Surgery in Armed Conflicts

Predicting surgical treatment needs and improving resource use in resource-constrained settings

Måns Muhrbeck

Surgery in Armed Conflicts

Predicting surgical treatment needs and improving
resource use in resource-constrained settings

Måns Muhrbeck

Department of Surgery in Norrköping, and Center for Disaster Medicine and
Traumatology, and Department of Biomedical and Clinical Sciences, Linköping
University, 581 85 Linköping, Sweden

Norrköping 2021

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.

<https://creativecommons.org/licenses/by-nc/4.0/>

The previously published papers were reproduced under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0>)

Cover: Photo of an abandoned operation table in Kindamba, Democratic Republic of the Congo, by author

Published by Linköping University

Printed by LiU-Tryck, Linköping, Sweden 2021.

© Måns Muhrbeck, 2021

ISSN 0345-0082

ISBN 978-91-7929-042-9

Surgery in Armed Conflicts

Predicting surgical treatment needs and improving resource use in resource-constrained settings

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Måns Muhrbeck

Principal Supervisor

Associate Professor Peter Andersson
Linköping University
Department of Biomedical and Clinical Sciences
Center for Disaster Medicine and Traumatology

Co-supervisors

Professor Andreas Wladis
Linköping University
Department of Biomedical and Clinical Sciences
Center for Disaster Medicine and Traumatology

Professor Johan von Schreeb
Karolinska Institutet
Department of Global Public Health
Division of Global Health

Opponent

Professor Ari Leppäniemi
University of Helsinki, Finland
Helsinki University Hospital
Department of Surgery
Division of Emergency Surgery

Examination Board

Professor Torbjörn Ledin
Linköping University
Department of Biomedical and Clinical Sciences

Professor Per Örtengren
University of Gothenburg
Sahlgrenska Academy
Institute of Clinical Sciences

Associate Professor Lisa Strömmer
Karolinska Institutet
Department of Clinical Science,
Intervention and Technology

To Lotta, Hampus, Mårten and Disa

*It is much easier to put existing resources to better use,
than to develop resources where they do not exist.*

George Soros

ABSTRACT

Background

In armed conflicts, civilian health care struggles to cope. Understanding the relationship between demographics, injury mechanism, and injury patterns to predict what surgical resources are needed is therefore vital. In the 1990s the International Committee of the Red Cross (ICRC) implemented the Red Cross Wound Score (RCWS) for the assessment of penetrating injuries. It is unknown to what extent RCWS can be used to predict surgical resource consumption and in-hospital mortality. A substantial portion of surgical resources in conflicts are used in the treatment of infected wounds, which entails antibiotics, debridements, and change of dressings. Many health care providers advocate for the use of honey as an adjunct due to its bactericidal and hyperosmotic properties. However, the scientific evidence to support this notion is insufficient. If efficacy for honey could be demonstrated, the need for repeated debridements and antibiotics could potentially be reduced, subsequently allowing for more efficient use of surgical resources.

Objectives

To increase knowledge regarding the relationship between demographics, injury mechanism, injury patterns, treatment received (*Paper I*), treatment trends (*Paper II*), and surgical resources consumption (*Paper III*). To identify predictive methods (*Paper III*) and practices (*Paper IV*) that can improve how surgical resources are used in resource-constrained conflict settings.

Materials and Methods

In *Papers I – III* routine clinical data from patients treated at three ICRC hospitals were used. The patients had weapon-related injuries from either the conflict around the Afghanistan – Pakistan border or the conflict in the Kivu region in the Democratic Republic of the Congo (DRC). In *Paper I*, the relationships between gender, injury mechanism, injury patterns, and treatment received were examined with univariate statistics. In *Paper II*, differences in treatment practices between 1992 – 1995 and 2009 – 2012 for extremity injuries were analysed with univariate statistics and logistic regression to adjust for differences in injury mechanism and severity. In *Paper III*, univariate statistics, logistic regression, receiver operating characteristics curves, and goodness of fit were used to determine the predictive ability of RCWS and commonly used trauma scores for surgical resource consumption. In *Paper IV*, 50 standardized wounds on two pigs were infected with *S. aureus* and separately treated with either topical honey or intramuscular gentamicin to determine if topical honey is non-inferior to systemic gentamicin treatment. Treatment efficacy was evaluated with quantitative cultures, wound area measurements, histological and immunohistochemical assays, as well as assessment of inflammatory response.

Main findings

In both conflicts, most of the patients were males in their mid-twenties with injuries to the extremities. Injuries from indiscriminate weapons (such as bombs, missiles, and explosives) were more predominant in the Afghanistan – Pakistan border conflict, and gunshot injuries were more frequent in the Kivu conflict. Soft tissue procedures were the most common type of surgery. Less than 20% of the patients were subjected to major surgery, and in-hospital mortality was under 5% (*Papers I – III*). Females were more frequently injured by indiscriminate weapons, had more severely affected vital parameters, received more blood transfusions, and were subjected to more extensive surgery than the males (*Paper I*). During the last decades, the risk for amputation remained unchanged, while the use of external fixation and split skin grafts decreased (*Paper II*). RCWS predicted high surgical resource consumption better than and in-hospital mortality at least equal to commonly used trauma scores (*Paper III*). Wound size was unchanged with topical honey and decreased with intramuscular gentamicin. However, there was no difference in bacterial count between honey and gentamicin at the end of treatment (*Paper IV*).

Conclusions

Females were more frequently injured by indiscriminate weapons, had more severe injuries, and had greater surgical treatment needs than the males (*Paper I*). Even though injuries to the extremities remain common in armed conflicts, the use of limb-preserving treatment techniques seems to have decreased during the last decades (*Paper II*). RCWS demonstrated a better predictive ability for surgical resource consumption compared to commonly used trauma scores (*Paper III*). Topical honey may be non-inferior to gentamicin in reducing *S. aureus* colonization on the wound's surface, but not in reducing wound size (*Paper IV*).

LIST OF PAPERS

This thesis is based on the following original articles, referred to in text by their Roman numerals.

- I. Peter Andersson, Måns Muhrbeck, Harald Veen, Zaher Osman and Johan von Schreeb. **Hospital Workload for Weapon-Wounded Females Treated by the International Committee of the Red Cross: More Work Needed than for Males.**
World Journal of Surgery (2018) **42**(1): 93–98.
- II. Måns Muhrbeck, Kaspar Holmgren, Zaher Osman, Johan von Schreeb, Andreas Wladis and Peter Andersson. **Trends in Demographics and Surgical Treatment of Weapon-Related Limb Injuries Over Two Decades in a Resource-Scarce Setting.**
World Journal of Surgery (2019) **43**(11): 2681–2688.
- III. Måns Muhrbeck, Zaher Osman, Johan von Schreeb, Andreas Wladis and Peter Andersson. **Predicting surgical resource consumption and in-hospital mortality in resource-scarce conflict settings: a retrospective study.**
BMC Emergency Medicine (2021) **21**: 94.
- IV. Måns Muhrbeck, Maria Lampi, Andreas Wladis, Peter Andersson and Johan Junker. **Efficacy of Topical Honey Compared to Systemic Gentamicin for Treatment of Infected War Wounds: A Non-inferiority Pilot Study.**
Article in press in *Injury*.

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
APM	Anti-personnel mine
ARRIVE	Animal research: reporting of <i>in vivo</i> experiments
AUC	Area under the curve
CFU/g	Colony forming units per gram of tissue
°C	Degree Celsius
CI	Confidence interval
COD	Change of dressing
DBR	Debridement
DPC	Delayed primary closure
DRC	Democratic Republic of the Congo
FDA	U.S. Federal Drug Administration
GSD	Geometric standard deviation
GSW	Gunshot wound
ICRC	International Committee of the Red Cross
ICU	Intensive care unit
IFRC	International Federation of the Red Cross and Red Crescent Societies
IQR	Interquartile range
IM	Intramuscular
ISIL	Islamic State of Iraq and the Levant
ISS	Injury Severity Score
KTS	Kampala Trauma Score
LMIC	Low- and middle-income countries
LOS	Length of hospital stay
MCAR	Missing completely at random
MDR	Multidrug resistance
MSF	Médecines sans Frontières
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
NGO	Non-governmental organization
RCWS	Red Cross Wound Score
ROC	Receiver operating characteristics
RTS	Revised Trauma Score
SD	Standard deviation
SSG	Split skin graft
STROBE	The strengthening the reporting of observational studies in epidemiology statement
TSP	Trauma stabilization point
UCDP	Uppsala Conflict Data Program
UN	United Nations
WHO	World Health Organization

LIST OF DEFINITIONS

Abbreviated Injury Score	Classifies each injury per body region on a 6-point scale ⁴
Asymmetric warfare	Warfare between opposing forces which differ greatly in military forces ⁵
Blast injury	Direct or indirect exposure to an explosion causing physical trauma ⁶
Civilian	A person who is not a member of the armed forces ⁷
Combatant	A person who is a member of the armed forces of a party to the conflict ⁷
Constrained setting	Also, low-resource or resource-scarce setting. Poorly defined term, but frequently used in literature. Definition used in this thesis: health care system that doesn't meet minimum standards set by WHO or other relevant organizations due to constraints in available resources ^{8,9}
Delayed primary closure	Closure of skin wound at a later timepoint to prevent accumulation of microorganisms in a confined space ¹⁰
Echelons	Structured levels of care
Essential surgical care	Interventions that are critical for specific conditions in preventing premature death and disability ¹¹
<i>Ex vivo</i> study	Study in or on tissues in an artificial environment outside the organism with minimal alteration of natural conditions ¹²
First-level hospital	District hospital with 50 – 250 beds, with resources to provide the majority of the care needed for the population in the catchment area ¹³
High contracting party	Representative of a state who have signed or ratified a treaty ⁷
High-resource setting	Poorly defined term, but frequently used in literature. Definition used in this thesis: health care system with an abundant capacity to meet minimum standards set by WHO or other relevant organizations ^{8,9,14}
<i>In vitro</i> study	Study on biological material performed, in a controlled environment, outside the normal biological context ¹²
<i>In vivo</i> study	Study done with or within an entire living organism ¹²
Jus in bello	Laws regulating the conduct of warring parties ⁷
Natural disasters	Catastrophic events with atmospheric, geological, and hydrological origins (e.g., droughts, earthquakes, floods, hurricanes, landslides) that can cause fatalities, property damage and social environmental disruption ¹⁵
Stakeholders	All groups or individuals that affect or are affected by an event ¹⁶

