.

Prescribing blood products constitutes a medical treatment which is always the responsibility of the prescribing physician. There are a lot of blood products that can be transfused in a transfusion therapy: the primary product for the WBB is whole blood.

The administration of whole blood is carried out only on medical prescription. The objectives are : 1/ to guarantee sufficient oxygenation at the cellular level, 2/ to preserve an efficient blood circulation volume and 3/ to maintain the haemostasis. The benefits and risks of a transfusion must always be weighed and then used for life-threatening haemorrhages.

The prescribing physician should always consider the patient's clinical condition, not just the laboratory results. The parameters used to decide to proceed with a transfusion are: the blood pressure, heart rate and the lactate. The prescribing physician must write down the indication to transfuse on the request form. Note that there is no threshold value that automatically requires a transfusion.

6.1. Therapeutic: shock patients

These values are part of the indication of the lethal triad and the haemorrhagic shock installation: a combination of multiple failures can lead to the decision to transfuse whole blood which allow a clinical benefit:

- Life threatening hemorraghe
- Blood pressure < 100 mmHg and pulse > 130 BPM (110 BPM after analgesia)
- Haemoglobin level is <8 g / dL

- Lactate > 3
- pH < 7,35 (less useful)

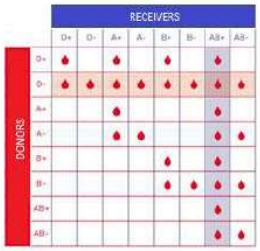
6.2. Massive haemorrhage

In case of a massive haemorrhage, a transfusion of RBC, plasma, and sometimes platelets, is necessary. Whole blood contains all of it. Moreover, the management of massive haemorrhages is very resource-intensive. The available stock of blood products may even not be sufficient to treat this type of patient. Thus, once again, the walking blood bank is a solution.

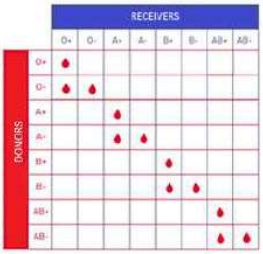
7. TRANSFUSION PRINCIPLES USING WHOLE BLOOD

These tables compare the compatibilities of transfusion with red blood cell concentrate or with whole blood.

Transfusion with ECL



Transfusion with whole blood



As shown in these tables, there are more possibilities with ECL than with whole blood.

Whole blood contains plasma and therefore contains naturally anti-A and/or anti-B antibodies.

For this reason we consider that the type-specific or compatible transfusion is the best solution. Moreover, by including A and O blood types in the donor population, 90% of the Eurasian population is covered with regards to the frequency of blood group expression. This is the best solution to increase the number of potential donors for small deployed teams (SF teams far forward).

Nevertheless, it entails more risks in case of several patients being taken in charge at the same time. Therefore, if the collected blood bags are not properly identified, a transfusion error may result in death due to hemolysis. In these case (R1/R2), Group O blood could be compatible with all other blood groups as we can select donors group O blood with a low titer of those antibodies. However, given the size of the

detachment, the number of potential donors will be sufficient by taking only LTOWBs. Type O people represent about 45% of the population.

7.1.1. Advantages and disadvantages

Advantages

- Mitigates the dilutional effect of crystalloid solution
- Limits the number of exposures to donor component blood products
- Replete with plasma clotting factors
- Reduces the risk of hypothermia because of storage at room temperature

Disadvantages

- Increases the potential risk of bacterial contamination or bacterial overgrowth
- Increases the risk of transfusion-associated graft-vs-host disease: no deleukocytation
- Transfusion response to RBC antibodies and HLA: diminished if we only collect whole blood from male donors
- No pathogen reduction treatment

8. SCOPE AND REQUIREMENTS

8.1. Responsibilities of blood establishment and blood bank

According to the Belgian functioning concerning blood management, there are two distinct institutions with their own tasks: the blood establishment and the blood bank.

The procedures and responsibilities (Belgian legislation) for a blood establishment (donation) and a blood bank are also applied abroad but they must be adapted, reviewed or delayed (a posteriori testing).

A Blood establishment is responsible for the collection with these detailed tasks:

- Information of donor candidate: pre-deployement
- Donor selection (Interview -Signature): pre-deployement and on-site
- Blood collection: on site
- Donor screening (NATesting): pré-deploy, on-site and a posteriori
- Product testing: not done
- Product processing: not done
- Product release: on-site
- Product labeling: on site
- Product storage: on site or not done
- Product transport: on site (MEDEVAC)
- Product distribution (→ to a bank if not used)

A blood bank is responsible to give the right blood product at a patient with these detailed tasks:

- Patient management: on site
- Pre-transfusion testing: on site
- Product delivery: on site

Concerning the adaptation of this concept for mission abroad and exceptional situations, part of these steps must planned and executed before deployment. Note that Defence do not have any accreditate establishment anymore.

8.2. Prior to deploy

8.2.1. Risk assessment of the mission

The level of risk of the engagement must be assessed prior to the beginning of the mission. If transfusion support is included in the medical support, the Defence Department organizing the mission must, in collaboration with COMOPSMED, establish a plan for the re-supply of blood products. If this is required, a WBB module must be provided.

Following this decision, the following steps should be organised and checked during pre-deployment training: medical responsibilities delegated on site, donation candidates in the deployed personnel, complete interview and a full screening of voluntary donors as prescribed by the law (NAT and serological), establish a list of donors and a medical record of the donation for each one, education and training of the medical staff dedicated to the implementation, set the responsibilities for the implementation.

Responsibility: ACOS OPS/Trg iccw COMOPSMED

Document: Define in the OPORDER

8.2.2. Blood donor Pre-screening

The first step is informing the candidate about blood donation

At the beginning, the national donor selection guide or interview is used to have a complete overview of the medical history of the donor.

A full interview of the donor to get a complete overview of his/her medical history is conducted to determine suitability for donation. At this stage, a questionnaire including a summary of international travel and diseases encountered is conducted in order to detect potential exposure to latent disease vectors. This interview also makes it possible to check any medical history. We can also determine if the candidate is a regular donor or not, if he has a risk status due to his lifestyle (homosexual or multiple partners), or any other contraindications like medications that will have a negative impact on the quality of the final blood product.

These indications may lead to either suitability to donate or temporary or permanent unsuitability.

All information will be included in the donor's initial file made available to the delegated medical authority on site.

At the end of this interview, the donor has to sign to give his consent and engage his responsibility (honesty to answer the questionnaire knowing the consequences of his decision).

Also at this stage, a blood sample is taken, which will be used to perform the steps of determining the blood type and a complete analysis of the donor's serological status and blood type. This includes ABO-typing and IAT (Irregular Agglutinin Test) and the research of Transfusion Transmissible Infections (TTI as prescribed by the law).
Two different independent blood determination are needed to confirm the blood group of each member of the detachment. The haemolysin titer will be determined for each O patient in order to indicate low titer O donors.
The TTI tests are serological analyses for HIV, HCV, HBcAb, HBsAg, HTLV, Syphilis, CMV and NAT testing for HIV, HCV and HBV.
HBcAb and HBsAg will be tested once, because we consider that, in order to be a blood donor, the candidate has to be vaccinated against HBV. This will simplify the screening on site and minimize the risk for the patient.

Aside from the before mentioned antigenic and/or serological tests, NAT tests will also be performed in order to fulfil the legal obligations incumbent on blood establishments. This step is necessary to ensure greater reliability of detection by reducing the window period for the virus.

Responsibility: lab tests executed by MMLC or delegated to a accredited establishment. Donor call and selection in close coordination between the unit's physician, COMOPSMED, the MMLC and the physician responsible during the operation.

Document: List of the suitable donors. A medical report containing the results of the screening for suitable donors: a complete version, to be kept by the MMLC, and a summary, to be kept by the detachment. A full interview document available for the physician of the unit.

Material: Full screening material at the MMLC. NAT testing by the Red-Cross.

8.2.3. Activation: Indications and prescription

Massive haemorrhage with Shock

Shortage of blood component

Need for platelets in case of a patient in shock

The indication has to be correctly reported to justify the activation of the walking blood bank. The reason for transfusion of whole blood has to be indicated on the prescription by the physician in case of activation of the protocol. The parameters or laboratory results, upon which the decision to activate were based, must also be mentioned on the prescription.

Responsibility: the physician, who is designated to be responsible, is identified before the beginning of the mission. He/she authorizes the activation of the protocol. In case of small team deployed far forward: an on call physician must be appointed, to authorise the activation and the transfusion by the medic on site.

Document: Activation and prescription sheet.

Material: diagnostics rapid tests and material.

8.2.4. Interview: Blood donor selection

We will have a preference for blood donors of the same blood type in small teams and we can go for a pool of LTOWB donors for larger detachments. These considerations will be established during the discussion prior to the development of the PDT, during the risk assessment.

With a female patient, a rhesus-compatible donor is essential in order to reduce the risk of damaging immunisation for a woman of childbearing age.

Similarly, in order to reduce the risk of transfusion reactions and to reduce the risk of anaemia, women in the detachment will not be considered as potential donors.

This is a shorter questionnaire used to complete the primary interview on the field and help to make a selection.

Based on the questionnaire proposed by doughty et al., which consists of a primary triage, secondary triage and a risk triage tool.

Primary triage: To verify the donor is willing to give blood with a preference for the regular donors

Secondary triage: To verify the health state of the potential donor

Risk Triage: To give a risk score regarding the lifestyle of the potential donor. Lowest score = lowest risk

Responsibility: Nurse or medic, after receiving appropriate training

Document: Questionnaire.

8.2.5. Donor testing

It would be ideal to be able to perform rapid tests before the blood is actually transfused.

Donor and recipient blood group verification tests MUST be performed prior to the transfusion of the product to the patient.

The type of test to be performed, will depend on the level of medical support that is available:

- Role 2: compatibility test
- Role 1: confirmation test of the ABO-group by a MD-Multicard

- Advanced teams without medical support: Eldon card determination card

NAT testing is not feasible on the field, but a sample taken at the time of donation will be returned back to the MMLC so that NAT testing can be later completed (lookback).

In order to reduce the risk of blood-borne infections, we are planning to carry out rapid tests for the antigenic detection of the relevant viruses: HCV, HIV. For long-term deployments, rapid tests should be used as a follow-up screening on site in order not to miss (IgM detection) a development of one of these infections on site.

It must be ensured that potential donors are suitable for donation only if they are vaccinated against HBV. In this way, the risk is considerably reduced.

Responsibility: the physician, who is designated to be responsible, is identified before the beginning of the mission and makes sure iccw MMLC that the tests are performed. The nurse to collect ASAP the samples. The lab technician to perform the test.

In case of advanced team, all the team members must be able to collect blood and perform the rapid tests.

Document: follow up sheet for medical report (app and QR code in the future)

Material: rapid tests oraquick HCV, HIV ½ early detect, Eldon card, / compatibility test materials and/or MD multicard.

8.2.6. Collection

Once the donor(s) have been selected and the blood samples collected for rapid testing, the nurse in charge can start the collection using the TERUMO collection bag containing the anticoagulant CPDA-1.

This bag will be placed on a scale in roles 1 and 2 to ensure the quantity collected (480g) and thus ensure the quality of the final product. Too much would be damaging to both the donor and the product, as the quantity of anticoagulant may be insufficient, and too little would be a loss to the patient and the product would be less good as it would be mixed with too much anticoagulant.

To ensure the quality of the product (clotting factors), the collection time cannot exceed 12 minutes.

For small teams deployed without medical support, the team medic will collect the blood and will respect the indications provided during the training in order to get as close as possible to the standard quantities (450ml).

In each situation, the operator must ensure that the collection bag is located under the donor as the bag will be filled by gravity.

The collector will also require assistance to wave the bag every 30 seconds to ensure that the blood is in homogenous contact with the anticoagulant contained in the bag.

Responsibility: The nurse to collect the whole blood may be assisted by other people in case of necessity

Document: follow up sheet for medical report (app and QR code in the future)

Material: blood collection bag Fresenius CPDA-1. standard sampling material

8.2.7. Labelling – medical report

The donor himself will be asked to indicate his name on the bag as well as the time of collection.

The blood group will also be registered as soon as it is verified.

The blood thus collected will be transfused immediately as it is collected for a specific purpose.

The non-transfused products can be stored for 6 hours at room temperature or 48 hours if placed in a blood bank fridge (still to be adapted).

Responsibility: The nurse and the donor

Document: follow up sheet for medical report (app and QR code in the future)

Material: marker

8.2.8. Medical report donor and patient

A simplified but complete medical record of the donation will be compiled for each donor and provided to the doctor responsible for the mission. This doctor will be responsible for the transmission of information to his successor in case of multiple rotations.

In the future, we propose the development of an app that would facilitate the monitoring and ensure the traceability of acts and products. Each staff member, deployed for a WBB mission, would be assigned a QR-code containing all the information collected before departure concerning their identification, blood group, donation frequency, ITT screening. The responsible medic would have the full application to assign each QR code to a donor or patient, browse the last screening elements registered, encode the current results (even a posteriori) and thus have a follow-up of the donation and the timing of each act performed. The operator could also assign each action to an operator (doctor, nurse, medic, laboratory technician) by following the order of the actions to be taken.