CONTENTS

ABSTRACT	9
LIST OF PAPERS	11
LIST OF ABBREVIATIONS	13
LIST OF DEFINITIONS	15
PROLOGUE	19
INTRODUCTION	21
BACKGROUND	23
History of surgical care in armed conflicts	23
Humanitarianism and armed conflict	23
Jus in bello, the legal framework for armed conflicts	26
Surgical care for civilians in armed conflicts	30
Wounds and wound treatment	36
THEORIES AND HYPOTHESES	39
AIMS OF THE THESIS	41
MATERIAL AND METHODS	43
Overview	43
Conflicts	45
Hospitals	47
Study designs and methods (<i>Papers I – III</i>)	48
Data collection and management (<i>Papers I – III</i>)	51
Statistical analysis (<i>Papers I – III</i>)	51
Study design and method (<i>Paper IV</i>)	53
Data management (<i>Paper IV</i>)	53
Statistical analysis (<i>Paper IV</i>)	54

MAIN FINDINGS AND DISCUSSION	55
Descriptive findings (<i>Papers I – III</i>)	55
Females were more likely to be injured by indiscriminate weapons than men and had more extensive surgical treatment needs (<i>Paper I</i>)	57
In low-resource conflict settings the risk of amputation was unchanged over time (<i>Paper II</i>)	58
RCWS predicted surgical resource consumption better than KTS and RTS (<i>Paper III</i>)	59
Honey may be non-inferior to gentamicin in reducing <i>S. aureus</i> colonization, but not in reducing wound size (<i>Paper IV</i>)	60
METHODOLOGICAL CONSIDERATIONS	63
Research on routinely collected data (<i>Papers I – III</i>)	63
The porcine wound model (<i>Paper IV</i>)	65
ETHICAL CONSIDERATIONS	67
Human research subjects (<i>Papers I – III</i>)	67
Animal research subjects (<i>Paper IV</i>)	69
CONCLUSIONS	71
CLINICAL IMPLICATIONS	73
FUTURE PERSPECTIVE	75
PERSONAL REFLECTIONS	77
FUNDING	79
ACKNOWLEDGEMENTS	81
SAMMANFATTNING PÅ SVENSKA	83
REFERENCES	85
PAPERS I – IV	95

PROLOGUE

2004, Kinkala, the Democratic Republic of the Congo. It was unclear when it had happened, but they came on a market day. Four men carrying a pregnant woman who had been shot with a high-velocity gun in the left leg. The leg had a strange S-shaped form. It was clear even without the nonfunctioning X-ray machine that both the tibia and fibula were shattered. Bone fragments and pus oozed out of the wound. Luckily, the circulation and innervation to the foot seemed to be intact. I only had a limited supply of gauze, sutures and anesthetics, and completely lacked the experience to manage this type of injury. Moreover, there also was a hernia and a caesarian section that needed to get done. Several questions and thoughts entered my brain: Where do I start? Can I save the woman's leg? Or would it be safer to amputate? At least I knew how to do that. What did the humanitarian organization think when they sent me, a first-year resident in gynecology and obstetrics, to do surgery in an area of armed conflict? After seeking comfort in Maurice King's book "Primary Surgery," I irrigated the woman's leg, administered antibiotics, and put the leg in a makeshift traction device. A couple of days later, I managed to get her transferred to a military hospital in Brazzaville. However, the thoughts about my inadequacy stayed with me for a while. Am I doing more harm than good? There must be better ways to match the provided surgical resources to the given context. What injuries should I know how to treat before going on a medical mission in an armed conflict? How can I put the surgical resources I have to optimal use?

A couple of months later, the mission ended. Back into the routine of hospital work and life in Sweden, the thoughts about the optimal use of surgical resources were drowned out by the abundance of the Swedish health care system. Several years went by, and Lotta and I had three children. One day Peter Andersson called and presented a research idea that had been passed on from Johan von Schreeb. It had something to do with predicting surgical resource use in armed conflicts. The pregnant woman with the gunshot wound to her leg re-entered my mind. Maybe, this was my chance to explore the thoughts I had in Kinkala. Peter said it wouldn't be that difficult. We would do it together. I was curious but realized that it would take large chunks of time away from the family. I, therefore, waited until Lotta was in a good mood and casually slipped the project into the dinner conversation with the argument that by doing research I would actually free up time for the family. I'm pretty sure that Lotta saw right through me but nonetheless gave her approval. Some of the needs of Lotta and the children have been put on hold for me to be able to complete this Ph.D. For this, I'm eternally grateful. Embarking on this journey together with Peter Andersson, Andreas Wladis, Johan von Schreeb, and all the other inspiring people I have encountered has been a gratifying experience and, as expected, a lot of hard work and frustration. In the end, I hope the findings of my research, however insignificant they may be, will add to a better understanding of what surgical resources are needed, especially for civilians, in armed conflicts.

INTRODUCTION

The English word “War” and the French word “Guerre” share their origin with the German word “Verwirren,” meaning “to confuse” or “to bring into confusion.”^{17, 18} War has been a perpetual companion to humans throughout history. Arguably, the first war recorded was between Sumer and Elam in Mesopotamia in c. 2700 B.C.¹⁸ According to the Uppsala Conflict Data Program (UCDP), there are currently more than 40 ongoing armed conflicts globally, mainly in low- and middle-income countries (LMIC)^{2, 19} In 2013, armed conflicts caused an estimated 100,000 deaths and 800,000 injured that required hospital treatment.^{2, 20} Additionally, Jawad *et al.* estimated in a panel regression analysis that armed conflict indirectly caused almost 30 million civilian deaths between 1990 – 2017 with the largest relative increase in the 0 – 5 age group.²¹ Unsurprisingly, the added burden of armed conflicts can result in a breakdown of the overstrained health care system that already exists in many LMIC, resulting in limited or no surgical resources to treat patients with life-threatening but survivable injuries.²² Furthermore, injuries from armed conflict are often more complex, and treatment frequently requires more extensive resources than injuries seen in a non-conflict setting.²³⁻²⁵ For civilian health care personnel, especially surgeons, with no previous experience of war injuries, this represents a major challenge, adding to already existing security and logistical constraints.^{26, 27} These challenges in delivery and access to surgical care emphasize the importance of understanding what surgical resources are needed in each context. This holds particularly true for non-local organizations with the intention of setting up medical interventions in armed conflicts. Sadly, there are several examples from recent years of medical interventions in armed conflicts and natural disasters that have been ill-equipped or unprepared to provide the surgical resources needed.^{22, 28-30} Several reports exist on injury patterns and mortality in civilians from different armed conflicts, but the association between injury mechanism, injury patterns, and surgical resource consumption is poorly understood. This thesis aims to explore this relationship with the intention of identifying predictive tools and treatment practices to improve how surgical resources are used in resource-constrained conflict settings.

BACKGROUND

History of Surgical Care in Armed Conflicts

Accompanying war and armed conflict is injury and death. Before the 19th-century, care of casualties of war varied greatly between cultures and countries: from every man for himself to the well-organized Roman army with medical corpsmen, casualties transport system, field hospitals, and protected water supplies.³¹ Sadly, these practices were not adopted outside the Roman army to any extent and therefore vanished with the collapse of the Roman empire. It was not until the Renaissance period that the practices from the Roman army were rescued from oblivion and were gradually incorporated into what would become modern war medicine.^{31, 32} Moreover, several other advances were made during the Renaissance period that would contribute to the formation of modern war medicine. The introduction of the tourniquet by Jean Louis Petit in 1718, John Pringle's textbook in military medicine in 1752, the use of forceps to remove bullets, and Pierre-Joseph's description of wound debridements are especially worth mentioning. Dominique-Jean Larrey (1766 – 1848), surgeon-in-chief of the French armies, 1797 – 1815, adopted many of these advances and also introduced the use of triage of wounded, evacuation by flying ambulances (horse-drawn carriages), and the organized use of field hospitals.³¹ He is considered by many to be the father of modern war medicine. The casualty care invented by Larrey can still be seen today with the use of structured levels of care (echelons) and flying ambulances, but now with helicopters and airplanes. The development of radiology, tetanus toxin, better anesthetics, endotracheal intubation, thoracic and vascular surgery, plasma and blood transfusions, intravenous fluids, and antibiotics have also been essential in the evolution of war medicine.³² These advancements have most likely been instrumental in decreasing the mortality from war wounds seen in the last 100 years, from 21% in World War I to 10% in present conflicts.^{33, 34}

Humanitarianism and Armed Conflict

“The will to alleviate the suffering of others.”