Responsibility: MMLC to provide and charge all adequate information in the system per mission. Medic in charge to scan and register each follow up needed regarding the transfusion and blood collection.

Document: QR code for each member of the detachment (app and QR code in the future)

Material: mobile phone and or tablet

8.2.9. Transfusion

The transfusion will be central or intraosseous depending on the ease of access.

The filter used will have a pore size between 150 and 250 µm and the transfusion should be done as quickly as possible without pushing the blood through the line.

Freshly collected and warm blood should not be heated, even if a blood warming device is available.

In case of a transfusion reaction, it must be referenced in the follow-up sheet of the medical file. The transfusion will be stopped immediately and the line should be disconnected and rinsed. It is however of utmost importance that the intravenous or intraosseous cannula remains in place!

Responsibility: the physician, who is designated to be responsible, is identified before the beginning of the mission. He/she makes sure that the tests are performed. The nurse to assist.

Document: follow up sheet for medical report (app and QR code in the future)

Material: transfusion set.

8.2.10. Follow-up

The patient's medical file will have to be copied. The copy will accompany the patient and the original will be sent back to Belgium to the MMLC address for follow-up.

The donor will eventually be supplemented with iron in case of regular donation.

The donor will be asked to report any undesirable event following the donation.

Samples taken from donor and patient for a posteriori NAT testing must be returned to the MMLC as soon as possible and in accordance with the appropriate transport conditions.

The patient will be assessed regularly to determine the need for further transfusion prior to evacuation to a surgical management site with adequate holding capacity.

In the event that evacuation is rapid concurrent with the patient's need for transfusion, whole blood collected for the patient will accompany the patient during evacuation to ensure a continuum of care.

Responsibility: The physician, who is designated to be responsible, is identified before the beginning of the mission, The nurse to assist and the donor to inform in case of ITT.

Document: follow up sheet for medical report (app and QR code in the future)

Material: Frigobox to send samples back to MMLC

9. RESPONSABILITIES

The members of the OPS laboratory included in the procedure and the protocols above are responsible for carrying out the tasks assigned to them. OPS military personal and staff members who perform any of the above tasks must comply with these guidelines.
The management of the OPS laboratory iccw CCMed must ensure the adequacy of the working method in relation to legal requirements and benchmarks, monitor the application of the described working method and if necessary, intervene at times and / or at the determined levels.

10. ASSESSMENT AND IMPROVEMENT

The revision of this document is carried out in response to a need and is the responsibility of the management of the OPS laboratory or every 3 years.

11. HISTORICAL

Edition	Revision	Date	Raison / Note
01	00	08/04/2020	Basic document
01	02	16/05/2024	New template

APPENDIX B :
QUESTIONNAIRE TRIAGE TOOL

WBB-ON FIELD QUESTIONNAIRE MHKA-LABO-OPS@MIL.BE
+32 2 443 21 02
+32 2 443 20 84

WORK

On Field Questionnaire

(DOUGHTY ET AL. 2016)

WBB

OPS

Validated by: Degueldre Julie

1. PRIMARY SELECTION: IN GROUP

Steps	Question	Yes	No	Action
1	Do you want to give blood?			Disqualified if not
2	Have you given blood before?			Consider early selection if yes

2. SECONDARY SELECTION: INDIVIDUALLY

Steps	Question	Yes	No	Action
3	Are you unwell now? Fever, Diarrhea, vomiting, chronic medical conditions and not well?			Disqualified if yes
4	Are you taking medication for blood pressure: stroke or heart, lung, kidney, cancer or blood conditions?			Disqualified if yes
5	Have you had a blood transfusion or blood products in the last year			Disqualified if yes, accept after 1year
6	Are you living with HIV/AIDS/Hep B or C or are you living with anyone with these conditions?			Disqualified if yes
7	Have you ever been refused as a donor or told not to donate blood? (past history of treated anemia may be accepted)			Disqualified if yes
8	Male donors only: have you ever had sex with another male?			Disqualified if yes
9	Have you ever taken illegal drugs with a needle? (even steroids)			Disqualified if yes
10	Are you currently pregnant or breast feeding?			Disqualified if yes
11	Conduct a physical examination: check Temperature, Rash, malnutrition, pallor, jaundice, cyanosis, shortness of breath, intoxication from alcohol or drugs, veins			Disqualified any potentially unwell donors with very difficult veins

Temperature: .....

Pulse: .....

Blood pressure: .....

Hb (if measured): .....

+32 2 443 21 02
+32 2 443 20 84

3. RISK TRIAGE: INDIVIDUALLY

1. Blood donation history

Score	Status	Subtotal	Notes
1	Regular donor		Optimum
2	Previous donor		
3	Non-donor		

2. Veins and body weight

Score	Status	Subtotal	Notes
1	Good lateral veins		Optimum
2	Poor or difficult veins		
3	Under 60kg		Risk of fainting

3. INFECTION

Score	Status	Subtotal	Notes
1	➢ 21 days well		Optimum
3	➢ 21 days well		

4. Travel

Score	Status	Subtotal	Notes
1	No travel in the countries below in the last 6 months		Optimum
2	South America		
4	Asia and Africa		

5. Lifestyle

Score	Status	Subtotal	Notes
1	Sex with one partner		Optimum
3	Sex with multiple partners but protected		
-	Sex with a sex workers or in exchange for money/drugs		Avoid for 12 months

6. SERIOUS MEDICAL CONDITIONS

Score	Status	Subtotal	Notes
1	None		Optimum
3	Past or present serious medical conditions but managed and well		
3	Untreated current medical conditions but well		

SCORE: .....

LOWER SCORE = LOWER RISK

APPENDIX C:
BELGIAN KITS

DONOR KIT

Kit component sheet

• Nitrile gloves L / XL	6515-01-565-3179/81
• Eldoncard (ABO)	6550-01-587-1889
• Oraquick HCV	6550-01-601-8094
• Oraquick HIV	6550-01-679-1860
• Blood collecting and sampling bag	6515-17-112-9557
• Tourniquet 25mm-600mm	6515-13-113-8060
• Soft-Zellin-C	6510-98-204-1062
• Compress 10 x 10	6510-13-113-7664
• Blood collection tubes (3X EDTA)	6640-41-001-5283
• Blood collection tubes (2X Serum)	6640-27-028-4177
• Lancet for finger prick	6515-17-055-7701
• Eclipse needle	6515-12-376-0068
• Tube holder	6630-14-516-3056
• Peha-haft	6510-12-341-0546
• Opsite	6510-99-605-9985
• Working procedures	
• On field questionnaire	

PATIENT KIT

Kit component sheet

• Nitrile gloves L / XL	6515-01-565-3179/81
• Eldoncard (ABO)	6550-01-587-1889
• Tourniquet 25mm-600mm	6515-13-113-8060
• Soft-Zellin-C	6510-98-204-1062
• Compress 10 x 10	6510-13-113-7664
• Opsite	6510-99-605-9985
• Lancet for finger prick	6515-17-055-7701
• Blood collection tubes (1X EDTA)	6640-41-001-5283
• Blood collection tubes (1X Serum)	6640-27-028-4177
• Three-way valve	6515-12-336-9775
• Catheters (16G)	6515-14-580-8771
• Catheters (18G)	6515-14-580-8782
• Catheters (20G)	6515-14-580-8780
• extension line (Heidelberger 75cm)	6515-12-328-4400
• Combi stopper	6515-14-486-1417
• 5 ml syringe	6515-01-428-3799
• Eclipse needle	6515-12-376-0068
• Tube holder	6630-14-516-3056
• Working procedures	

ANNEXE D:
PUBLISHED ARTICLES

PATIENT BLOOD MANAGEMENT

Review

A systematic review of indications when and how a military Walking Blood Bank could bridge blood product unavailability

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Background - Blood supply problems in remote areas are well known. To overcome this shortage, many countries have developed innovative Walking Blood Bank (WBB) protocols. However, no common standards have yet been set for their use and common actions. Given that these procedures involve a certain risk, it would be interesting to analyse the activating criteria that lead to using this unusual protocol. Thus, this review aimed to identify indications for a WBB and the common risk mitigation measures.

Material and methods - This PRISMA-compliant review only included studies published from 1985 to 25th of January 2023 that describe adult male military casualties requiring blood transfused locally using a walking blood transfusion protocol. All relevant data (i.e., activation and contextual factors and risk mitigation measures) were tabulated to retrieve information from the selected military studies.

Results - Our results indicated that activation criteria were homogeneous across the 12 reviewed studies. Whole blood was collected from a WBB when there was a shortage of blood products and when platelets were needed. In the literature reviewed, the main risks associated with such a protocol, namely hemolytic adverse events and transfusion transmitted diseases, are mitigated by the use of typing and screening measures if they are reported. However, there is less consistency in the implementation of those risk mitigation measures.

Discussion - This unusual protocol needs to be integrated into the medical support plan until conventional transfusion support can take over, and should include on-site blood collection from a donor, whether a WBB or an emergency donor panel. The benefits of such a protocol outweigh the risks in a life-threatening situation, especially since these risks can be anticipated and minimised by planning to pre-screen all potential donors before their deployment. Finally, educating and training the staff who must implement this unusual procedure can also improve the safety and survival rate of future patients.

Keywords: *Walking Blood Bank, whole blood, emergency donor panel, indications, risk mitigation measures.*

Arrived: 26 July 2023
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INTRODUCTION

Over the last decade, transfusion medicine has evolved towards fractionated whole blood components such as red blood cells, platelets, or plasma, to improve the efficiency of storage and use in a standard hospital environment[1]. However, in austere environment (e.g., combat zones), military medical support must also provide the most appropriate product for the treatment of shock and coagulopathies, as hemorrhage remains a major cause of death among combat casualties[2]. Nevertheless, logistical constraints limit access and/or storage of these blood products[3]. The medical support system has been forced to adapt by developing innovative solutions that improve combat casualty care (e.g., DCR)[4]. They have therefore developed techniques, such as walking blood bank (WBB) protocol, to sufficiently access blood anywhere to support combat casualties until their evacuation[5] and thereby increase their survival rate[6]. A WBB is a pool of donors available "on call" to donate whole blood (WB) in the event of an emergency[7]. These donors are among those deployed and consent to be registered as prospective donors prior to deployment[8].

In addition to its essential role in increasing the survival rate of hemorrhagic patients, WB also offers biological advantages by providing all the blood components in a single transfusion to counteract the lethal triad observed in hemorrhage patients[9,10]. Essential blood components are often in short supply on the battlefield, especially platelets. Due to their short shelf life –between 5 and 7 days depending on the country– platelets are usually unavailable. This is why the use of WB, which contains platelets, can be essential for the treatment of certain hemorrhagic patients in extreme environments. Whole blood transfusion seems to be the only accessible solution in logistically challenging situations. This solution would address the need for platelets and logistical issues[5]. Any disadvantages that may arise seems far outweighed by the benefits of such a transfusion[11]. While risks will always exist, we can control and mitigate them. The literature shows that if the donor is pre-screened and a clear protocol is followed[11], WB transfusion from a WBB is safe and effective. WBB implementation currently appears to rely on several different protocol-driven techniques[11]. There is no existing interoperable protocol for the use of WBB even within the NATO coalition based on different national regulations.

The aim of this review is to identify situations where the benefits exceed the risks of resorting to a military WBB by focusing on these two questions:

1. What military context leads to the activation of a WBB (when/where)? and
2. What measures can be taken to minimize the inherent risk of such an implementation on the battlefield?

MATERIALS AND METHODS

This systematic review was conducted according to Preferred Reporting Items in Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Literature search and screening criteria

PubMed and Scopus databases were searched using the following keywords: "Walking blood bank"; "Walking AND blood AND bank"; "Emergency whole blood"; "Buddy transfusion"; "Blood far forward"; "Walking donor"; "Emergency donor panel" and "Warm fresh whole blood". All articles published from 1985 (after HIV appearance in blood transfusion) until 25th of January 2023 were considered.

Selection of studies (exclusion and inclusion criteria)

First, the lead investigator identified relevant studies by reviewing the abstracts according to the inclusion and exclusion criteria. In addition, two authors independently assessed all the full texts, and then the full list of eligible studies was agreed by all the authors. The exclusion and inclusion criteria for study selection are described in **Table I**. Studies were included if they described male military adults who were injured and required transfusion of blood collected in the field according to a WBB protocol. Our research focused specifically on adult male military patients, who make up over 95% of our deployable at-risk-population. Furthermore, studies in women tend to reflect transfusion in a perinatal setting, which is not representative of managing bleeding patients in the military. Moreover, studies reporting field-tested protocols and information on at least two of the three outcomes of interest (see **Table I**) may be considered even if they did not include patients. A flowchart illustrating the selection procedure is presented in **Figure 1**.