Humanitarianism is not a modern invention and is integrated into all major religions. In Judaism, *Tikkun Olam* is the concept that Jews bear responsibility for the wellbeing of others.³⁵ Charity to alleviate suffering, *Zakat*, is one of the five pillars of Islam.³⁶ It is therefore not surprising that the early development of medical missions is strongly intertwined with faith-based organizations' missions.³⁷ Some of the earliest recorded were the Jesuit Christian's missions to Goa (1542) and Macau (1563) to convert people into Christianity. The missionaries quickly understood that providing medical aid made people more willing to convert to Christianity. Therefore, providing medical aid became an essential part early on in these missions.³⁷ For this reason, the American missionary Elijah Bridgman commissioned Peter Parker, an American surgeon and theologian, in 1834 to China as the first Protestant medical mission. Parker is therefore said to have “opened China to the gospel at the point of a scalpel.”³⁸

The non-religious, i.e., the modern humanitarian movement, was born in the 18 – 19th century, during the Age of Enlightenment, from the beliefs of human autonomy and equality. These early humanitarian missions were nationalistic by nature as they focused on aiding their nationals abroad. For example, the London-based Committee for Relieving the Distressed Inhabitants of Germany

and Other Parts of the Continent provided relief to Sweden during the Napoleonic wars of 1808 and 1809. Money was collected from expatriates in the United Kingdom to support war widows, orphans, and returning Swedish soldiers after the battles with the Napoleonic army in the Swedish Pomerania.³⁹ A more famous example is Florence Nightingale and her team of 38 nurses aiding British soldiers in the Crimean War (1853 – 1856), where ten times more soldiers were dying of disease than from war wounds.⁴⁰

The International Committee of the Red Cross

The formation of the International Committee of the Red Cross (ICRC) in 1863 is considered the start of modern international medical humanitarianism.³⁷ In 1859, the Swiss businessman Henry Dunant traveled to Solferino, Italy, to negotiate water rights in Algeria with Napoleon III. In Solferino, Napoleon's army was engaged in the war between Piedmont-Sardinia and Austria. On arrival in Solferino, Dunant witnessed the ruins, many injured and dead after one of the most violent battles of the 19th century. He completely abandoned the intention of negotiating water rights with Napoleon and instead supported the local women in providing care to the injured and sick soldiers. Care was given to all wounded regardless of what side they had fought on, under the device "*Tutti Fratelli*" (all are brothers). After returning to Geneva, Dunant wrote a three-part book about his experience, *A memory of Solferino*. In this book, Dunant outlines a plan of national relief societies that would provide care for war wounded. *A memory of Solferino* was distributed to leading political and military figures in Europe. On February 7, 1863, the *Société genevoise d'utilité publique* (Geneva Society for Public Welfare) appointed the Committee of the five: Henry Dunant, Swiss army general Henri Dufour, jurist Gustave Moynier, physicians Louis Appia and Théodore Maunoir to evaluate the possibility of putting Dunant's plan into action. In the subsequent meeting, on February 17, 1863, the Committee was renamed the International Committee for Relief to the Wounded and this date is therefore considered to be the founding date of the ICRC. This newly formed Committee organized a conference in October 1863 where delegates from 12 states attended. Several important resolutions were adopted at this conference: the establishment of national relief societies for wounded soldiers, neutrality, and protection for wounded soldiers, and the use of volunteer forces for relief assistance on the battlefield are especially worth mentioning.^{41, 42}

ICRC guiding principles and mandate

From early on, the ICRC derived its mandate from the principles of impartiality, political, religious, and economic independence, the universality of the Movement, and the equality of its members. However, it was not until 1921 that these principles were incorporated into the Statutes of the ICRC.⁴³ In 1965, the principles were reformulated and adopted as the seven fundamental principles of the ICRC: humanity, impartiality, neutrality, independence, voluntary service, unity, and universality.⁴³ These principles are incorporated in the ICRC's current mandate: "*the ICRC is an independent, neutral organization ensuring humanitarian protection and assistance for victims of war and armed violence. It takes action in response to emergencies and promotes respect for international humanitarian law and its implementation in national law.*"⁴⁴ The United Nations (UN) general assembly has endorsed humanity, impartiality, neutrality, and independence as the core principles for humanitarian action.⁴⁵

The International Federation of the Red Cross and Red Crescent Societies (IFRC)

The League of the Red Cross Societies, later to be renamed IFRC, was formed on 5 May 1919 as a response to the evident need for cooperation between national relief societies after World War I. The IFRC and the ICRC share the same fundamental principles, but IFRC has adopted a broader mandate than the ICRC, by also providing relief assistance to emergencies not related to armed conflict, such as natural disasters. The IFRC and the ICRC have no hierarchical relation, but in the event of an armed conflict ICRC will take on a coordinating role of the national Red Cross societies. IFRC is currently the largest humanitarian organization with national societies in 190 countries.^{46, 47}

Jus in Bello, the Legal Framework for Armed Conflicts

“Inter arma leges silent” – in times of war the laws are silent (Marcus Tullius Cicero, 106 – 43 B.C., Roman statesman and philosopher).

Laws and rules of conduct aiming to protect civilians from the horrors of war have existed with various impacts throughout human history.⁴⁸ However, many consider the Lieber Code by Frances Lieber, from 1863 to be the first modern laws of war. A central issue in the Lieber Code is the justification of when the use of military force is allowed. Frances Lieber considers the use of military force justified if it is an indispensable measure to bring an end to the war. However, the use of military force must always be restricted by the principle of humanity. Several other principles for modern warfare are also established in the Lieber Code, such as acceptable methods of war, the distinction between civilians and combatants, and the definition of prisoner of war.⁴⁸ The Lieber Code was later used in the development of the Hague Conventions in 1899 and 1907.⁴⁹ The Hague Conventions stipulate the rules of conducting war but do not address the protection of victims of war, which is at the center of International Humanitarian Law (IHL) (Box 1).

Box 1 International Committee of the Red Cross (ICRC) definition of International Humanitarian Law

International humanitarian law is part of the body of international law that governs relations between states. It aims to protect persons who are not or are no longer taking part in hostilities, the sick and wounded, prisoners and civilians, and to define the rights and obligations of the parties to a conflict in the conduct of hostilities.³

The adaptation of the First Geneva Convention “for the Amelioration of the Condition of the Wounded and Sick in Armed Forces in the Field” on August 22, 1864 (revised in 1906, 1929, and 1949) marks the birth of modern IHL (Box 1).⁴¹ In this agreement, adopted by 12 states, the Red Cross is recognized as a neutral organization and allowed to provide aid in war zones. Following the First Convention, three additional Geneva Conventions have been adopted by 196 states and constitute the core of IHL:

- The Second Geneva Convention for the Amelioration of the Condition of the Wounded, Sick and Shipwrecked Members of Armed Forces at Sea” (adopted in 1907)^{7, 48}
- The Third Geneva Convention relative to the Treatment of Prisoners of War (adopted in 1929, revised in 1949)^{7, 48}
- The Fourth Geneva Convention relative to the Protection of Civilian Persons in Time of War (adopted in 1949)^{7, 48}

Furthermore, three amendment protocols have been ratified in addition to the four Conventions:

- Protocol I, relating to the Protection of Victims of International Armed Conflicts (amended in 1977)^{7, 48}
- Protocol II, relating to the Protection of Victims of Non-International Armed Conflicts (amended in 1977)^{7, 48}
- Protocol III, relating to the Adoption of an Additional Distinctive Emblem in which the Red Crystal is added as a protective sign for medical services, as an alternative to the Red Cross and Red Crescent emblems (amended in 2005)^{7, 48}

There is somewhat of an antagonistic relationship between the two regulating bodies of armed conflict, the Hague Conventions and IHL, as both the intentions and basis for enforcement are different. The Hague Conventions are designed to regulate how armed conflict is conducted, and the enforcement is based on the principle of reciprocity while IHL aims at protecting victims of armed conflict and enforcement relies on the warring parties respecting IHL.⁵⁰ Furthermore, the full extent of IHL can only be applied when an armed conflict fulfills the definition stated in IHL. Three types of armed conflicts are recognized by IHL:

- International armed conflict
All cases of declared war or any armed conflict that may arise between two or more states, even if the state of war is not recognized, IHL shall also apply to all cases of partial or total occupation of the territory of a high contracting party even if the said occupation meets with no armed resistance.^{7, 51}
- Internationalized armed conflict
All cases of war between different fractions fighting internally but supported by different states.^{7, 51}
- Non-international armed conflict (NIAC)
All cases of war between government forces and non-governmental groups, or between such groups only.^{7, 51}

The definition of a non-international armed conflict (NIAC) is further clarified in the Geneva Convention Article 1 of Additional Protocol II: “*internal disturbances and tensions, such as riots, isolated and sporadic acts of violence and other acts of a similar nature do not qualify as a NIAC.*”⁷ This clarification unfortunately limits the possibility to apply IHL in many situations where its application could have had a substantial impact for civilians affected by systematic armed violence. The Khmer Rouge regime in Cambodia (1975 – 1979) can be used to illustrate why the current definition of NIAC is problematic. Before the Vietnamese army’s invasion, more than 1 million civilians were killed by the Khmer Rouge. However, as the regime did not encounter an armed organized resistance it was not considered to be a NIAC. Consequently, IHL could not be applied to protect civilians.⁵²

Several alternatives of a more unifying definition of what constitutes an armed conflict have been proposed that would allow IHL to be more broadly applicable. Common for many of these definitions is that they are based on the reason for the conflict and threshold-levels of intensity of the violence and how organized the warring parties are (Box 2, for example).⁵¹ However, so far none of the suggested definitions have been adopted mutually by the world's political leaders. The difficulties of applying IHL in NIAC are increasing as the number of asymmetric conflicts is increasing worldwide, and new automated weapon systems have been implemented that put more civilians at risk for the consequences of armed conflict.⁵³

Box 2 Uppsala Conflict Data Program's (UCDP) definition of an armed conflict

An armed conflict is a contested incompatibility that concerns government and/or territory where the use of armed force between two parties, of which at least one is the government of a state, results in at least 25 battle-related deaths in one calendar year.²

International Humanitarian Law and Weapons in Armed Conflicts

IHL states two articles that restrict the use of weapons: Article 35 prohibits weapons that cause superfluous injury or unnecessary suffering and Article 51 prohibits the use of indiscriminate weapons that are defined as weapons that are incapable of discriminating between civilian and military objects and whose effect cannot be limited.⁷

In a legal sense, these principles cannot be applied without considering the context where the weapons were used, as almost any weapon can be used with both discriminate and indiscriminate intentions.⁵⁴ There are, however, conventions within IHL that inhibit the use of specific types of weapons that are indiscriminate by nature:

- The Geneva Protocol (1925) prohibits the use of biological and chemical weapons.^{7, 54}
- The Ottawa Treaty (1997) prohibits the use, production, stockpiling, and transfer of anti-personnel mines.^{7, 54}
- The Convention on Cluster Munitions (2008) prohibits all use, production, transfer, and stockpiling of cluster munitions.^{7, 54}

In general, and for the purpose of this thesis, indiscriminate weapons are defined as weapons whose effects cannot be limited to the intended objective, such as bombs, missiles and explosives.⁷

Surgical Care for Civilians in Armed Conflicts

Access to Essential Surgical Care

The World Health Organization (WHO) defines emergency and essential surgical care as critical interventions for specific conditions in preventing premature death and disability.¹¹ The access to surgical care varies extensively globally.⁵⁵ It has been estimated that two-thirds of the world's population do not have the possibility to get safe surgical care when needed. Nine out of ten people in LMIC, especially in sub-Saharan Africa and South Asia, do not have access to basic surgical care. Each year 77.2 million disability-adjusted life-years (DALYs) are lost due to conditions treatable with surgery.⁵⁵ An additional 143 million surgical procedures are estimated to be needed in LMIC to save lives and mitigate disabilities.⁵⁵ However, improving access to surgery is not as simple as increasing the number of surgeons, anesthesiologists, and hospitals. Economic reasons, infrastructural limitations, religious and cultural beliefs, and education level are also essential factors that influence why people do not have access to surgical care.⁵⁵ In the last decade, several important steps have been taken to bring the problems of the uneven distribution of access to surgical care into the focus of the global health discourse:

- The formation of the Lancet Commission on Global Surgery in January 2014 with its description of the problems, core indicators, and suggestions for future solutions.⁵⁵
- In May 2015, WHO passed resolution A68/15 at the 68th World Health Assembly, which aims to strengthen emergency and essential surgical care and anesthesia as components of universal health coverage.⁵⁶
- The adaptation of the United Nation's Sustainable Development Goals by member states in September 2015, which stress the importance of strengthening health care systems and universal health coverage.⁵⁷
- In June 2016, Katherine O'Neill *et al.* described caesarian section, laparotomies, and surgical treatment of open fractures as potential procedures for planning and monitoring essential surgical care in LMIC, the Bellwether procedures (Box 3).¹

Box 3 The Bellwether procedures

A first-level hospital's ability to perform caesarian sections, laparotomies and surgical treatment of open fractures was closely associated with the ability to perform all necessary procedures needed for essential surgical care.¹

Access to Surgical Care in Armed Conflicts

The onset of an armed conflict can rapidly diminish the capabilities of vital societal structures, such as access to medical care, infrastructure, the justice system, monetary system, and trade.²² Furthermore, many of the current conflicts are located in areas and countries where these structures were fragile even before the onset of the armed conflict.⁵⁸ It is therefore not surprising to find that many of the countries where access to surgery is poor also are involved in an ongoing armed conflict (Fig. 1).^{2, 59, 60}

Fig. 1 **A** Global distribution of access to surgery for civilians.⁵⁹ Reproduced with permission from Paul Farmer and under the terms of CC BY 4.0. **B** Ongoing armed conflicts in 2018.² Reproduced with permission from the Uppsala Conflict Data Program (date of retrieval: 15 October 2019).

Surgical care providers in these low-resource settings not only have to handle the inflow of patients generated by the conflict but also conditions that are not conflict-related that require surgical treatment, such as incarcerated hernias, obstructed labor, traumas, and burns.⁶¹ Furthermore, elective cases will build up over time as demonstrated by my co-supervisor Johan von Schreeb *et al.* in a conceptual model of the need for hospital resources following sudden onset disasters.⁶² For health care providers planning on delivering health care in an armed conflict, these issues need to be taken into consideration.

Epidemiology of Civilians Injured in Armed Conflicts

Several reports have been published describing who, when, and how civilians are injured in armed conflicts.⁶³⁻⁶⁵ However, most of these reports have limitations in their methodology, as they are based on data from a single treatment facility, retrospectively collected, used variables are not consistently defined, or treatment needs in relation to gender are not explored. These limitations are effectively inhibiting the possibility to do objective comparisons. Moreover, given the insufficient infrastructure and access to health care seen in conflicts, it is reasonable to assume that these reports rarely provide the complete epidemiological picture. Nonetheless, similar findings have been reported from different conflicts. This may be best summarized in a review article by Wild *et al.* in 2020, where 58,578 patients from 49 contemporary and recent armed conflicts in 18 countries were analysed.⁶⁶ The patients consisted of civilians and local combatants. A majority of

these patients were male (79%, range 73 – 87%) with a median age of 26 years (IQR 22.8 – 27.6 years). A substantial portion were under 18 years (35%, range 17% – 38%). Mechanism of injury varied depending on whether the conflict took place in an urban area or not. In urban conflicts, gunshot wounds (GSW) constituted 42%, and blasts from missiles, bombs, and explosives constituted 19% of the mechanism of injury. Conversely, in conflicts primarily located in rural areas, the reversed relationship was seen, GSW 9% and blast 70%. Injuries to the extremities were the most frequent (34%, range 20 – 40%). The frequency of thoracic and abdominopelvic injuries was low (10%, range 4% – 17% and 8%, range 2% – 12%, respectively), even though blasts and other indiscriminate weapons caused a substantial portion of all injuries treated. This, together with the low in-hospital mortality observed (6%, range 2% – 16%), could indicate a survivorship bias, where those with life-threatening injuries, such as thoracic injuries, die before reaching the hospital.⁶⁶ However, it is difficult to confirm this assumption given the lack of available data on how many lose their lives at the injury site.

Surgical Resource Consumption

“War surgery is the management of an epidemic of trauma.” (Nikolai Ivanovich Pirogov, 1810 – 1881, Russian anatomist and surgeon)

Surgical workload, i.e., surgical resource consumption, in armed conflicts is often discussed in terms of the number and type of surgical procedures, amputations, use of blood products, and mortality.^{65, 67-70} The ICRC War Surgery textbook defines surgical workload as the number of operations and blood transfusions per patient and the length of hospital stay.⁶⁸ Whether these variables best reflect surgical resource consumption is of course debatable. However, to my knowledge, there is currently no universally accepted definition of what surgical resource consumption should entail. This is not surprising as the definition could vary depending on the context and available resources (Fig. 2).

Fig. 2 Total amount of surgical equipment, medications, and operation theater available at a first-level hospital in Pool-region, the Democratic Republic of the Congo during 2005 – 2006. Photos by author.

Nonetheless, much would be gained if context-specific surgical resource consumption could be assessed, especially in resource-constrained settings. This would facilitate planning, monitoring of surgical care and enable comparisons of treatment outcomes between hospitals. Trauma scores used in non-conflict settings could potentially be used for this purpose. Studies on trauma patients, mostly from high-resource settings, have demonstrated that trauma scores intended to be used for mortality predictions also can be used to predict resource-related outcomes, such as the need for

admission and surgical intervention, massive haemorrhage, and prolonged intensive care (ICU) stay (Table 1).⁷¹⁻⁷³

Table 1 Commonly used trauma scores and their intended predictive abilities

Score	Components	Intended to predict
Kampala Trauma Score (KTS)	Age Systolic blood pressure Respiratory rate Neurological status Number of severe injuries ^a	Injury severity and mortality following trauma in LMIC settings ⁷⁴
MGAP	Mechanism of injury Glasgow coma scale Age Systolic blood pressure	Mortality following trauma ⁷⁵
Revised Trauma Score (RTS)	Glasgow coma scale Systolic blood pressure Respiratory rate	Probability of survival following trauma ⁷⁶
Injury Severity Score (ISS)	An anatomical scoring system that combines the most severe injury from each body system using the 6-point Abbreviated Injury Scale (AIS)	Probability of survival following trauma ⁷⁷
Trauma Injury Severity Score (TRISS)	Combination of RTS and ISS	Probability of survival following trauma ⁷⁸

^aAbbreviated Injury Scale (AIS) of 2 or above for any given anatomical site.

However, the predictive abilities for these trauma scores could be diminished in armed conflicts due to the survivorship bias previously discussed. Furthermore, injury patterns in armed conflicts are different from those seen in civilian traumas. Penetrating injuries are more prevalent in conflicts, whereas blunt injuries are more frequent in civilian traumas.^{66, 79} It is therefore reasonable to question the predictive abilities of trauma scores when applied in the context of armed conflicts.