Table I - *Exclusion and inclusion criteria for military study selection*

		Inclusion criteria	Exclusion criteria
Screening	Language	Papers written in English	Papers written in all other languages
	Study design	Prospective (including feasibility studies) or retrospective studies in international peer-reviewed journal	Unpublished material, communication, letter to the editor, reviews, and conference abstract
	Publication year	Papers published from 1986 onwards	Papers published up to 1985 to include the ITT related risk
	Participants	Military males adults if patients are involved	Females and children
Eligibility	Outcomes	At least 2 of 3: • indication of resorting to a Walking Blood Bank • donor safety • recipient/patient safety	Analysis of donor, patient, or use of the Walking Blood Bank apart
	Setting	Military setting	Civilian setting

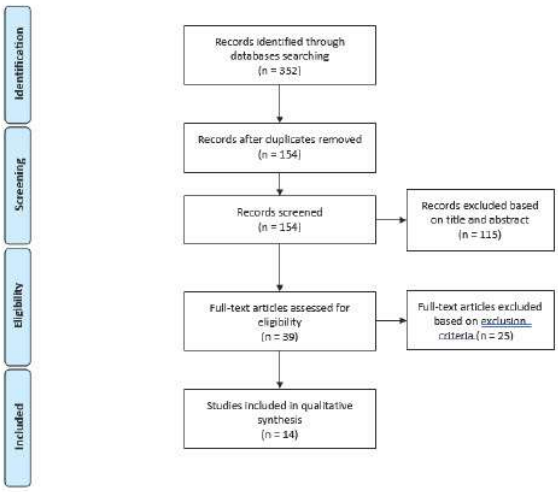

Figure 1 - PRISMA flowchart illustrating the entire selection process, from the literature search to the selection of studies of interest based on the inclusion and exclusion criteria
The screening process allows the rejection of duplicates and papers that do not meet the inclusion criteria based on titles and abstracts (i.e., language, year of publication, study design and participants). The eligibility process involves full-text analysis of the remaining papers based on specific outcome criteria, namely the setting and reporting of at least 2 of the following 3 aspects: activation criteria, donor safety or/and patient safety.

Data extraction and analysis

The data were extracted by the lead author and checked by a second author to ensure accuracy. Disagreements were discussed and decision was taken by a third author. The literature review was divided into two steps: activation indicators and risk mitigation measures.

To retrieve information from the selected studies, several tables were created. All relevant data regarding the activation factors of a WBB are compiled in **Table II**. The following contextual factors were determined:

1. availability of a blood bank and type of product in stock,
2. type of patient injury,
3. type of emergency situation (i.e., massive transfusion, mass casualty, remote, or combinations of the above).

In addition, this table also included the activation criteria of the WBB as well as information on the type of WB used (i.e.: cold-stored WB or fresh warm WB).

All the mitigating and protective measures implemented in each study to minimize the risk associated with the use of a WBB were summarized in **Tables III** to **V**. These countermeasures were grouped into two categories: donor-related and patient-related. The latter were likely to occur at two different times, before deployment and on-site during blood collection. Information on donor-related activities is provided as follows:

1. donor screening before deployment and
2. donor screening at blood collection.

Table II - *Summary of the indications of activation of a Walking Blood Bank*

Authors	Basic situation			Walking blood bank activation	
	Blood bank product available?	Type of Injuries	Situation	Activation indicator	WB used
Lewis *et al.*, 2020	Yes (CSWB + Full CT)	Blast injury, hemorrhage	Mass casualties, massive transfusion	Depletion of CSWB/evacuation impossible or delayed	FWB
Miller *et al.*, 2018	Yes (frozen pRBC + FFP)	No specific injury described: Helicopter crash	Mass casualties, massive transfusion	Platelets needed/severe coagulopathy	FWB
Bassett *et al.*, 2016	Yes (Full CT)	Traumatic amputations, blast injury, shrapnel injury	Massive transfusion	Combat injured patients likely to require massive transfusion (benefits from early activation)	FWB
Strandenes *et al.*, 2015	No (no blood bank available)	No specific injury described: Feasibility study for Norwegian frigate conducting antipiracy operations	Remote situation	Planning	CSWB for banking
Garcia Hejl *et al.*, 2015	Yes (pRBC, FDP)	No specific injury described: Feasibility study	Mass casualties, massive transfusion	Platelets needed/severe coagulopathy	FWB
Hrezo and Clark, 2003	No	Rectal bleeding	Remote situation	Shortage of blood products	FWB
Gaspary *et al.*, 2020	Yes (CSWB + Full CT)	No specific injury described: feasibility study	Mass casualties, massive transfusion	Shortage of blood products (CS LTOWB serve to start massive transfusion until FWB become available from the WBB)	FWB
Hakre *et al.*, 2013	Not reported	IED Blast	Mass Casualties, massive transfusion + remote situation	Shortage of blood products	FWB
Malsby *et al.*, 2005	Not reported	Gunshot wound	Massive transfusion + remote situation	Shortage of blood products	FWB
Liu *et al.*, 2014	Yes (RBC, FFP, PLT)	No specific injury described: Hit by a ship cable	Massive transfusion	To correct coagulopathy when all other blood products failed	FWB
Gaddy *et al.*, 2021	No (any products available at POI)	Gunshot wound	Remote situation	Absence of blood products (transfusion after extraction before evacuation, POI)	FWB
Song *et al.*, 2021	Yes (CSWB)	Blast injury	Remote situation	No access to stored blood product at the POI: Delay for evacuation	FWB

CSWB: cold stored whole blood; pRBC: packed red blood cells; FDP: freeze-dried plasma; CT: components therapy; FFP: fresh frozen plasma; RBC: red blood cells; PLT: platelets; POI: point of injury; IED: improvised explosive device; CS LTOWB: cold stored low titer O whole blood; FWB: fresh whole blood; WB: whole blood.

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This distinction was made because fully equipped laboratories and remotely accessible laboratories differ greatly in terms of resources, procedures, availability, as well as the sensitivity and specificity of the tests used. The blood grouping, the type of screening (i.e., infectious disease screening using questionnaire, nucleic acid testing, serology, or rapid test) and the virus tested were reported if mentioned. Donor screening included questionnaires and/or tests, and we considered both as one. The tests might differ depending on national

Table III - *Summary of the "typing" risk mitigation measure*

Authors	Type of WB	Pre-deployment	At collection
Lewis *et al.*, 2020	Type sp. & LTOWB	Not detailed	Not reported
Miller *et al.*, 2018	Type sp.	Only a 10% sample of on board personal	Confirmation
Bassett *et al.*, 2016	Not reported	Refer to CPG	Refer to CPG
Strandenes *et al.*, 2015	LTOWB + AWB	National standard procedure for regular donor in civilian health care: Grouping + titer	Confirmation (rapid test)
Garcia Hejl *et al.*, 2015	Type sp.	No reported	Type
Hrezo and Clark, 2003	Type sp.	Only a 10% sample of population	Type + Crossmatch
Gaspary *et al.*, 2020	LTOWB	Not reported	Samples collected on site and send back to homeland for titer analysis
Hakre *et al.*, 2013	OWB + AWB	Not reported	Not reported
Malsby *et al.*, 2005	OWB	Not detailed	Not reported
Liu *et al.*, 2014	Not reported	Not reported	Not reported
Gaddy *et al.*, 2021	Type sp. LTOWB prehospital	Yes: blood ID card	Confirmation by rapid test required but not executed due to tactical limitations - use of blood ID card
Song *et al.*, 2021	LTOWB	Not reported	Not reported

WB: whole blood; Type sp.: ABO type specific; LTOWB: low titer O whole blood; OWB: O whole blood; AWB: A whole blood; LTOWB: low titer O whole blood; CPG: clinical practice guidelines; ID: identification.

Table IV - *Summary of the "screening" risk mitigation measure*

Authors	Pre-deployment	At collection
Lewis *et al.*, 2020	Not detailed	Not reported
Miller *et al.*, 2018	Only a 10% sample of on board personal HBV - HCV - Syphilis - malaria	Rapid tests
Bassett *et al.*, 2016	Refer to CPG	Refer to CPG
Strandenes *et al.*, 2015	National standard procedure for regular donor in civilian health care	Combined rapid test
Garcia Hejl *et al.*, 2015	No reported	Questionnaire Rapid tests HIV, HCV + complete HBV vaccination
Hrezo and Clark, 2003	Only a 10% sample of population. Questionnaire Serologic tests: HIV, HCV, HBV, HTLV	Rapid testing
Gaspary *et al.*, 2020	Recommanded JTS CPG but not executed	Rapid testing
Hakre *et al.*, 2013	Questionnaire Screening (90 days): HIV, HCV, HBV, Syphilis, HTLV, West Nile virus (sample back to the US). Complete HBV vaccination.	Rapid tests: HIV, HCV, HBV
Malsby *et al.*, 2005	Not detailed	Not reported
Liu *et al.*, 2014	Not reported	Not reported
Gaddy *et al.*, 2021	Not reported	Not reported
Song *et al.*, 2021	Not reported	Not reported

HBV: hepatitis B virus; HCV: hepatitis C virus; CPG: clinical practice guidelines; HIV: human immunodeficiency virus; HTLV: human T-lymphotropic virus; JTS: Joint Trauma system; TTD: transfusion transmitted diseases.

Table V - *Summary of the patients' follow-up parameters*

Authors	Patient follow-up/Measured indicators
Lewis *et al.*, 2020	TACO - Surgery - Recovery
Miller *et al.*, 2018	HR - Blood Pressure - pH - Lactate - Hb - PLT count
Bassett *et al.*, 2016	pH - BE - Hb. 30 days follow-up: survival + transfusion reaction/blood borne pathogens transfer - OR time - time to transfer
Strandenes *et al.*, 2015	Not reported
Garcia Hejl *et al.*, 2015	Sample for immunoassays infectious agents: HTLV, HIV, HBV, Syphilis + Nucleic Acid Testing: HIV, HCV, HBV
Hrezo and Clark, 2003	Blood count - PT/PTT - Hb - HR - BP - sO_2. Sample for future serologic testing. 48 h follow-up - Surgery
Gaspary *et al.*, 2020	Sample back for pre-screening to add donor to register
Hakre *et al.*, 2013	Transfusion associated adverse events. TTD's: HTLV - WBC - Temperature
Malsby *et al.*, 2005	Pulse - BP - Surgery. Follow-up 4 weeks
Liu *et al.*, 2014	Temperature - HR - Respiratory rate - BP - Hb - PT- INR - PTT - PLT count - Calcium level - Surgery - Acute lung injury - Respiratory distresses
Gaddy *et al.*, 2021	sO_2 - BP - HR - Respiration - Pulse - Glasgow score - Surgery
Song *et al.*, 2021	Survival - Surgery

TACO: transfusion-associated circulatory overload; HR: heart rate; Hb: hemoglobin; PLT: platelets; BE: base excess; OR: operating room; HTLV: human T lymphotropic virus; HIV: human immunodeficiency virus; HBV: hepatitis B virus; PT/PTT: prothrombin time/partial thromboplastin time; BP: blood pressure; sO_2: oxygen saturation; TTD: transfusion transmitted disease; WBC: white blood cells; INR: international normalized ratio.

requirements. Regarding the risk associated with the product, a distinction was made between the studies using only O WB and using type-specific blood or both depending on the situations. The tables also listed if the authors did consider the titer of hemolysins (low or not) in the product. All medical and related laboratory parameters helping to assess the patient's status were reported in the tables. Finally, the data concerning the patient's follow-up after transfusion were also included when available.

Assessment of the quality of evidence

The quality of evidence was assessed using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach to rate the reliability of evidence from each included study. This was assessed by the lead author and independently verified by two others.

RESULTS

Search results

The literature search identified 352 records, of which 154 were assessed for eligibility after removing 198 duplicates. Based on title, abstract and article type, 115 studies were also excluded according to the inclusion and exclusion criteria defined in **Table I**. There was also one study exclusion on language grounds. The lead investigator identified 39 relevant studies through a review of abstracts against the exclusion criteria. Twelve papers agreed by all authors were included in the review[12-23]. A summary of the results of the literature search is shown in **Figure 1**.

Quality of evidence

Nine of the 12 included articles were case reports and series[12,13,16-22]. Therefore, they were all graded "very low" according to the GRADE system. There were also three prospective observational studies[14,15,23]. They were all graded as "low" quality according to the GRADE system. Clustering by repeat authors did not appear to be an area of potential bias. These low-quality gradings were mainly due to the observational design of all studies, putting them at risk of bias, imprecision, inconsistency and indirectness. There was no disagreement between the reviewers with regard to the risk of bias and the GRADE rating.

Analysing results

Activation indicators of a WBB

Based on the situations considered (see **Table II**), the literature review identified four studies that only referred to a remote environment to support the use of a WBB[17,21-23]. Another reported having the WBB protocol ready to provide blood during an event combining remote situations, mass casualty and massive transfusion[18]. The remaining studies supported the activation of the WBB, either for massive transfusions[16-20], or for a combination of mass casualties and massive transfusions[12-15], or for a combination of massive transfusions and remote situations[19].