Red Cross Wound Score

In the early 1990s, Mr. Robin Coupland, at the time Chief surgeon for ICRC, devised a system for classifying penetrating wounds, the Red Cross Wound Score (RCWS). RCWS was intended as a descriptive tool to facilitate triage and the surgical management of wounds in armed conflicts.^{68, 80} To classify a wound according to RCWS, the extent of the wound (RCWS grade) and type of tissue involved (RCWS type) is assessed through visual inspection and if needed x-ray (Table 2). The grade component corresponds to the energy transfer to the tissue at the time of injury.^{68, 80}

Table 2 Red Cross Wound Score

Type	Grade 1	Grade 2	Grade 3
ST Soft tissue wounds	Small simple wound E+X<10cm C0 F0 V0 1ST	Medium sized soft tissue wound E+X<10cm C1 F0 V0 2ST	Large soft tissue wound E+X≥10cm C1 F0 V0 3ST
F Wounds with fractures	Small wound with simple fracture E+X<10cm C0 F1 V0 1F	Medium sized wound with important fracture E+X<10cm C0 F2 V0 2F	Large wound with significant fracture E+X≥10cm C1 F2 V0 3F
V Vital wounds	Small wound threatening life E+X<10cm C0 F0 V=N, T, A, OR H 1V	Medium sized wound threatening life E+X<10cm C1 F0 V=N, T, A, OR H 2V	Large wound threatening life E+X≥10cm C1 F0 V=N, T, A, OR H 3V
VF Vital wounds with fractures	Small wound threatening limb and/or life E+X<10cm C0 F1 V=N, T, A, OR H 1VF	Medium wound threatening limb and/or life E+X<10cm C1 F2 V=N, T, A, OR H 2VF	Large wound threatening limb and/or life E+X≥10cm C1 F2 V=N, T, A, OR H 3VF

E=Entry wound in centimeters; X=Exit wound in centimeters; C=Cavity >2 fingers wide; F=Fracture (0 = No fracture, 1 = Simple fracture, hole, or insignificant comminution and 2 = Clinically significant comminution); V=Vital structure (0= No penetration of vital structure, N=Penetration of the dura of the brain or spinal cord, A=Peritoneum, T=Pleura and H=Major peripheral vessels extending to the brachial or popliteal or carotid arteries)

The use of RCWS as a predictive tool for resource-related outcomes and mortality is poorly examined. However, there are compelling reasons to study if such a relationship does exist. The RCWS is a robust classification system that does not rely on advanced diagnostic equipment or calculation, making it suitable for use in trauma care and especially in resource-constrained settings. Similar to the Injury Severity Score (ISS), RCWS also provides data on injury severity, which enables measurements of injury- and treatment-specific outcomes. Unlike many commonly used trauma scores RCWS does not rely upon vital parameters, which may reduce the influence of survivorship bias.

Quality Assessment of Surgical Care in Armed Conflicts

Surgical care in low-resource armed conflicts is predominantly experienced-driven. The scientific body of evidence that supports treatment practices and subsequent outcomes is limited.⁶⁶ Several stakeholders in the humanitarian surgical field have therefore advocated for the implementation of a universal data set that would enable systematic evaluations of the care provided.^{30, 55, 66, 81} Parallels have been drawn to the importance trauma registers in high-resource settings have had to improve trauma care.^{82, 83} In an article from 2020, the Stanford humanitarian surgical response in conflict working group, a consensus framework for humanitarian surgical response to armed conflict is outlined.⁸² This framework also includes a suggested universal minimum data set that would enable systematic data collection that could be used for quality assessment (Table 3).

Table 3 Suggested minimum data set for humanitarian trauma system by Stanford humanitarian surgical response in conflict working group.⁸²
 Reproduced with permission from Sherry Wren and under the terms of CC BY 4.0.

Required

- Age
- Gender
- Mechanism and anatomic location of injury
- Estimated time of injury
- Time of arrival at each point of care
- Mode of transport
- Vital parameters
- Triage category ⁸⁴ and Kampala Trauma Score ⁸⁵
- Procedures performed
- Status at discharge (dead/alive)
- Time of discharge
- Intended destination at discharge

Desirable

- Geographic place of injury (if not risking patient safety)
- Clinical complications
- Functional status at discharge
- Any available data about long-term patient outcomes

Wounds and Wound Treatment

"Small wounds and poor friends should not be despised." (Swedish proverb).

Several definitions, with minor differences, exist on what constitutes a wound and a wound infection. For the purpose of this thesis, the following definitions are used:

- Wound: a wound is a breakdown in the protective function of the skin; the loss of continuity of epithelium, with or without loss of underlying connective tissue⁸⁶
- Wound infection: >100,000 bacteria per gram of tissue have been demonstrated to impair healing significantly^{87, 88}

For surgeons working in low-resources armed conflict settings, a substantial portion of the workload is related to wound care and to achieve wound closure.⁶⁸ This is due to the complexity of weapon-related wounds. At the time of injury, high amounts of energy are transferred to the tissue leading to substantial tissue loss that is not immediately evident.⁶⁸ The wounds are frequently embedded with foreign material.⁶⁸ Patients often have multiple wounds and due to insufficient transportation means present late.⁶⁸ All of these are factors that predispose for wound infection.^{89, 90} Therefore, it is not surprising that studies from armed conflicts have demonstrated culture verified wound infections rates of 10 – 30%.^{89, 91, 92} Commonly found bacteria are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Clostridium*, and *Klebsiella* species.^{89, 90} A wound infection left untreated can lead to systemic infection and further wound progression, i.e., more devitalized tissue, potentially resulting in more extensive surgical treatment needs, such as repeated debridement.⁹⁰

Treatment

Treatment of weapon-related wounds is primarily surgical. The objective is to prevent infection and to achieve wound closure.^{68, 93} Debridement is the first step of this treatment, where devitalized tissue and foreign material are excised (Fig. 3). Several factors will influence if a successful debridement is achieved: the timing of the debridement in relation to the time of injury, vital anatomical structures that limit the possibility of excising all devitalized tissue, the surgeons experience of debridements, and familiarity of the anatomical location that is subject to the debridement are some worth mentioning. The wound is then left unsutured and packed with fluffy dry gauze. After 4 – 7 days, the wound is inspected, and if the wound appears clean, wound closure is undertaken. Limited studies have reported that if wound closure is done prior to this time infection will often follow, and the wound has to be reopened.^{94, 95} Wound closure can be achieved through several techniques: suturing (delayed primary closure, DPC), flaps, split skin grafts (SSG) or a combination thereof.⁶⁸ A study with 16,172 patients with weapon-related wounds treated at ICRC hospitals demonstrated that 66% of the patients could be treated with two surgical procedures or less.⁶⁸

Fig. 3 Debridement of a stump infection in the Norwegian Red Cross field hospital in Cox's Bazar, Bangladesh.
Photo by author.

ICRC treatment protocols for weapon-related wounds state that all patients should be given prophylaxis against tetanus and Penicillin for five days against *Clostridium perfringens* and β -hemolytic streptococcus.⁶⁸ The rationale behind the choice of Penicillin is that the bacteria mentioned above historically have been the source for the most lethal infections following weapon-related injuries.⁶⁸ The length of the Penicillin treatment is based on the assumption that an infection already is established, as a majority of the patients treated at ICRC hospitals arrive more than six hours after the injury.⁶⁸ However, ICRC is currently considering revising the choice of Penicillin due to the reported increase of multidrug resistant (MDR) bacteria seen in patients injured in contemporary armed conflicts.^{89, 91}

Honey in Wound Treatment

The use of honey in wound care has a long history. Archaeological findings indicate that honey has been used in wound treatment at least since ancient Egypt. The Ebers Papyrus, 1550 B.C., describes a dressing combining honey, linen, and grease as an effective treatment for infected wounds.⁹⁶ This empirical knowledge has led health care providers, including the ICRC, to advocate for the use of honey dressings as an adjunct for treatment of infected wounds and wounds intended to heal by secondary intention.^{68, 97, 98} Furthermore, wound dressings and gels containing Manuka honey were approved by the U.S. Federal Drug Administration (FDA) in 2007 as an alternative and a natural

material for the treatment of wounds.⁹⁹ Honey's antimicrobial and hyperosmotic properties have been established during the last decades in several, mostly *in vitro*, studies.¹⁰⁰⁻¹⁰³

The WHO has stated that: *"the global community must encourage sustainable investments in new medicines, diagnostic tools, vaccines, and alternative interventions. The majority of pharmaceutical companies are no longer researching a new antibiotic which is a global concern for human and animal health. Research and development are needed to produce new treatments that can be deployed against multidrug resistant infections."*¹⁰⁴ As a result of the ever-spreading antimicrobial resistance, an increasing number of non-healing wounds globally are infected with MDR bacteria causing morbidity and adding burden to already strained health care systems, especially in armed conflicts.^{89, 90, 105} A recent cohort study of Syrian patients with conflict-related injuries treated in Jordan found MDR bacteria in over 70% of the infected wounds, which also were associated with a less favorable outcome and higher resource consumption.⁹¹ This highlights the need to identify treatment strategies that do not rely on antibiotics. Honey's antimicrobial properties, low cost, almost indefinite shelf life, and readily availability worldwide would make it an ideal candidate. However, a Cochrane review from 2015 stated that: *"it is difficult to draw overall conclusions regarding the effects of honey as a topical treatment for wounds due to the heterogeneous nature of the patient populations and comparators studied and the mostly low quality of the evidence."*¹⁰⁶

THEORIES AND HYPOTHESES

Two theories constitute the foundation for this thesis:

- I. Mechanism of injury and injury patterns can be used to predict surgical resource consumption in resource-constrained conflicts settings.
- II. Locally available products can be used to improve how surgical resources are used.

Based on these theories, the following hypotheses were generated and tested in this thesis:

- Civilians are to a larger extent injured from indiscriminate weapons and require more surgical resources than combatants.
- Surgical treatment practices of extremity injuries in low-resource conflict settings have not changed during the last decades.
- RCWS has a better predictive ability for surgical resource consumption and mortality than trauma scores commonly used in high-resource settings.
- Honey and antibiotics have comparable efficacy as treatment for infected wounds.

AIMS OF THE THESIS

Overall

This thesis aims to generate knowledge regarding the relationship between demographics, injury mechanism, injury patterns, and surgical resource consumption with the intention to identify predictive factors and treatment practices that can improve how surgical resources are used in armed conflicts.

Specific

- I. To investigate injury mechanism, injuries and surgical treatment needs in resource-constrained conflict settings. (Understanding)
- II. To investigate trends in demographics and surgical practices of weapon-related limb injuries in a resource-constrained setting between 1992 – 1995 and 2009 – 2012 focusing on amputation rates. (Understanding)
- III. To determine if RCWS, KTS, and RTS have predictive ability to assess surgical resource consumption and in-hospital mortality in resource-constrained conflict settings. (Predicting)
- IV. To assess the efficacy of topical honey as treatment for *S. aureus* infected war wounds compared to systemic antibiotics. (Improving)

MATERIAL AND METHODS

Overview

The material, method, and statistical analyses used in each paper are summarized in Table 4 and the following sections and described in detail in the attached papers. The material used for this thesis consists of routinely collected clinical data (*Papers I – III*) and data from an animal model (*Paper IV*). The routinely collected clinical data were obtained from three different ICRC hospitals in two different armed conflicts. The data were prospectively collected by health care staff that was specifically trained for the task. However, at the time of data collection, the intended use was not specified, more than vaguely to be used for quality control purposes. In *Paper III* we also utilized data extracted from patient charts located in the ICRC archives in Satigny, Switzerland. The data collected was applied in observational cohort studies (*Papers I – III*) and a non-inferiority experimental study (*Paper IV*) with the intention to test the hypothesized cause-effect relationships.

Table 4 Overview of included papers in the thesis

	<i>Paper I</i>	<i>Paper II</i>	<i>Paper III</i>	<i>Paper IV</i>
Study periods	2009 – 2012	1992 – 1995 and 2009 – 2012	2009 – 2012 and 2012 – 2014	2020 – 2021
Aims	Understanding	Understanding	Predicting	Improving
Designs	Retrospective cohort study	Retrospective cohort study	Retrospective cohort study	Non-inferiority experimental study
Participants	Patients with conflict-related injuries	Patients with conflict-related extremity injuries	Patients with conflict-related injuries	Pigs
Hospital locations	Peshawar	Peshawar, Quetta	Peshawar, Goma	
Number of study subjects	3,028 patients	998 patients	834 patients	50 wounds on two pigs
Data sources	Routinely collected clinical data	Routinely collected clinical data	Routinely collected clinical data	Porcine wound model
Main statistical analysis	Descriptive, Chi- square, and Mann- Whitney <i>U</i>	Logistic regression models	Logistic regression models and ROC curves	ANOVA with Greenhouse-Geisser correction to avoid violation of sphericity
Main outcome measures	Differences in surgical treatment needs for females compared to men	Differences in surgical practices in 1992 – 1995 compared to 2009 – 2012	Trauma score's predictive ability for surgical resource consumption and mortality	Reduction of <i>S. aureus</i> over time in wound tissue with topical honey compared to intramuscular gentamicin

ROC: receiver operating characteristics; ANOVA: analysis of variance

Table 5 demonstrates which variables from the Stanford humanitarian surgical response in conflict working group’s suggested minimum dataset are used as main variables in *Papers I – III* of this thesis.⁸²

Table 5 How variables included in the minimum data set for humanitarian trauma systems are applied as main variables in *Papers I – III* ⁸²

	<i>Paper I</i>	<i>Paper II</i>	<i>Paper III</i>
Age	Independent		Independent
Gender	Independent		Independent
Mechanism of injury	Dependent		
Anatomic location of injury			
Estimated time of injury			Independent
Time of arrival at each point of care			
Mode of transport			
Vital parameters			Independent
Triage category ⁸⁴ and KTS ^{a, 85}			Independent
Procedures performed	Dependent	Dependent	Dependent
Status at discharge (dead/alive)	Dependent	Dependent	Dependent
Time of discharge		Dependent	
Intended destination at discharge			

^a Kampala Trauma Score

For all papers (*Papers I – IV*), a statistician was consulted regarding appropriate statistical methods. Statistical analysis was done using IBM SPSS Statistics software version 23 – 25 (IBM Corporation, Armonk, NY, USA) (*Papers I – III*) and GraphPad Prism 8.4 (GraphPad Software, San Diego, CA, US) (*Paper IV*). Non-overlapping confidence intervals (CI) and p-values (two-tailed) less than 0.05 were considered significant in all analyses.

Conflicts

In this thesis, data from patients injured in two different armed conflicts are utilized. Both these conflicts are still ongoing. For *Papers I – II*, we analysed data from patients injured in the border conflict between Afghanistan and Pakistan (the Durand Line conflict). For *Paper III* we also analysed data obtained from patients injured in the Kivu conflict in the eastern part of the Democratic Republic of the Congo (DRC). A conflict that also involve neighboring countries and Rwanda in particular.

Afghanistan, DRC, and Rwanda are low-income countries, and Pakistan a lower middle-income country.¹⁰⁷ Life expectancy and income per capita at the time of data collection are demonstrated in Fig. 4. Total health expenditure per capita for these countries was in the lower quarter of the world's countries.^{107, 108} Moreover, the proportion of the population that has access to safe, affordable surgery and anesthesia is estimated to be less than 10%.⁵⁹

Fig. 4 Life expectancy, gross national income (GNI) per capita and income thresholds for 2012. Arranged by country. Data compiled and provided free from www.gapminder.org (date of retrieval: 5 May 2021). Income thresholds according to the World Bank.¹⁰⁷

The Durand Line and Afghanistan Conflict (Papers I – III)

The borderline between Afghanistan and Pakistan, the Durand Line, was a result of an agreement between the British diplomat Sir Henry Mortimer Durand and the Amir of Afghanistan, Abdur Rahman, in 1893 (Fig. 5).^{109, 110} The incentive for the British empire to establish this border was to protect British India from Russia that was steadily expanding at the time.^{109, 110} There are several reasons why the Durand Line has been a constant source of tension between Afghanistan and Pakistan ever since its creation. It divided the ethnic group Pashtun's land and forced Afghanistan

to give up the province of Baluchistan. Furthermore, it effectively prevents Afghanistan from access to the Arabian sea.¹⁰⁹ No government of Afghanistan has therefore recognized the legitimacy of the Durand Line.^{109, 111} The Pakistan government, on the other hand, views the Durand line as a necessity for Pakistan's existence.¹⁰⁹ Consequently, the Durand line has been the reason for recurring clashes between Afghan and Pakistani security forces, resulting in injured and killed civilians.¹⁰⁹ The Durand Line conflict can best be described as an asymmetric war, with civilians being injured by the use of explosives, grenades, attacks by gunmen, and remote-controlled aerial bombings on both sides of the Durand Line.^{5, 69}

The Durand line conflict is closely interlinked with the complex internal conflict in Afghanistan. The Soviet invasion of Afghanistan in 1979 provided an opportunity for Pakistan to intervene in Afghanistan's internal affairs.¹⁰⁹ Supported by the Central Intelligence Agency (CIA), the Pakistan Inter-Services Intelligence Agency (ISI) aided mujahideen groups to oppose the Soviet invasion, which subsequently led to the fall of the Afghan government in 1992.¹⁰⁹ The Taliban rule that followed was supported by Pakistan. Despite this the Taliban refused to recognize the Durand Line as the legitimate border.¹⁰⁹ Pakistan's attempt to stop Pashtuns from crossing the Durand Line spurred the Taliban to carry out attacks inside Pakistan.¹⁰⁹ The nature of the internal conflict in Afghanistan changed again with the U. S. invasion in Afghanistan in 2001 and establishment of a democratic government, which forced Taliban members to escape to remote mountain areas of Afghanistan and to Pakistan. However, this change of government had little impact on the Durand line conflict as clashes continued with various intensity.¹¹² As of August 30, 2021, the U. S. has withdrawn its military forces from Afghanistan and the Taliban have once again seized power over Afghanistan. At the time of writing this thesis it is unclear what position the new Taliban government will take concerning the Durand line and in their relations with Pakistan. Most likely the Durand Line conflict will continue as the underlying reasons for the conflict remains unsolved.

The Kivu Conflict (Paper III)

The conflict in the Kivu province of DRC has its roots in the First Congo War 1993 – 1997 and the Rwanda Civil War 1990 – 1994.¹¹³ In 2004, following increased tensions, an armed conflict broke out between the Forces armées de la République démocratique du Congo (FARDC) and the Hutu group and the Democratic Forces for the Liberation of Rwanda (FDLR) in the southern Kivu province of eastern DRC.¹¹⁴ At later stages, Tutu rebel forces (National Congress for the Defence of the People, CNDP) and several local armed groups, the largest group being the Mouvement du 23 Mars (M23), got involved.¹¹³ The conflict is driven mainly by rights to mineral resources in the region.^{113, 115} The use of children as soldiers and systematic rapes have been frequently reported.^{116, 117} Compared to the conflict along the Durand Line, fewer injuries from indiscriminate weapons such as grenades, bombs, and anti-personnel mines have been observed.¹¹⁸⁻¹²⁰

Hospitals

The Durand Line and Afghan Conflict (Papers I – III)

In response to the conflict in Afghanistan and at the Durand Line, the ICRC opened its first hospital 60 kilometers from the Afghanistan border in Peshawar, northwestern Pakistan, in 1981. This was followed by the opening of another hospital in Quetta, 80 kilometers from the border and 840 km south of Peshawar, in 1983 (Fig. 5). Both hospitals closed in the 1990s, but due to the intensification of the conflicts in the border area, the Peshawar hospital was reopened in 2009 and remained operational until 2012, and finally closed in 2014. The hospital in Peshawar had 116 beds, three operating rooms, a laboratory, X-ray service, blood bank, and basic ICU.¹¹⁹ The hospital in Quetta had 150 beds, one operation theatre with three operation tables, a laboratory, X-ray service, and blood bank.¹¹⁹ Both hospitals had access to rehabilitation centers for training and prosthesis fitting.¹¹⁹ Patients were not asked whether they were combatants or civilians. Together with some small facilities run by non-governmental organizations (NGOs), the ICRC hospitals were the only hospitals at the time providing surgery for weapon-wounded regardless of origin, completely free of charge. Public hospitals did provide initial surgical care for free but were difficult to access for others than Pakistanis.

Fig. 5 The Durand Line, i.e., Afghanistan – Pakistan border, and locations of ICRC hospitals.

Copyright Google Maps.