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Accordingly, apart from the study by Strandenes and colleagues[23], all studies justified the use of a WBB as a response to shortages of blood products, and/or delays in evacuation (see **Table II**)[12,13,22,14-21]. Shortages were either contextual or caused by the depletion of available supplies due to acute point-in-time demand[12,17-19,22]. Some of the authors also pointed out the shortage of a specific blood component: blood platelets[13,14,20]. As platelets were often scarce on the battlefield, they could only be obtained from WB. Whole blood has made the difference in the stabilisation and recovery of coagulopathic patients with certain types of injuries resulting in bleeding casualties[13,14,20].

All 9 retrospective studies described hemorrhagic patients with either uncontrollable bleeding or coagulopathy due to various traumatic injuries as the cause of injury leading to activation of a WBB (see **Table II**)[12,13,16-22]. Among the remaining three prospective studies, two studies evaluated the feasibility of setting up a WBB and the supply potential generated by implementing the protocol[14,15], while the third one described the protocol they used to collect and bank WB from a pool of identified donors to anticipate potential needs on board[23]. It was also the only study to specify the use of cold-stored WB as a means of accessing and maintaining a "blood bank" without having home blood[23].

Risk mitigation measures of a WBB

Linked to the donor

Two measures are reported to be used to limit donor-related risks, namely blood typing (**Table III**) and donor screening (**Table IV**). Both can be performed in early pre-deployment planning and/or on-site at the time of collection.

Across studies, blood typing prior to deployment and its confirmation at the time of collection were often combined with the aim of establishing a registry of potential donors and their blood groups that could be confirmed at the time of collection[13,16,17,21,23]. Three studies focusing on patient or donor screening failed to report pre-deployment or on-site blood group typing[18,20,22]. The study by Song and colleagues reported on the use of a donor registry, but did not provide any details on the potential risk reduction measures that were taken either prior to deployment or at the time of collection[22]. Nevertheless, it seemed to be a relatively important measure as most authors reported it, even though the protocols were quite different, and the lack of reporting did not mean that it was not done. The use of WB from only O donor, rather than type-specific or compatible blood, was reported in only 3 studies[15,19,22]. Furthermore, two studies did not even address this issue and did not specify the product used[16,20].

For donor screening risk mitigation measures (i.e., tests or questionnaires), all details provided by authors are shown in **Table IV**. Eight studies reported pre-deployment screening as part of the donor registry planning in the preparedness phase[12,13,15-19,23] and seven at the time of collection[13-18,23]. Six studies performed pre-deployment and on-site screening[13,15-18,23]. Two studies did not report on-site testing but did report pre-deployment testing[12,13,16-19], and one reported on-site testing but did not report pre-deployment testing[14]. Despite this, only two studies reported no screening at least once during the process[20,21]. In their study, Song and colleagues did not report any screening before or at the time of collection, but specified that the protocol was to "call" donors from a registry[22].

Linked to the patients

It was not possible to identify only one or even a few important parameters for patient follow-up, as all authors used different parameters (see **Table V**), except for the prospective study by Strandenes et al, which used no parameters for follow-up[23]. From a transfusion perspective, the parameters reported in these studies can be divided into two main types:

1. the medical parameters, where the most commonly reported were blood pressure, heart rate, survival rate, surgery, transfusion reactions and laboratory parameters reflecting the status of the patient (e.g.: hemoglobin or pH, lactate)[12,13,16,17,19-22] and
2. adverse events related to TTDs or screening on sample return to the home country[14-18].

Patient follow-up for potential TTDs was reported in five studies[14-18]. Hakre and colleagues focused their analysis on one patient's seroconversion following an on-site walking blood transfusion[18].

DISCUSSION

This review aimed to identify activation criteria for military WBB as well as the risk mitigation measures associated with their use.

Our first research question investigated the rationale for

its application. Two main trends have been identified in the literature to justify the use of WBB protocols:

1. access to blood products in case of shortage (i.e., logistical indication of activation)[12,15-23] and
2. access to blood products for the treatment of a hemorrhagic patient when a required specific component is not available (i.e., clinical indication of activation)[13,14,20].

All but two of the studies[21,23] reported on the use of fresh WB to overcome the shortage of blood products[12-23]. Gaddy *et al.* reported collecting blood for a casualty during a combat assault and withdrawing it at the site of injury. There was no shortage of blood, but blood was not immediately available on site[21]. Strandenes and colleagues, however, chose a different strategy, collecting blood to build up an emergency bank[23]. These two different strategies are equally acceptable and can be chosen according to the initial situation: collecting to meet a specific need based on a shortage or creating a bank based on an absence. Yet, both strategies are named differently: one is called a "Walking Blood Bank" while the other is called an "Emergency Donor Panel" (EDP). The NATO Blood Panel recently discussed this difference[24]. It was decided that the WBB refers to WB collected for banking. In contrast, the emergency donor panel refers to a pool of pre-screened donors who are ready to give blood for immediate use without banking[24]. One may notice that this distinction is not yet clear in the literature. Therefore, to ensure that all studies were included, we decided to extend our search to the most used terms in the literature. Furthermore, all authors reported using this protocol to avoid overwhelming their designated transfusion system for highly demanding patients presenting with uncontrolled bleeding leading to massive transfusion or hemorrhagic shock. As previously reported in the literature, WB is an essential resource for DCR, e.g., at sea, it offers operational flexibility as the use of component therapy, the ratio "1:1 RBC": FFP" is not always and everywhere sustainable[33]. Our analysis led us to the same conclusion. The use of FWB collected on site could become, in exceptional situations, the only solution to access blood and save lives. While this review focuses only on the military setting, it was also used in isolated and large geographical areas presenting blood supply challenges comparable to military theatres (e.g., [25-28]). The Norwegian Preparedness Plan is the more developed and published model for using WBB/EDP in the civil world when geography or supply is difficult to secure[29].

Finally, some authors reported choosing to use FWB in order to obtain a clinical advantage[20], as FWB offers a better survival rate in hemorrhagic shock[29]. However, it is still a highly controversial topic as the purported benefits of FWB are still not clearly evidence based[30,31].

Concerning our second research question, while the awareness of risk is common to all articles, the protocols differ in their implementation regarding the use of risk mitigation measures, both in terms of the type of test and the timing of its implementation. Our review showed that risks related to both donors and products need to be considered. It is well established in the literature that FWBs should come from pre-screened donors to reduce the higher risk of TTD[29]. However, in our review, even if both TTD screening and blood typing are considered to reduce the risk, the techniques used, and the timing of the interventions varied widely and did not allow standardization of practice. There are two main explanations for this. The first one would be the national regulation, which is quite specific to each country. Therefore, because all requirements and protocols are different (Germany, USA, UK, Canada)[32], interoperability in the use of WBB cannot be adopted by all NATO members. As it also depends on the prevalence in the home country, there are no standards for TTD screening[29]. The second one relates to the bias inherent to the design. Most of the reviewed studies were case studies. This implies that the data used are those that are available a posteriori and some of the data may be missing without necessarily indicating that the procedure such as testing was not carried out. Furthermore, it is also possible that some information is missing because the authors choose to omit reporting some data and not because the full test was not carried out. Not reporting did not mean that it did not happen. Regarding the product used, it would be more convenient in terms of the risk of transfusion reactions to use only O donors. However, our results do not reflect this. Most authors reported using ABO type specific WB, but unfortunately did not rationalise their choice[12-14,17,18,21,23]. Indeed, O donors represent approximately 45% of the Caucasian population, whereas A donors represent

approximately 45% of the same population. By limiting the sample to O donors, an important part of the donor pool is excluded. This may be important for obtaining sufficient resources. Nonetheless, this presupposes that the typing has been determined pre-deployment or at the time of donation. In addition, some authors report also considering the hemolysin titer in O WB[12,15,21-23]. However, there is no consensus on titer determination, either from a technical point of view or from a cut-off point of view. Therefore, not every nation would consider a donor as a low titre donor using the same levels. This is part of the limitation of the use of low titers in an international setting[33]. This would lead to complications in communication, monitoring and interoperability decisions. Finally, patient outcomes were also considered in the studies reviewed, but there was no evidence of a consensus on these and their reporting was inconsistent. Nevertheless, all efforts should be made to assess patients' stability according to the resources available.

CONCLUSIONS

A blood collection protocol, whether a WBB or an emergency donor panel, must be part of the transfusion support concept because it provides access to resources that are otherwise inaccessible. Obviously, this will only be implemented in exceptional situations due to the associated risk. Most stakeholders are aware of these risks, which, if mitigated, are outweighed by benefits. Therefore, measures are taken to prevent, monitor and minimize the risks entailed by such protocol. To ensure a comprehensive selection of donors for the registry, it is essential to include this comprehensive protocol in the medical support planning process of operation. The key to success are donors, their education and regular follow-up. Based on this review, there is a clear need for such a protocol in the military operational setting, but it can also be applied in the civilian world, particularly in remote locations. However, it must be part of the country's preparedness plan to ensure the best possible care for patients.

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AUTHORS' CONTRIBUTIONS

JD proposed the research question, performed the literature search, the data extraction and analysis, assessed the quality and drafted the manuscript. ED checked the accuracy of the literature search, participation to the analysis, independently verified the quality assessment, contributed to the writing of the manuscript. FT checked the accuracy of the literature search, participation to the analysis, independently verified the quality assessment, resolved discrepancies between JD and ED during data extraction and revised the manuscript. VD is responsible for the supervision and revised the manuscript. All Authors contributed to the final manuscript and approved its final version. JD is guarantor.

The Authors declare no conflicts of interest.

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SUPPLEMENT ARTICLE

TRANSFUSION

Minimal tactical impact and maximal donor safety after a buddy transfusion: A study on elite soldier performances in both laboratory and field environments

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Abstract

Background: The major causes of death of combat casualties in austere environments are related to hemorrhage and occur early after injury. The implementation of a walking blood bank may overcome the logistical issues raised using blood component therapy. Nonetheless, it is important to ensure that this buddy transfusion is not going to compromise the mission success by altering the donor's performance. The results available so far cannot rule out this issue with certainty. Therefore, this study aimed at investigating the immediate effect of a 450-ml blood donation on the performances of elite soldiers in laboratory and field environments.

Study design and methods: This double-blind, randomized controlled study included two experiments. For both experiments, subjects were randomly assigned either to a control group ($n_1 = n_2 = 7$) or to a 450-ml-blood-bag donation group ($n_1 = 7$ and $n_2 = 8$). All participants underwent before and after a potential blood donation a multifactorial assessment including adapted physical tasks, hematological variables, vigilance parameters, and subjective assessments.

Results: No significant results were evidenced in this study. There was no impact of blood donation on the participants' performances in both the hospital and the combat-like environments.

Conclusion: From a donor's point of view, a 450-ml blood donation has no impact on the required abilities of our elite soldiers to fulfill a demanding tactical mission. Thus, the results of this study support the fact that buddy transfusions could be part of the operational clinical armamentarium in austere environments for elite soldiers when no blood components are available.

KEYWORDS
buddy transfusion, donor safety, performance, walking blood bank, whole blood

Abbreviations: ANOVA, Analysis Of Variance; d', Cohen's d; η^2, Partial eta-squared; PVT, Psychomotor Vigilance Task; RT, Reaction Time; SF, Special Forces; SFGP, Special Forces Group; SPSS, Statistical Package for the Social Sciences; UZ-Brussel, Universiteit Brussel; VAS, Visual Analog Scale; VO_2Max, Maximal oxygen uptake/consumption; VUB, Vrije Universiteit Brussel; WB, Whole Blood; WBB, Walking Blood Bank.

1 | INTRODUCTION

Hemorrhage is the leading "preventable" cause of death from combat injuries.[1,2] Survival rates in the hemorrhaging patient depend on rapid and adequate management of the patients[3] as well as early initiation of balanced resuscitation.[4] Unfortunately, most of them die before reaching a Military Treatment Facility.[5] Therefore, the immediate availability of blood in prehospital conditions can save life and improve prognosis. However, blood supply and storage in austere environments remains an important logistical challenge. To compensate the unavailability of blood components in exceptional operational circumstances, on-site collection of whole blood (WB) from a "buddy" deployed on the same site[6] is successfully used in operational settings.[7] This method is known as a walking blood bank (WBB).

Because of the well-known biological advantages of WB and the logistical issues raised using components, most of the North Atlantic Treaty Organization countries are developing their own WBB procedures in accordance with their national requirements. This is also the case in Belgium; we are currently developing WBB guidelines to aid our medical staff deciding when to trigger a buddy transfusion while having minimal tactical impact and ensuring maximal donor safety. Therefore, we study the Belgian Special Forces (SF) operators, our most exposed and at-risk population, to evaluate the effect of a blood donation on their vigilance and physical performances. This study aims at guaranteeing that our SF operators will still be able to fulfill their mission and return to a safe place.