The Kivu Conflict (Paper III)

In November 2012, due to the escalation of the armed conflict, the ICRC deployed surgical teams to support the N'Dosho hospital in Goma, Northern Kivu (Fig. 6). The hospital had at the time 65 beds, one operating theatre, X-ray service, and a laboratory.¹¹⁹ The hospital had access to a rehabilitation center for training and prosthesis fitting.¹¹⁹ Care at the hospital was free of charge. Patients were not asked whether they were combatants or civilians.

Fig. 6 The border area of the Democratic Republic of the Congo and Rwanda and the location of the ICRC hospital. Copyright Google Maps.

Study Designs and Methods (*Papers I – III*)

Paper I

This thesis aims to examine the relationship between injury mechanism, injury patterns, and surgical resources consumption for civilians in armed conflicts. A reasonable first step to explore this aim would be to examine this relationship in a strictly defined civilian cohort without preconceptions. However, what constitutes a civilian and a combatant can vary in different conflicts and can be especially difficult to determine in asymmetric warfare, as a person can be both a combatant and a civilian depending on their activity. Furthermore, ICRC staff, consistent with their guiding principles and mandate, never ask or record if a patient is a combatant or a civilian. Therefore, to ensure that we were studying civilians, we define all females together with all males aged less than 16 years old or more than 49 years old as civilians. This definition was based on known characteristics of the Durand Line and Afghan Conflict, and consistent with previous reports from conflict.^{69, 121, 122} The database used for this study contains clinical data from 3,028 patients (376 females) that were consecutively admitted to the ICRC hospital in Peshawar 2009 –

2012. Only weapon-related injuries were treated in the hospital during the study period. Entries in the database were done, mainly prospectively, by Zaher Osman, co-author of *Papers I – III*, at the time stationed at the ICRC hospital in Peshawar. All patients in the database, except for readmissions, were included in this cohort study. Injury mechanism, vital parameters, surgical treatment needs, and in-hospital mortality were compared between females, males, and age groups.

Paper II

This retrospective cohort study is a result of opportunity and the need for me to understand how surgical treatment practices for civilians in armed conflict have evolved over time. During the initial phase of our collaboration with the ICRC, I was presented with a report written by an intern at ICRC, Grace Bauhahn. This report examined the completeness of the handwritten patient charts stored in the ICRC archive in Satigny, Switzerland. Reading through this report I realized that the handwritten charts were complete enough to allow for comparisons with the database used in *Paper I*. We therefore decided to compare surgical treatment practices of extremity injuries for patients treated 1992 – 1995 and 2009 – 2012, with a focus on amputation rates. The reason to focus on extremity injuries were that these are the most frequently treated injuries at ICRC hospitals and represent a substantial part of the surgical workload. Additionally, focusing on extremity injuries with the dichotomous outcome of amputation would allow for relevant comparisons with studies published from non-conflict settings. We also included the use of external fixation, SSG, debridements (DBR), change of dressing (COD), blood transfusion, length of hospital stay (LOS) and in-hospital mortality as outcomes to get a better understanding of the differences between the two periods.

Data from the handwritten patient charts were manually extracted using a randomization process created by Kaspar Holmgren, a medical student at the time. The patients were treated during 1992 – 1995 at ICRC hospital in Peshawar and neighbouring ICRC hospital in Quetta, also located in Pakistan. Patients treated in Quetta were included to accelerate the exhaustive data extraction process. The collected data were merged with the database containing patients treated 2009 – 2012. Inclusion criteria were all patients admitted to the ICRC hospitals in Quetta or Peshawar, during 1992 – 1995 and 2009 – 2012, respectively, with weapon-related penetrating injuries to the limbs. Exclusion criteria were if patients had a significant injury to other sites than limbs requiring medical attention other than a basic dressing, injuries older than seven days, traumatic amputations including partial or total avulsion of the limb, or missing data on confounders. In total, 463 patients treated during 1992 – 1995 and 535 patients treated 2009 – 2012 were included.

Paper III

The protocol for this study is an adaptation of a protocol developed by my co-supervisor Johan von Schreeb's research group at Karolinska Institutet. The original intention was to assess RCWS's predictive ability for mortality using routinely collected patient data extracted from the ICRC archive in Satigny, Switzerland. However, to align the study protocol with the hypothesis developed for this thesis, I revised the aim, material, and methods. This was done to allow for comparison of RCWS and commonly used trauma score's predictive abilities for surgical resource consumption and in-hospital mortality. During my Ph.D. halftime seminar, concerns were raised regarding the difficulties of studying the proposed cause-effect relationship using such a heterogeneous study population and an unestablished definition of surgical resource consumption. I was, therefore, recommended to instead compare RCWS and Gustilio-Anderson's (GA) predictive abilities for the need of debridement and risk for amputation following weapon-related extremity injuries. GA is a well-established classification system for open fractures in high-resource settings. GA has been demonstrated to have a good predictive ability to predict outcome measures such as complications, need for wound revision, and risk for amputation.¹²³⁻¹²⁵ I therefore, together with two medical students, compared RCWS and GA predictive abilities for these outcomes using 304 patients treated at ICRC hospital in Peshawar with open fracture as the most severe type of injury. We found that RCWS and GA were equally good in predicting risk for amputation and high surgical resource consumption, and similarly poor predictive ability for the need of debridement. However, RCWS had a better discriminative ability within its classification system than GA (unpublished data). This is probably due to how GA is constructed, where all patients with injuries from high-velocity weapons are classified into one of three subgroups of the highest classification group IIIa-c.¹²⁴ This leaves little room for any clinically relevant predictive differentiation for patients with open fractures following injuries received in armed conflicts. We therefore decided not to pursue this idea any further.

How to define high surgical resource consumption was problematic and a matter of extensive discussion. After reviewing literature and discussing with senior ICRC surgeons, we decided on a definition based on how ICRC characterizes surgical workload.^{67-69, 120, 126, 127} High surgical resource consumption was therefore defined as three or more surgical procedures under anaesthesia, three or more blood transfusions, or in-hospital amputation proximal to the metacarpophalangeal joints of the hand or the midtarsal joints of the foot.

Instead of extracting data from handwritten patient charts, as intended in the initial study protocol by Johan von Schreeb's research group, we used the database from the ICRC hospital in Peshawar (used in *Paper I – II*), containing data from 3,028 patients consecutively treated between 2009 – 2012. We also utilized an identically constructed database from ICRC hospital in Goma, DRC, containing data from 689 patients consecutively treated between 2012 – 2014. The reason to include patients from Goma in the study was to increase the generalisability of the study by analysing patients from different conflict settings. Inclusion criteria were adult patients with weapon-related penetrating injuries, assessed RCWS, and known discharge status. Adults were defined as age 15 or older in accordance with previous studies from similar settings.^{119, 128} Patients with unspecified gender, time of injury, and vital signs were considered incomplete cases and excluded in the primary analysis. In total, 834 patients were included in this observational cohort

study. The structure of the report for this study followed the STROBE guidelines for reporting observational studies.¹²⁹

Data Collection and Management (*Papers I – III*)

All data used in *Papers I – III* was stored on protected servers provided by Linköping University. All participating researchers had access to data. Patient confidentiality was assured by randomly assigned identification numbers, and the patient's name or place of residence was not known. The data consists of two databases with clinical data from ICRC hospitals in Peshawar 2009 – 2012 and Goma 2012 – 2014. The data in these databases (Excel® spreadsheets) were collected prospectively for quality control purposes, by health care staff specifically trained for the task. For *Paper III* we also created a database (Excel® spreadsheet) with data extracted from handwritten patient charts from patients treated at ICRC hospitals in Quetta and Peshawar during 1992 – 1995. The three databases were transferred to IBM SPSS® Statistics software and merged. The final database was validated with the original Excel® spreadsheets by comparing 150 randomly selected entries from each hospital. All data were encrypted and stored in the Linköping University cloud storage. No paper copies exist. All participating researchers had access to the collected data. The data will continue to be available for participating researchers and upon reasonable request until 2028, when the data will be destroyed, as stipulated by Linköping University.

Statistical Analysis (*Papers I – III*)

Paper I

This study is in essence a comparative descriptive study. Chi-square test (categorical variables) and Mann-Whitney *U*-test (continuous variables) were used to analyse if a relationship between age, gender, injury mechanism, and surgical treatment needs existed.

Paper II

For the univariate comparisons of categorical variables in this study, we decided to use Fisher's exact test instead of Chi-square test. The reason for this is that Fisher's exact test is, as stated in the name, an exact test, whereas Chi-square is an approximation. Furthermore Fisher's exact test is better suited for analyses of a small sample size, which we anticipated to have in this study.¹³⁰ Mann-Whitney *U*-test was used for the univariate analysis of continuous variables. The results from the univariate analyses and a literature search were used to identify potential confounding factors, that would interfere in the comparison between the two periods.^{131, 132} The identified confounding factors were then included in binary logistic regression models to allow for adjusted comparisons between the two periods for our outcomes (in-hospital amputations, use of external fixation, SSG, and ≥ 3 DBR/COD, blood transfusion, ≥ 21 days LOS and in-hospital mortality). Forward Wald was performed to obtain final regression models to be able to examine the difference in frequencies for our outcomes and were presented as odds ratios (OR) with 95% CI.

Paper III

There is an abundance of studies assessing trauma score predictive ability for different outcomes and in different settings.¹³³⁻¹³⁵ In most of these studies receiver operating characteristic (ROC) has been used to determine the predictive ability of the scores tested. The result of a ROC-analysis is a curve where the true positive rate (sensitivity) is plotted against the false positive rate (1-specificity) at different threshold levels. The area under this curve (AUC) determines the score's total ability to distinguish between true positive findings and false positive findings for a binary outcome.¹³⁶ There are some issues that need to be considered prior to using ROC AUC analysis. A ROC AUC analysis does not tell you if a relevant relationship between the studied variable and outcome exists. Consequently, a ROC AUC analysis will not stop you from testing if you can predict cancer by looking at the appearance of coffee grounds. Therefore, a statistical and clinical relationship between the studied variable and outcome must be established prior to performing a ROC AUC analysis. This is usually done through goodness of fit tests and confirmed by findings in previous reports. Furthermore, ROC AUC does not adjust for the clinical impact different false negatives and false positives will have at different threshold levels.¹³⁶ A patient with a small wound that was falsely predicted to be amputated will have the same impact on the tested probability as a patient with an extensive wound that was falsely predicted not to be amputated.