To fulfill this objective, we focus on the immediate effect of a 450-ml blood donation (i.e., a standard blood donation) on physical performance, vigilance, hematological parameters, and psychological aspects (e.g., stress, fatigue, well-being) of SF operators. Over the past few decades, a few studies have arisen in the literature investigating some of these parameters among a military population.[8–10] With regard to vigilance, no effects of the blood donation were reported.[11] Yet, several studies reported a detrimental effect of standard blood donation on physical performance in a laboratory environment.[9,11–13] These studies reported that standard blood donation reduced hemoglobin level, maximal oxygen uptake (VO_2max), and maximal exercise capacity in a laboratory environment.[9,11,12] Unfortunately, only a few studies investigated the effect of a standard blood donation on performance in field scenarios.[8,9] These studies showed that the combat abilities of the participants are preserved immediately after a 450-ml blood donation.[10] However, a recent meta-analysis highlighted important limitations in these studies, precluding the possibility of establishing a clear effect of blood donation on performance.[12] Indeed, all these studies used different populations, procedures, designs, or/and variables.[12] Thus, the results were not readily transposable to the operational world for such a specific population. Indeed, the increased circulating blood volume of our highly trained SF operators[14] could decrease the visible impact of the donation on performance.[9]

We aim to evaluate precisely the potential immediate impact of the donation on the SF operators' performances in two different setups. First, we conduct a study in a laboratory and secure environment in order to verify the results of the literature. Then, we study our target population in an ecologically valid environment (i.e., a field exercise in a desert environment) to ensure the transferability of our results to real-life operational settings. In each setting, we evaluate in a double-blind randomized controlled experiment, the effect of a single 450-ml blood donation through a multifactorial assessment including hematological parameters, vigilance, and physical performance measures. Moreover, even if psychological parameters such as the expected effect of the blood donation were ignored in previous research studies, we consider these factors in both setups as they could significantly impact the participant's performance[15] through placebo effects.

2 | MATERIALS AND METHODS

This double-blind, randomized controlled study consisted of two distinct experiments, first in a laboratory setting and then in the field. The study was approved by the Medical Ethics Committee of the University Hospital (UZ-Brussel) and the Vrije Universiteit Brussel (VUB) (B.U.N.: 143201835663). All participants provided written informed consent. The article was written and edited according to the Consolidated Standards of Reporting Trials statement. The study was made in accordance with the guidelines for good clinical practice and the Declaration of Helsinki. No changes to trial design and methods were made following trial commencement.

2.1 | Study design

There were 14 Belgian male SF operators included in the laboratory experiment and 15 in the field experiment. Participants received an oral and written explanation concerning the study and, if they expressed a wish to participate in the study, they signed an informed consent. Inclusion and exclusion criteria as well as criteria for discontinuation of the study are provided in Table 1.

TABLE 1 Inclusion, exclusion, and discontinuation criteria

Inclusion criteria	Physically active operator of the Belgian Special Forces who volunteer to participate in the study. Military operational (i.e., Med Ops cat A) according to their yearly medical examination at the military hospital. Medically fit to donate blood (based on their medical questionnaire and measurement of vital signs). Right after a strenuous training period for both experiments.
Exclusion criteria	Does not meet blood donation criteria (according to Belgian law for blood donation). Donated blood within the last 3 months. Takes antihypertensive therapy. Suffers any physical injuries before the start of the study.
Discontinuation criteria	Any medical condition or physiological reaction that arises during testing deemed unfit by the supervising medical doctor. Decision of the participant to interrupt his participation.

FIGURE 1 During the blood donation, the participant was lying down with his legs raised and his arms alongside the body. He wore blacked-out goggles and noise-canceling headphones while listening to his favorite playlist for approximatively 10 min. This specific setup ensured that he remained blind about his group attribution. The most suitable arm was chosen to setup the sterile single collection bag and start the blood donation. Control group participants donated a small blood sample (5–10 ml) while donation group participants gave a 450-ml blood bag. The collection bag was placed on a scale to guarantee that the same amount of blood was collected from each participant (approximately 475 g)

For both experiments, subjects were randomly assigned either to a control group or to a donation group under the supervision of the study coordinator. To avoid compensatory mechanisms and the potential effect of blood loss awareness, participants wore blacked-out goggles and earmuffs during the blood donation (see Figure 1). Moreover, the field researchers who collected the data were not aware of the group distribution, as the donation procedure was conducted by a different team. All the materials and procedures were identical among both groups to guarantee a double-blind design. Moreover, all the instruments were calibrated, controlled, and used according to the manufacturers' requirements.

The laboratory experiment took place in the military hospital Queen Astrid, Neder-Over-Heembeek, Belgium. The field experiments took place during an exercise abroad under similar conditions to those experienced in operations (e.g., gear, climate). All participants underwent a multifactorial assessment including measures of hematological parameters, vigilance, and physical performances as well as subjective assessments. Only physical performance measurements differed between both experimentations. During the laboratory experiment, the potential effect of blood donation on performance was determined by comparing the results of the baseline with the results of the postdonation measures (see Figure 2). During the field experiment, the population performed one strenuous ecologically valid physical task (i.e., the warrior competition) after the blood donation (see Figure 3).

2.2 | Outcomes

As the main study objective was to assess the impact of a 450-ml blood donation on tactical capacity, and thus

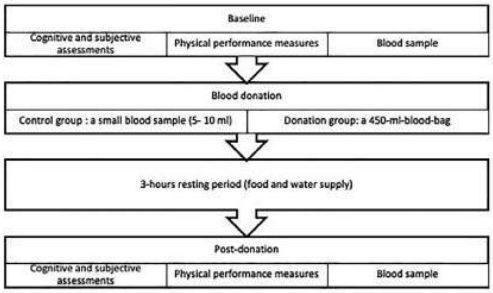

FIGURE 2 Summary of the testing sequence followed by each participant of the laboratory experiment in the Military Hospital. At their arrival, participant received detailed information about the experimental setup and the planning of the day, and they filled in the first subjective questionnaire. Then, they were interviewed by the doctor responsible for the blood collection to attest that they were fit to be a blood donor. Once the medical interview was finished, blood samples were collected. Then, participants performed the baseline psychomotor vigilance task (PVT) as well as the first incremental graded exercise tests until exhaustion. At the end of the first exercise test and before the blood donation, participants filled in a second subjective questionnaire and performed a second PVT. For the blood donation, control group participants donated a small blood sample (5–10 ml) while donation group participants gave a 450-ml blood bag (see Figure 1). A 3-h resting period followed the blood donation. During this period, participants must drink at least 500 ml water and performed a standardized intellectual assessment with a trained psychologist in the framework of a unit's internal project, completely independent from our study. After the resting period, they performed their second exercise test followed by the last PVT. They finished the assessment by filling in a third subjective questionnaire and a last blood sample was drawn

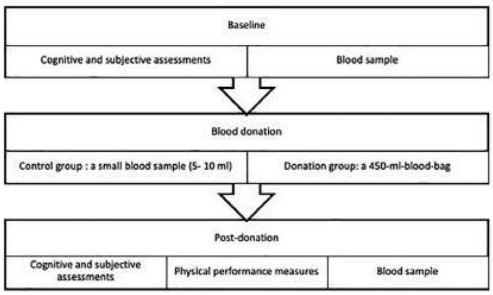

FIGURE 3 Summary of the testing sequence followed by each participant of the battlefield experiment. Upon their arrival, participant received detailed briefing about the study and explanation about the tasks of the day. Then, they were interviewed by the doctor responsible for the blood collection to attest that they were fit to be a blood donor. Once the medical interview was finished, blood samples were collected, and participants filled in their first subjective questionnaire. Before starting the blood donation, participants performed the baseline psychomotor vigilance task (PVT). For the blood donation, control group participants donated a small blood sample (5–10 ml) while donation group participants gave a 450-ml blood bag in a double-blind setup (see Figure 1). Then, participants started directly after blood donation the strenuous military circuit (i.e., the warrior competition), which was followed by the second PVT as well as a subjective questionnaire. Finally, a last blood sample was drawn

vigilance and physical performance right after a strenuous training period, these criteria were defined as the primary outcomes while subjective assessments and hematological variables were considered secondary outcomes.

2.2.1 | Blood parameters

The 5-ml venocapured blood samples were collected at the start and at the end of the testing day in both experiments. Unfortunately, due to logistical problem, these samples were not analyzed for the laboratory experiment. For the field experiment, blood parameters were immediately measured on an i-STAT handle analyzer with the chemistry (Chem8+) and the blood gas (CG4+) cartridges (ABBOTT, Chicago, IL).

Blood donation

Blood donation procedures were the same in both experiments. Upon arrival, the doctor responsible for the blood collection evaluated all the participants. He performed a medical interview through a standardized blood donor questionnaire to verify the participant's eligibility to be a donor as required by Belgian law. Moreover, the doctor also assessed their vital parameters (including body mass and height) to attest that they were fit to donate blood and could be included in the study. Then, participants were randomly assigned to either a control group or a donation group. Control group participants donated a small blood sample (5–10 ml) while the others gave a 450-ml blood bag. Every participant was connected to a sterile single collection bag with anticoagulant solution adequate for whole blood (i.e., citrate phosphate dextrose-adenine) (TERUMO BCT Inc.).

2.2.2 | Vigilance and subjective assessments

The 10 min-computerized Psychomotor Vigilance Test (PVT) recorded reaction times (RT) to visual stimuli that occurred at random inter-stimulus intervals.[16] Participants were instructed to monitor a screen and click as fast as possible once a millisecond counter appeared in the box and starts incrementing (from 0 to 1000 ms). Reaction speed, lapses (reaction time over 500 ms), and misses (missed stimuli) were recorded. This vigilance test was performed three times, before and after each physical task.

At the beginning of each experiment, participant rated their subjective levels of stress, mood, alertness, mental, and physical fatigue, as well as the quality of the previous night on a 10-cm visual analogue scale (VAS). After each physical evaluation, participants rated again on a VAS their subjective levels of physical and mental well-being, muscle pain, training intensity, stress, mood, alertness, mental, and physical fatigue. Scores ranged from 0 to 100, as physically measured on the VAS. A higher score indicated a greater intensity of the subjective feeling being measured.[17–19] At the end of both experiments, participants were asked about the eventual impact of the blood donation on their vigilance and physical performances, the perceived side-effects as well as if their evaluation regarding whether they were in the donation or the control group.

2.2.3 | Physical assessment

Laboratory experiment

Two incremental graded exercise tests until exhaustion[20] were performed, with 3 h of rest in between, in the Sports lab of the Military Hospital under medical supervision. One hour before each test, the participants received a standardized meal. The exercise test was performed on a treadmill associated with an exercise testing system (Ergocard Clinical, Medisoft, Belgium) according to a protocol specially designed and currently used at the exercise lab for the SF population. The protocol started at 5.4 km/h and consisted of 3-min stages at increasing running speeds, with an increase of 1.8 km/h and with a total maximum speed of 23 km/h. Treadmill inclination was kept constant at 0% for all the candidates. At the completion of each stages, blood was drawn from the right fingertip to evaluate blood lactate concentration; moreover, the maximal heart rate (HR) was determined using a HR monitor linked to the computer (Polar Sporttester, Kempele, Finland). The exercise was stopped when participants reached complete exhaustion. Gas exchange data with the candidate's oxygen intake and carbon dioxide output were measured using an automated breath-by-breath system (Ergocard Clinical, Software Medisoft, Belgium). After the test, the relative maximal oxygen consumption (VO_2max) was transcribed from the report of the device.[20]

Field experiment

Physical performance was evaluated, after the blood donation, by a strenuous military circuit (i.e., the warrior competition) while carrying approximately 27 kg of personal equipment and weaponry. Participants were involved in a competition throughout all the circuit. They had to perform as fast and as accurately as possible all the following tasks: a basic obstacle run, a 25 m-shooting range, a 100 m-shooting range, an 8-storey climb of a commando tower, a rappelling descent, and a close quarters battle house run, as well as an urban climbing parkour. In our study, we considered the score computed by the instructors based on the individual results on each task, the time to perform the obstacle run, and the score obtained for each shooting task. Moreover, the HR was determined at rest and within a

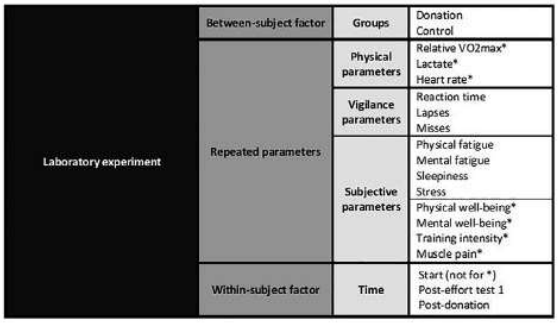

FIGURE 4 Overview of all laboratory experiment variables included within the statistical analyses and the results section

3-min interval after finishing the circuit using a fingertip pulse oximeter (Onyx II, Nonin, Minneapolis, MN).

2.3 | Statistical analysis

All statistical analyses were performed using IBMS Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 25.0 for Windows. For all statistical tests, statistical significance was accepted at the $p \leq .05$ level. Partial eta-squared (η^2) and Cohen's d (d) were used to measure the effect size.

Regarding the laboratory experiment, a 3 (Time [start, post-effort test 1 and post-donation]) × 2 (Group [control and donation]) mixed ANOVA, with Time as within-subjects factor and Group as between-subjects factor, was used to test the results of the repeated parameters (see Figure 4). To investigate the difference between the measures of the other repeated subjective parameters of the two groups (i.e., non-donor vs. donor), a 2 (Time [post-effort test 1 and post-donation]) × 2 (Group [control and donation]) mixed ANOVA with time as within-subjects factor and Group as between-subjects factor when the assumptions of normality and homogeneity were met (see Figure 4). Greenhouse–Geisser epsilon corrections were used when sphericity was violated. The assumptions of normality (Kolmogorov–Smirnov with a Lilliefors significance correction) and homogeneity (Levene's test) were tested before performing these statistical analyses.

With regard to the field experiment, a parametric independent-samples t-test (two-tailed) was used to investigate the difference between the measures of the physical parameters as well as the nonrepeated subjective parameters of the two groups (see Figure 5). Moreover, the repeated parameters were tested using a 2 (Time [pre, post]) × 2 (Group [control and donation]) mixed ANOVA (see Figure 5). Time was used as within-subjects factor and Group as between-subjects factor. Greenhouse–Geisser epsilon corrections were used when sphericity was violated. These statistical analyses were performed as the assumptions of normality and homogeneity were met. When the assumptions of normality and homogeneity were not met, scores on pre- and post-test were compared for each group separately using a nonparametric Wilcoxon signed-rank test.

3 | RESULTS

3.1 | Laboratory experiment

One participant of the donor group was injured and was not able to perform the physical and vigilance assessments; therefore, he was excluded from all the analysis. The ANOVA analysis of physical parameters during the laboratory experiment revealed only a significant decrease over time for the variable relative $V0_2$max [F (1, 12) = 7.455, p = .018, η^2 = 0.383]. No significant interaction effect between time and group was evidenced for any of the physical parameters (see Table 2).

Regarding the vigilance assessment, data recording on the PVT was problematic for two participants, they were excluded from the analysis ($n_{\text{non-donor}} = 6$ and $n_{\text{donor}} = 6$). The ANOVA analysis of vigilance during the laboratory experiment revealed only a significant main effect of time for the variable reaction time [F (2, 20) = 4.101, p = .0327, η^2 = 0.291] (see Table 2). However, no other significant effects were evidenced.

The analysis of the four subjective measures (i.e., physical fatigue, mental fatigue, sleepiness, and stress) with independent samples t-tests showed no significant differences between both groups at the start of the experiment. The ANOVA analysis of these four repeated

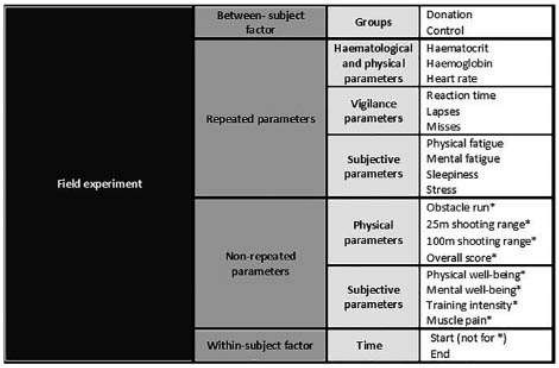

FIGURE 5 Overview of all field experiment variables included within the statistical analyses and the results section

TABLE 2 Means and SDs for the physical, vigilance, and subjective parameters during the laboratory experiment

		Start		Posteffort test 1		Postdonation	
Parameters	Measures	Control ($n = 7$)	Donation ($n = 7$)	Control ($n = 7$)	Donation ($n = 7$)	Control ($n = 7$)	Donation ($n = 7$)
Physical parameters	Relative VO_2-max (ml/kg/min)	—	—	54.38 (4.79)	53.96 (7.66)	53.30 (3.79)	49.81 (6.63)*
	Maximal heart rate (beats/min)	—	—	196.86 (3.29)	198.14 (13.21)	197.43 (4.83)	197 (11.27)
	Lactate (mmol/L)	—	—	9.52 (1.89)	9.13 (3.14)	9.41 (1.16)	7.63 (1.92)
Subjective parameters	Physical fatigue	40.31 (24.83)	55.82 (19.69)	35.74 (19.15)	52.65 (20.08)	57.74 (13.02)	49.20 (22.42)
	Mental fatigue	36.90 (24.95)	27.59 (20.45)	23.24 (21.19)	35.76 (19.84)	33.25 (25.13)	38.58 (27.50)
	Sleepiness	35.40 (10.57)	33.77 (20.94)	32.14 (26.7)	39.15 (22.89)	37.40 (24.33)	32.06 (28.40)
	Stress	13.37 (12.34)	15.63 (8.94)	12.43 (17.79)	19.61 (5.82)	11.28 (14.34)	14.02 (10.47)
	Physical well-being	—	—	65.86 (17.01)	63.93 (18.07)	67.13 (18.44)	68.91 (27.89)
	Mental well-being	—	—	84.99 (16.51)	81.52 (7.99)	85.43 (14.74)	80.02 (8.76)
	Training intensity	—	—	75.98 (10.36)	61.10 (21.29)	69.46 (12.92)	63.74 (20.08)
	Muscle Pain	—	—	29.75 (25.86)	3.99 (25.21)	27.81 (27.81)	46.18 (27.89)
Cognitive parameters	Reaction time (ms)	298.95 (18.51)	298.06 (18.01)	296.43 (21.77)	297.60 (24.65)	304.23 (23.81)	311.88 (34.07) *
	Lapses	1.50 (1.52)	0.33 (0.52)	2.17 (1.94)	0.67 (0.82)	1.33 (1.97)	1 (1.09)
	Misses	0.33 (0.52)	0 (0)	0.17 (0.41)	0 (0)	0 (0)	0 (0)

Note: Values are mean (SD).
The symbol * indicates that there was a significant difference in the statistical analysis.

subjective parameters during the laboratory experiment revealed no significant main effect of time or interaction effect between time and group. The analysis of the four subjective measures at the end of each effort test (i.e., physical well-being, mental well-being, training intensity, and muscle pain) showed no significant main effect of time or interaction effect between time and group (see Table 2). Moreover, only two out of the seven donors correctly indicated being part of the donor group (see Table 3). These two candidates were the only one to report a minor effect (average of 12,44%) of the blood donation on their physical performance.

TABLE 3 Crosstab representing the real group distributions versus the participant's expectations in laboratory experiment

		Participant expectations		
		Control	Donation	
Real group distributions	Control	4	3	7
	Donation	5	3	8
		9	6	

Note: Values are number of subjects (*n*).

3.2 | Field experiment

Four independent-samples *t*-test were conducted to evaluate the impact of the 450 ml blood donation on the physical performance during the field experiment. No significant differences were evidenced between the nondonor and the donor groups. However, the nondonors obtained a better performance than the donors in both the obstacle run and the 25-m shooting range while the donors performed better than the nondonors for both the overall score and the 100-m shooting range (see Table 4).

The ANOVA analysis of vigilance parameters during the field experiment revealed no main effect of time and no significant interaction effect between time and group.

Hematological parameters were not normally distributed; therefore, two nonparametric Wilcoxon sign-rank tests were performed. These tests indicated that post-test ranks for the donor group were significantly lower than the pretest ranks for hematocrit ($Z = -2456$, $p = .014$) and for hemoglobin ($Z = -2388$, $p = .017$) while no differences were evidenced for the nondonor group.

The analysis of the four subjective measures at the start of the experiment with independent-samples *t*-tests showed significant differences between both groups for the mental fatigue level (t (13)$= -3.286$, $p = .006$, $d' = 1.701$), the physical fatigue level (t (13)$= -2.875$, $p = .013$, $d' = 1.488$), and the stress level (t (12) $= -2.875$, $p = .014$, $d' = 1.528$). The donors reported to be significantly more stressed and mentally and physically tired than the nondonor group at the start of the experiment (see Table 5). The ANOVA analysis of the four repeated subjective parameters during the field experiment revealed only a significant main effect of time for the physical fatigue [F (1, 12) $= 8.016$, $p = .01$, $\eta^2 = 0.40$] and the sleepiness [F (1, 12) $= 7.454$, $p = .018$, $\eta^2 = 0.383$]. However, no significant interaction effect between time and group has been evidenced. The analysis of the four subjective measures at the end of the testing day showed no significant differences between both groups. Moreover, only three out of the eight donors correctly indicated to be part of the donor group (see Table 6). These three candidates were the only one to report a minor effect (average of 19.33%) of the blood donation on their physical performance.

4 | DISCUSSION

Our double-blind randomized controlled study examined the immediate impact of a 450-ml blood donation on SF donor performances in two distinct experiments: a laboratory experiment and a field experiment. In each trial, participants were randomly assigned to either a control group or a donation group. Then, they were submitted to a multifactorial assessment including hematological measures, vigilance, and physical performance measures as well as subjective assessments. This study aimed at evaluating precisely the potential immediate impact of the donation on the SF operators' performances. First, we analyzed the results of the laboratory experiment to confirm the assumptions of the literature and to guarantee the safety of the participants for a blood donation in an operational-like environment. Indeed, to the best of our knowledge, no double-blinded randomized controlled study so far has examined the effects of a blood donation after a strenuous battle-like task in this context. This is why we adapted the settings for the field experiment.

Regarding the hemoglobin and hematocrit, even if technical reasons prevented us from analyzing the blood samples in the laboratory setting, the analysis of the field data evidenced only significant immediate effect of the 450 ml donation in the donor group. Our results were consistent with the literature[13,21] even if other studies focused only on the effect 24 h after donation.[13,21]

With regard to the impact of blood donation on performance, we did not find any significant effect of the blood donation on the physical performance in both setups, which corroborates the results obtained by Nadler and colleagues.[8] Moreover, our results evidenced no significant effect of the blood donation on the vigilance level, which is consistent with the litterature.[11,22]

This absence of significant differences in performance between both groups at the end of the testing days was evidenced regardless of individual differences (e.g., level of fatigue, stress, or expected effect of blood donation). Indeed, we could have expected an impact on performances due to the significant higher fatigue and stress levels of the donors in the beginning of the field experiment. Furthermore, their expectations regarding their group distribution could have impact their physical

TABLE 4 Physical performance assessment during the field experiment

	Control ($n = 7$)	Donation ($n = 8$)	p. *t*-test	Effect size
Obstacle run (s)	158 (18.01)	175 (19.05)	0.100	0.915
Shooting range—25 m	101.43 (13.96)	96.00 (11.02)	0.415	0.436
Shooting range—100 m	74.29 (7.34)	78.50 (11.73)	0.428	0.423
Overall score	49.43 (14.89)	63.88 (16.29)	0.098	0.923

Note: Values are mean (SD).

TABLE 5 Means and SDs for the hematological, vigilance, and subjective parameters during the field experiment

		Start		End	
Parameters	**Measures**	**Control ($n = 7$)**	**Donation ($n = 8$)**	**Control ($n = 7$)**	**Donation ($n = 8$)**
Hematological and physical parameters	Hematocrit (%PCV)	41.14 (2.19)	42.63 (2.77)	42.00 (2.00)	40.50 (2.726)
	Hemoglobin (g/dl)	13.99 (0.75)	14.49 (0.92)	14.27 (0.69)	13.77 (0.93)
	Heart rate (beats/min)	68.86 (12.24)	68.63 (10.51)	146.43 (31.59)	160 (13.51)
Subjective parameters	Physical fatigue	18.57 (15.80)	46.25 (20.71)*	43.00 (21.82)	54.75 (21.87)*
	Mental fatigue	19.00 (14.34)	43.88 (14.86)*	24.57 (16.79)	39.63 (18.98)
	Sleepiness	14.71 (14.20)	31.00 (18.45)	11.86 (13.73)	16.38 (11.38)*
	Stress	11.29 (12.16)	32.29 (15.16)*	8.71 (8.54)	28.25 (26.75)
	Physical well-being	—	—	84.14 (15.04)	66.00 (17.65)
	Mental well-being	—	—	90.57 (11.46)	78.13 (9.75)
	Training intensity	—	—	66.57 (26.18)	73.75 (15.39)
	Muscle pain	—	—	19.43 (14.57)	41.25 (28.09)
Vigilance parameters	Reaction time (ms)	303.62 (13.95)	314.27 (19.59)	298.68 (20.29)	304.38 (21.49)
	Lapses	0.43 (0.53)	1.87 (1.64)	1.57 (2.07)	1.75 (1.49)
	Misses	0.14 (0.38)	0.12 (0.35)	0 (0)	0 (0)

Note: Values are mean (SD). The symbol * indicates that there was a significant difference in the statistical analysis.

TABLE 6 Crosstab representing the real group distributions versus the participants' expectations in the field experiment

		Participant expectations		
		Nondonor	**Donor**	
Real group distributions	Nondonor	5	2	7
	Donor	5	3	8
		10	5	

Note: Values are number of subjects (n).

performance (i.e., "placebo" effect) or at least the SF operators could have allowed themselves to have a diminished physical performance (i.e., motivational effect).

Despite our attempts to counteract the limitations reported in the literature, certain limitations are inherent to our target population and must be accepted. The major weakness of our study is obviously the rather small sample size, which may lead to the impression of an underpowered study. Nevertheless, even if our sample size seems to be limited, it is still representative of our target population. Indeed, our research focused on an elite military unit composed only by a really restricted number of highly trained male individuals. Therefore, by agreeing to compromise on the sample size rather than on the ecological validity of our field setting, we ensure that the guidelines are tailored to the specificities of our target population. Moreover, it also offered the actual future "client" to this exceptional procedure, the opportunity to safely experience the potential effect of a blood donation on their performances.

5 | CONCLUSIONS

A 450-ml blood donation has no significant impact on the SF operator performances even for a strenuous exercise in an ecologically valid field environment. Thus, a 450-ml blood donation has no immediate effect on their capacities to fulfill their demanding mission in tactical circumstances. Therefore, from a donor point of view, we are in favor of allowing under strict medical supervision the use of a buddy transfusion in exceptional operation life-threatening situations when no blood components are available.

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CONFLICT OF INTEREST

This study is funded by a HFM19-06 (Belgian Department of Defense) grant. Neither the coauthors nor the author have any conflicts of interest. This submission is an original manuscript that has not been previously published and is not under concurrent considerations elsewhere. No similar manuscript was ever published by any of the authors.

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ORIGINAL RESEARCH

TRANSFUSION

When do benefits turn to risks? Impact of a 900 mL whole blood donation on Special Forces performance

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Abstract

Background: Special Forces (SF) teams operate in remote environments with limited medical support. As a result, they may need to rely on buddy transfusions to treat bleeding teammates. Considering that 450 mL has no direct impact on their combat performances, it might be tempting to take more blood from a compatible donor to save a hemorrhaging teammate. This study investigates the effect of a 900 mL blood donation on SF operator performance and recovery time following this donation.

Study Design and Methods: Participants underwent a multifactorial assessment including measures of physiological parameters, vigilance, and physical performance. Results from the day of blood donation were compared with baseline values obtained 1 week earlier (i.e., immediate effect), as well as repeated testing at 7, 14, and approximately 30 days after blood donation (i.e., recovery period).

Results: Hemoglobin levels and heart rate were affected by giving blood. The participants also experienced a significant decrease in physical performance of more than 50% immediately after blood donation. Recovery was slow over the following weeks, remaining significantly different from baseline until full recovery around day 30. However, participants were still able to respond to a simple stimulus and adjust their response, if necessary, even immediately after donating blood.

Discussion: A 900 mL blood donation greatly affects the physical fitness of SF operators. A donation may be worthwhile if it is the only life-saving procedure available and does not endanger the donor's life. The donor would then become a patient and unable to complete the mission.

KEYWORDS
buddy transfusion, donor safety, elite soldier, performance, walking blood bank

1 | INTRODUCTION

Special Forces (SF) operate with limited logistical and personal footprints in remote and dangerous areas. In such austere conditions, they might be required to take care of a teammate with a life-threatening hemorrhage while awaiting evacuation.[1–4] Compensating for large blood loss may require considering a buddy transfusion.[5,6] The immediate collection of whole blood from a compatible donor may be the only way to obtain blood, as there are no components available in such circumstances.[7] To ensure the safety of our SF operators who

need to donate blood to optimize patient survival, we must identify and quantify the potential impact of whole blood donation.

Previous studies showed no significant effect of a 450 mL blood transfusion either on the vigilance of the operators or on the physical performances.[8,9] This means that a blood donation of 450 mL has no impact on the ability of the donor to carry out the mission and should not have any tactical consequences.[8,10] Therefore, in an emergency situation where there are few compatible donors available, it may be tempting to increase the amount of blood collected from a matched donor, especially considering the above findings.[8–10]

The maximum volume of whole blood that can be collected is strictly limited so as not to exceed the proportion of the circulating volume that might affect a regular donor.[11] Consequently, research on the effects of large blood donations is relatively sparse and dated. Reports of performance studies after donation of more than 450 mL are very limited.[12,13] One of these studies examined physical performance immediately after donating 800 mL and showed an immediate significant 13% reduction in hemoglobin (Hb) and 30% reduction in time run at maximal capacity.[12] The authors also looked at the long-term effects of such a large blood donation and found a progressive recovery that began 4 days after donation and continued for 4 weeks, with no return to baseline values. However, Hb level returned to the baseline level after 14 days.[12] Another study found that a blood loss of 1 liter leads to a decrease in Hb level, which affects maximal oxygen uptake and therefore working time.[13]

This raises the question of how the SF operator feels after this 900 mL exceptional but potentially strategic collection. According to Convertino and colleagues, total blood volume increases by 100 mL per 10 kg of body weight in athletes.[14] Since the SF operators are comparable to the elite athletes, it is expected that they will also have a higher circulating blood volume.[15] In this specific population, this means that a double donation would remain below the legal percentage threshold.[11] However, the tactical implications of such a medical decision must be assessed in terms of performance and duration of effect. This assessment could determine whether the donor should be considered as an additional patient or whether, from a tactical point of view, he would still be able to carry out the mission. According to the literature, physical performance is likely to be affected immediately and significantly.[12,13] In addition, the return to normal self may take several weeks and is likely to depend on the hemoglobin level.[12,13]

Our study aims to investigate: (1) the immediate effect of 900 mL blood donation on physical performance, vigilance, and executive functions, and (2) the recovery time of potentially altered capacities.

2 | MATERIALS AND METHODS

This prospective observational study was approved by the Medical Ethics Committee of the University Hospital (UZ-Brussel) and the Vrije Universiteit Brussel (VUB) (B.U.N.: 143201941913). The manuscript was written and edited according to the Consolidated Standards of Reporting Trials statement. The study was conducted in accordance with the guidelines for good clinical practice and the Declaration of Helsinki. There were no changes to the study design or methods since the start of the study.

The study was explained to the Belgian SF operators both verbally and in writing, and the participants signed an informed consent form if they agreed to take part. Inclusion, exclusion, and withdrawal criteria are shown in Table 1. To ensure donor safety, operational restrictions were added to the usual criteria of the Belgian Blood Transfusion Service, in particular no diving or skydiving for 15 days after blood donation. Seven Belgian male SF operators, who fulfilled all the inclusion criteria, were included in the experiment.

2.1 | Study design

Fourteen days before donating blood, participants came to the Military Hospital Queen Astrid (MHQA) to perform an incremental graded exercise test to exhaustion (i.e., VO_2max test) and to become acquainted with the testing protocol (i.e., familiarization). The participant completed a baseline test 7 days after the familiarization. The study design is shown in Figure 1.

On day 0, before the start of the test, participants completed a medical questionnaire and were interviewed by a doctor who assessed whether the participant was medically fit to be a blood donor. The blood donation occured only on D0. The first blood unit was collected using a sterile single collection bag with a 16-gauge needle and filled with CPDA (Citrate-phosphate-dextrose-adenine) (TERUMO BCT). After a 30-min resting period, a second bag was filled. The blood donation was followed by another 30-min resting time before beginning the testing sequence. The collected blood was not stored and was destroyed immediately after the donation. After the blood donation, a physician assessed their well-being and cleared them for testing. The whole procedure was supervised by an anesthesiologist. To reduce as much as possible potential dizziness due to dehydration, the participant had to drink at least 900 mL of water and eat a standardized salted snack during the whole blood donation. The participant received a hot meal for lunch from the hospital canteen and had to eat it at the end of the testing phase before leaving.

TABLE 1 Inclusion, exclusion, and withdrawal criteria of the study.

Inclusion criteria	• Being a physically active operator of the Belgian Special Forces. • Being military operational (i.e., Medical Operational category A) according to the yearly medical examination at the military hospital. • Meeting the Belgian Blood Transfusion Service criteria. • Being medically fit to donate blood (based on medical questionnaire and vital sign measurements). • Sufficient circulatory volume calculated using Nadler's formula $[(0,3669 * (Height\ in\ m)3) + (0,03219 * (Weight\ in\ Kg)) + 0,6041]$ which should ensure that collected volume remains below 20% of circulatory volume.
Exclusion criteria	• Failed to meet blood donation criteria. • Have donated blood in the last 3 months. • Antihypertensive therapy intake. • Any physical injury before starting to study. • Heart disease history. • Diabetic. • Hemoglobin level lower than 14 g/dL.
Withdrawal criteria	• Any medical condition or physiological reaction that arises during testing deemed unfit by the supervising physician. • Decision of the participant to interrupt his participation. • Operational constraints.

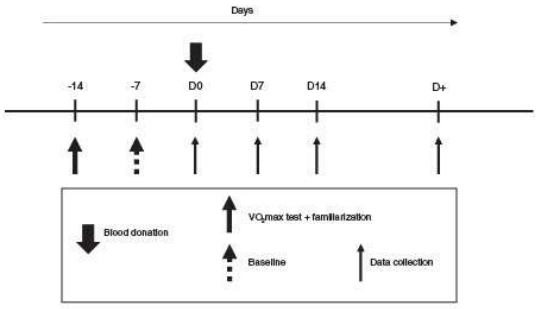

FIGURE 1 Study design and testing plan. VO_2max testing was performed 14 days before blood donation, followed by baseline testing 7 days later. On day 0, the test sequence performed at baseline was repeated after donation to measure the immediate effect of the 900 mL blood donation. It was repeated on days 7, 14, and + to assess potential recovery over time. D+ is an average of 30 days after donation, depending on the availability of the donor.

The testing phase (Figure 2) was repeated 7, 14, and approximatively 30 days (28 or 35, depending on the availability of the participants) after blood donation. All participants underwent this multifactorial assessment including measures of physiological parameters, vigilance, and physical performances as well as subjective assessments.

2.2 | Outcomes

As the main study objective was to assess the impact of a 900 mL blood donation on tactical capacity, the study design included physiological parameters, vigilance, and physical performance measurements.

2.2.1 | Physiological parameters

Participants had vital signs measurements (e.g., heart rate) at the beginning and end of each day of the trial. On day 0, the parameters were also measured before each test to ensure participants remained healthy. The anesthetist released the participant from test to test based on these parameters.

Blood samples (EDTA tube 3 mL) were taken at the beginning and end of the testing sequence on days 0, 7, 14, and + (The D+ refers to the testing day 28 or 35, depending on the availability of the participants). A complete blood count was performed on a Sysmex XP-300 automated hematology analyzer. This study only reported hemoglobin level. On the day of donation, there

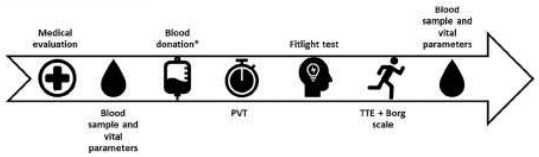

FIGURE 2 Sequence of a testing day from the baseline to D+. The test sequence is standardized and repeated in the same order each time the participant completes it. It consists of a medical interview with blood sampling, the blood donation (*only performed on D0), a vigilance test (i.e., psychomotor vigilance test), a fitlight test, a physical performance test (i.e., the maximal run measured by a time to exhaustion and the Borg Scale) and, finally, another blood sample is taken at the end of the sequence.

was a gap of 5 hours between the collection of the first sample and the collection of the end-of-test sample.

2.2.2 | Vigilance assessment

The 10 min-computerized psychomotor vigilance test (PVT) recorded reaction times (RT) to visual stimuli that occurred at random inter-stimulus intervals. Participants were instructed to monitor a screen and click as fast as possible once a millisecond counter appeared in the box and starts incrementing (from 0 to 1000 milliseconds).

2.2.3 | Fitlight test

The visuomotor task requiring the Fitlight-hardware and software (http://www.fitlighttraining.com/) developed by Van Cutsem was used.[16] This task measured simple and complex reactive time to visual stimuli. Seven lights were placed against a wall and lit in different colors for 2 s, one behind another in a random order. If a light turned red, green, or yellow (i.e., simple stimuli), participants had to put out the light as fast as possible by passing before the light with the left or right hand within a range of 5 cm. However, if a light turned blue (i.e., complex stimuli), participants were instructed not to respond to the stimulus on the wall. Instead, they had to turn around and put out anotheight lying behind them on the floor (1m50). After each stimulus, participants had to return to the indicated starting position and focus again on the fixation cross. Each color was presented 16 times, yielding a total of 64 stimuli. The inter-stimulus time varied between 3 to 6 s and each inter-stimulus time was randomly used 16 times. The total test lasts approximately 6 min 30 s. RT was measured for both simple and complex stimuli. Furthermore, we calculated the interference effect by measuring the difference between the RTs to simple and complex stimuli. This measures the ability to inhibit an automatic response (i.e., the simple task) to provide another, more appropriate response to a complex task.

2.2.4 | Physical assessment

To assess the effect of the 900 mL blood donation on physical performance, the participants performed a maximal running test to exhaustion after the blood donation.[12,17] The "running test to exhaustion" (TTE) was performed on a treadmill (Ergocard Clinical, Medisoft, Belgium) at a speed determined by the individual VO_2max results from the preliminary incremental maximal exercise test (VO_2max protocol).[18] The TTE started after a 5-min warm-up on the treadmill. Participants run until volitional exhaustion at a pace corresponding to 90% of the speed associated with their VO_2max (ranging from 13.8 to 15.4 km/h). Time to exhaustion is measured with a manual chronometer and expressed in seconds. The participants were not informed on their recorded times until completion of the whole study and no watches were viewable during the maximal run. Blood lactate concentration was measured at the beginning and at the end of the TTE. Immediately after the end of the TTE, the subjects completed a perception of exertion questionnaire, the Borg scale. This is a self-rated scale ranging from 6 to 20. 6 corresponds to no exertion and 20 to maximum perceived exertion.[19]

2.2.5 | Logbook

Participants were required to complete a daily questionnaire in addition to the test sequence. The questionnaires were designed to assess how people felt day-to-day and over time in terms of exercise tolerance, fatigue, and experienced symptoms. This allowed us to better interpret the effects of the donation and to record unmeasured but perceived information.

2.2.6 | Statistical analyses

All statistical analyses were performed using IBMS Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 28.0 for Windows. A parametric paired sample t-test was used to test the results of the repeated parameters compared with the baseline at every step of moment of the measurement. Except for the physiological parameters, which are compared to those measured when D0 started. The assumptions of normality (Kolmogorov–Smirnov with a Lilliefors Significance Correction) and homogeneity (Levene's test) were tested before performing these statistical analyses. For all statistical tests, statistical significance was accepted at the $p \leq .05$ level. Cohen's d (d') was used to measure the effect size.

3 | RESULTS

3.1 | Physiological parameters

This section analyzed two key metrics. The first was the change in hemoglobin and heart rate on the day of the donation (Table 2). This was based on samples and measurements taken at the start and end of the D0 test (5 h delayed). The second was the hemoglobin level variation on each test day to measure recovery over weeks (Table 3 and Figure 3).

The hemoglobin level of the participants was significantly lower 5 h after 900 mL blood donation than before donation. The heart rate of the donors remains significantly higher 5 h after the 900 mL blood donation than right before (Table 2).

Statistical analysis of hemoglobin levels showed that hemoglobin levels were significantly lower 7 and 14 days after blood donation, while returning to normal at 28 days (Table 3).

3.2 | Performance parameters

Exhaustion reached significantly faster after the blood donation compared to baseline. Moreover, the 900 mL blood donation still had a significantly negative effect on the physical performance of the donors 7 days and 14 days after the donation (Table 4 and Figure 3). After approximately 30 days, their performance had normalized. Regarding the vigilance and fitlight testing, donating 900 mL of blood had no significant negative effect on participants' reaction time (Table 4 and Figure 3).

3.3 | Logbook

Logbook analysis showed that five participants reported significant shortness of breath on days 1 to 4, two on day 7, and one on day 14. Orthostatic disturbances occurred in three participants on day 1 or by day 4 and 7. Finally, increased exercise intolerance was reported by two participants up to day 7 and by two more up to day 14.

4 | DISCUSSION

This observational study examined the immediate effects of a 900 mL blood donation on the performance of seven Belgian SF operators and the time required for recovery.

TABLE 2 Mean values and standard deviations of the hemoglobin level and of the heart rate at the beginning and at the end of the D0.

Measures	Pre-donation ($n = 7$)	Post-donation (+5 h) ($n = 7$)	*p*. *t*-test	Effect size
Hemoglobin (g/dL)	15.41 (1.21)	14.33* (1.14)	.005	1.653
Heart rate (bpm)	69.29 (7.91)	94.14* (8.13)	.003	−1.812

Note: *p*. *t*-test = *p*-value of the Student's *t*-test. Values are mean (standard deviation). The symbol * indicates that there was a significant difference in the statistical analysis (i.e., $p < .05$).

TABLE 3 Means and standard deviations for the hemoglobin level at D0, D7, D14, D+.

Measures	D0 ($n = 7$)	D7 ($n = 7$)	D14 ($n = 7$)	D+ ($n = 5$)
Hemoglobin (g/dL)	15.41 (1.21)	14.00* (0.75)	14.20* (1.07)	14.96 (0.74)
p. *t*-test	-	.003	.030	.511
Effect size	-	1.860	1.069	0.322

Note: D + = testing day 28 or 30 depending on the availability of the participant; *p*. *t*-test = *p*-value of the Student's t-test. Values are mean (standard deviation). The symbol * indicates that there was a significant difference in the statistical analysis (i.e., $p < .05$).

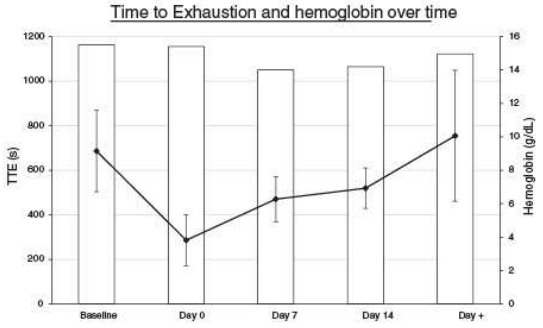

FIGURE 3 Graphical comparison between time to exhaustion (TTE) and hemoglobin changes over time. The mean values of hemoglobin are represented by the bar graph and are related to the right axis. TTE mean values are plotted on the left axis with their associated standard deviations.

TABLE 4 Means and standard deviations for the performance's parameters: time to exhaustion, psychomotor vigilance test, and fitlight test at D0, D7, D14, D+.

	Measures	B ($n = 7$)	D0 ($n = 7$)	D7 ($n = 7$)	D14 ($n = 7$)	D+ ($n = 5$)
Physical performance	TTE (s)	686.71 (182.93)	285.71* (115.00)	470.29* (101.64)	519.57* (91.19)	755.20 (294.27)
	p. *t*-test	-	< .001	.004	.007	.990
	Effect size	-	2.932	1.720	1.510	−0.006
	Borg scale Score	17.57 (0.787)	18 (1.15)	17.43 (1.40)	17.57 (1.51)	17.40 (1.52)
	p. *t*-test	-	.200	.689	1.000	0.477
	Effect size	-	−0.545	0.159	0.000	0.351
	Lactate (mM/L)	11.98 (2.27)	10.63 (2.72)	12.95 (1.78)	12.51 (0.87)	11.64 (0.41)
	p. *t*-test	-	.418	.388	.606	.378.328
	Effect size	-	0.328	−0.352	−0.206	0.443
PVT	RT (ms)	293.18 (20.24)	288.58 (24.77)	283.70 (15.41)	288.97 (23.74)	283.43* (19.98)
	p. *t*-test	-	.549	.068	.352	.030
	Effect size	-	0.240	0.841	0.381	1.477
Fitlight test	RT blue (ms)	1317.96 (82.09)	1332.41 (112.56)	1305.57 (93.79)	1350.20 (104.68)	1342.78 (146.65)
	p. *t*-test	-	.546	.649	.277	.276
	Effect size	-	−0.242	0.181	−0.452	−0.563
	RT color (ms)	1066.05 (117.77)	1044.93 (142.91)	1019.10* (106.97)	1034.15 (122.69)	1014.03 (153.61)
	p. *t*-test	-	.131	.003	.099	.224
	Effect size	-	0.661	1.825	0.739	0.643
	Interference effect (ms)	251.91 (69.03)	287.48 (85.83)	286.47 (105.16)	316.05* (103.29)	328.74* (71.19)
	p. *t*-test	-	.096	.113	.017	.008
	Effect size	-	−0.744	−0.701	−1.236	−2.223

Note: Values are mean (standard deviation). The symbol * indicates that there was a significant difference in the statistical analysis (i.e., $p < .05$).
Abbreviations: D+, testing day 28 or 35 depending on the availability of the participant; *p*. *t*-test = *p*-value of the Student's *t*-test; PVT, psychomotor vigilance test; RT, reaction time; TTE, time to exhaustion.

The results showed that a 900 mL blood donation had a significant and immediate effect on participants' physiological parameters. This was demonstrated by comparing heart rate and hemoglobin data at the beginning and end of the day. After the 900 mL blood donation, hemoglobin levels were lower and resting heart rate was higher. Several studies have already reported decreased hemoglobin levels following standard donation.[10,20–23] However, an increase in resting heart rate after donating 450 mL has only been reported twice.[20,24] In addition, the effect of such an important blood donation on the heart rate was to be expected on the basis of the ATLS classification of hypovolemic shock.[25] The fact that our study reported both effects can be explained by the large volume of blood collected. Removing an average of 15% of circulating volume means removing 15% of available red blood cells, resulting in a significant reduction in hemoglobin. As cardiovascular function is known to be strongly influenced by circulating volume, cardiac output must be increased to maintain oxygen delivery and compensate for the significant volume loss.[14,26] The volume loss was apparently not fully compensated for by the fluid intake. This volume was replenished at a later stage by the application of the Frank-Starling forces.[10,14] However, as we observed a significant decrease in the hemoglobin levels at the end of D0, it can be assumed that the volume was at least partially recovered, allowing for dilution.[10,14] The orthostatic intolerance experienced by our participants in the days following donation may be explained by these same significant variations in circulating volume.

In terms of physical performance, the immediate effect of the 900 mL blood donation was rather severe. All participants experienced at least a 50% reduction in exercise capacity as measured by the time to exhaustion test. Recovery was slow over the following weeks. It remained significantly different from baseline values until complete recovery at D+. The findings can be compared to Ekblom and colleagues in 1972.[12] They observed a loss of 30% in the maximum running time immediately after an 800 mL blood donation. They also observed a gradual recovery in the weeks following the blood donation. However, they did not observe a complete recovery after donation, whereas the hemoglobin levels were reported to be comparable to baseline values 14 days after the donation.[12] The results also showed that the lactate inflection point, measured at the end of the maximal effort, was far exceeded. In addition, the participants completed the Borg Scale after the physical task to assess perceived fatigue. There was no difference in perceived exhaustion during recovery, and we can confirm that participants stopped at the point of physical exhaustion and not because of a motivational bias. This potential motivational bias was already minimized by our experimental design.[12,17] In our setting, the relative bias was limited by the fact that the participants had to perform a maximal run of about 5 minutes at 90% of their maximal speed. The motivational aspect would have become more relevant in a submaximal exercise of longer duration and could have influenced the interpretation of the results. Moreover, the maximal exercise mimics a rapid response to outrun the enemy or launch an attack. This may be the least well-described in the literature, yet the one most likely to be influenced by donating blood, and thus have the greatest tactical impact on mission success or personal safety.[27]

In terms of vigilance, no effect of a 900 mL blood donation could be demonstrated, either immediately or in the long term. These results are in line with our previous findings after a standard double-blind blood donation.[9] Overall, reaction times improved over time. There was also no significant effect on complex responses involving executive functions. Thus, participants remained able to respond to a simple stimulus and adapt their response pattern to a more appropriate response when needed.

Although the design was robust, given the repeated measurements and the homogeneity of the sample, this study presents some limitations that must be addressed. The sample size is the most obvious limitation. This limitation is inherent to the population studied. We are dealing with a small elite unit with significant operational constraints. The number of missions, exercises, and constraints associated with maintaining their professional skills made significant and sustained participation more difficult. Nevertheless, despite the small sample size resulting from these constraints, it has been possible to present a very consistent, convergent, and overall strong set of results. We would like to emphasize that these results can certainly not be generalized to other members of the military or to the average civilian population. The population studied has already demonstrated tolerance to donating 450 mL of blood and was able to perform combat tasks in the same way.[8,9] In addition, this population has been shown to possess a higher circulating volume, comparable to highly trained athletes. Working with this specific population allowed us to ensure that the volume withdrawn remained below the 20% total blood volume limit. For this reason, our results are only intended as a recommendation to ensure the safety of the SF operators in exceptional operational circumstances. Regrettably, the study protocol did not mimic the operational environment. The study was carried out in a standardized and controlled environment, without being exposed to real life, to anticipate the consequences of a hypovolemic response. Therefore, it was impossible to

take fully into account the realities of this population, which include strenuous exercise under extreme conditions, stress, dehydration, fatigue, and the enormous weight of the material to be carried. As a result, our conclusion cannot include any specific recommendations for the "usual" battlefield environment.

5 | CONCLUSION

In conclusion, a 900 mL donation could be considered for Belgian SF operators in exceptional life-saving circumstances in remote environments with scarce resources and delayed evacuation. This would require a reassessment of the mission. The donor would become a patient and would no longer be able to carry out the mission as his physical capabilities would be severely compromised. A donation of 900 mL of blood should only be considered as a last resort in an absolute emergency and under strict medical guidelines.

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CONFLICT OF INTEREST STATEMENT

The authors have disclosed no conflicts of interest.

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