To assess the feasibility of using our databases to evaluate the predictive abilities of trauma scores in a ROC AUC analysis, a sample size calculation was done. 592 patients would be needed to detect a difference of 0.10 in the trauma scores' ability to detect high surgical resource consumption in a ROC AUC. Nonetheless, all eligible patients were included in the analysis. Similar to *Paper II*, Fischer's exact test, Mann-Whitney *U*-test, and binary logistic regression with Forward Wald was used to adjust for confounders influence on the trauma score's ability to predict the studied outcomes, surgical resource consumption, and in-hospital mortality.^{71, 120, 131, 137, 138} The obtained models were tested for goodness of fit with Akaike Information Criterion (AIC). The sensitivity and specificity for the predictive abilities of RCWS, KTS, and RTS for our outcomes were then assessed using ROC AUC analyses. AUCs and 95% CI were used to determine the differences in the trauma score's ability to predict surgical resource consumption and in-hospital mortality. Overlapping CI were interpreted as non-significant differences. Little's MCAR test and imputation with fully conditional specification was done to enable the analysis of incomplete cases and thereby assessing the impact of exclusion of patients with unknown gender, time of injury and vital signs. Mann-Whitney *U*-test with Fisher's exact test was used to evaluate why a patient ended up in the high-resource consumption group.

Study Design and Method (Paper IV)

The starting point for *Paper IV* was that both my supervisor Peter Andersson and I had experienced difficulties with getting a surgical debridement just right. One either tended to be too limited or too overzealous in one's excision, especially if you have limited experience of war surgery. For the surgeon, a suboptimal debridement will result in the need for repeated surgical procedures to achieve wound closure, i.e., suboptimal use of surgical resources. For the patient, repeated debridements can result in unnecessary suffering, and in the worst case, lifelong disabilities. Consequently, there is much to gain if methods could be identified that can increase the efficacy of the debridement. We initially had the idea of evaluating the Tissue Viability Imager (TiVi®) for this purpose. The TiVi® is an image-based method of measuring microcirculation in skin developed at Linköping University. The technology is easy to learn and can be incorporated in handheld, low power devices equipped with cameras, e.g., smartphones. However, in the pilot study, we found that TiVi® was not sensitive enough to assess changes in microcirculation in wounds. At the same time my co-supervisor Andreas Wladis and co-author Johan Junker had started to outline a project to evaluate the use of honey in wound care. Reading up on honey, I found that its antimicrobial properties, low cost, almost indefinite shelf life, and readily availability worldwide would make it an ideal candidate for making treatment of wounds more efficient, especially in resource-constrained conflict settings, thereby potentially reducing the need for repeated debridements. Therefore, the original study proposal was revised to a non-inferiority experimental study of the efficacy of topical treatment with Manuka honey compared to systemic treatment with intramuscular (IM) gentamicin for *S. aureus* infected wounds. *S. aureus* was chosen as it is one of the most commonly detected bacteria in infected wounds in armed conflicts and is prone to develop antimicrobial resistance.^{90, 91, 139, 140} Several types of honey exist depending on the floral source. The Manuka honey was chosen due to its established broad antimicrobial spectrum, including commonly found wound pathogens in armed conflicts.^{90, 102, 141-143} The Manuka honey is a monofloral honey produced by the *Apis mellifera* honeybees that collect nectar from the flowers of the Manuka tree (*Leptospermum scoparium*). Gentamicin was chosen as it has bactericidal properties against *S. aureus*, is generally available in LMIC and is recommended treatment for severe cutaneous infections by organizations that provide health care in armed conflicts.^{68, 144, 145} The porcine wound model was chosen as it is a well-established experimental model for human wound healing that would allow us to study the efficacy of honey with a minimum of confounding factors.¹⁴⁶⁻¹⁴⁸ Response to treatment was evaluated with quantitative cultures of *S. aureus*, wound area measurements, histological and immunohistochemical assays, as well as assessment of inflammatory response. The report for this study was written according to the ARRIVE guidelines for reporting on animal research.¹⁴⁹

Data Management

All data obtained from the experiment were entered into Excel® spreadsheets, encrypted, and stored in the Linköping University cloud storage. No paper copies were stored. All participating researchers had access to the collected data. The data will continue to be available for participating researchers and upon reasonable request until 2030, when the data will be destroyed, as stipulated by Linköping University.

Statistical Analysis

Crucial for this study was to ensure that we had enough wounds that would allow for sufficient statistical power to demonstrate if topical honey was non-inferior to IM gentamicin as treatment for *S. aureus* infected wounds. Therefore, substantial consideration was given to the sample size calculation. For primary endpoint, we chose reduction of bacteria in tissue after eight days of treatment. Reduction to $\leq 3 \log_{10}$ CFU/g of tissue after eight days of treatment was considered successful. The rationale being that the potential of wound healing is significantly impaired, i.e., infected, with bacterial bioburden of $>5 \log_{10}$ CFU/g and the bacterial bioburden of non-infected skin is $2 - 3 \log_{10}$ CFU/g.^{87, 88} With a non-inferiority margin of 0.21, to allow for error in the application of honey, we calculated that 19 samples were needed in each of the two treatment arms to demonstrate a non-inferiority at 90% power. Therefore, five samples were needed at each of the four time points to achieve a total sample size of 20 for each treatment arm and to allow for some margin of error. An additional ten wounds were created on the pig predetermined for topical honey treatment as we expected wounds to be excluded due to leakage of the applied honey. Nonetheless, data were collected from all wounds. Results were given as mean $\pm$ standard deviation (SD), except for the quantitative cultures of *S. aureus* in wound tissue over time, where instead geometrical mean and geometric standard deviation (GSD) were used. This was done to include all observations and to diminish misleading visual effects caused by the $\log_{10}$ scale. Comparisons between treatment groups were performed using a mixed-model analysis of variance (ANOVA) with Greenhouse-Geisser correction to avoid violation of sphericity.

MAIN FINDINGS AND DISCUSSION

Descriptive Findings (*Papers I – III*)

Characteristics of the patients included in *Papers I – III* are shown in Table 6. Consistent with other reports from other armed conflicts, most patients were male in their mid-twenties and arrived at the hospital more than six hours after the injury.^{66, 119, 150} Injuries caused by indiscriminate weapons were more frequent in patients treated in Peshawar compared to Goma. This finding is coherent with the previously described differences in weapons used in these conflicts. Even though a substantial portion of the patients treated in Peshawar 2009 – 2012 were injured by indiscriminate weapons, only 5% of the patients had injuries caused by anti-personnel mines (APM). This figure contrasts sharply with figures reported from the beginning of the 1990s, where 48% of all weapon-wounded from the same area in northwestern Pakistan and neighboring Afghanistan were injured due to mines.⁶³ Consistent with this we also noted a higher number of APM-related injuries in the cohort of the patients treated for extremity injuries in Quetta 1992 – 1995 analysed in *Paper II* (19% of the patients). The potential decline in APM-related injuries between 1992 – 1995 and 2009 – 2012 is most likely a favorable result of the global work against landmines, launched by the ICRC, resulting in the Ottawa treaty in 1997, prohibiting their use.¹⁵¹ Less than 20% of the patients analysed in *Papers I – III* were subjected to any major surgery, and in-hospital mortality was under 5% in all the studied cohorts. The low in-hospital mortality, absence of need of major surgery, coupled with that most patients arrived more than six hours after the injury, indicates potential survivorship bias. Consequently, the early but potentially preventable, deaths in the trimodal distribution of trauma death proposed by Trunkey is not seen in our material.^{68, 152}

Table 6 Overview of patient characteristics per hospital site

	Hospital site and period				Total <i>n</i> = 3,668 (%)
	Quetta 1992 – 1995 <i>n</i> = 419 (%)	Peshawar 1992 – 1995 <i>n</i> = 44 (%)	Peshawar 2009 – 2012 <i>n</i> = 3,028 (%)	Goma 2012 – 2014 <i>n</i> = 177 (%)	
Included in <i>Paper</i>	<i>II</i>	<i>II</i>	<i>I – III</i>	<i>III</i>	
Injury location included	Extremity	Extremity	Any	Any	
Gender					
Male	405 (97)	44 (100)	2,622 (87)	149 (84)	3,220 (88)
Female	14 (3)	0	376 (12)	28 (16)	418 (11)
Missing	0	0	30 (1)	0	30 (1)
Median age (IQR ^a)	26 (10)	25 (10)	24 (14)	28 (13)	25 (14)
Injury mechanism					
Bullet	254 (61)	26 (59)	1,119 (37)	156 (88)	1,555 (42)
APM ^b	79 (19)	12 (27)	166 (5)	0	257 (7)
Indiscriminate weapon ^c	80 (19)	6 (14)	1,679 (56)	17 (9)	1,782 (49)
Other	6 (1)	0	32 (1)	3 (2)	41 (1)
Missing	0	0	32 (1)	1 (1)	33 (1)
Time since injury					
≤6 hours	13 (3)	12 (27)	401 (13)	35 (20)	461 (13)
7 – 24 hours	205 (49)	25 (57)	830 (27)	78 (44)	1,138 (31)
>24 hours	201 (48)	7 (16)	1797 (60)	64 (36)	2,069 (56)
Major surgery					
Craniotomy	0	0	61 (2)	2 (1)	63 (1.5)
Thoracotomy	0	0	24 (1)	0	24 (0.5)
Laparotomy	0	0	270 (9)	14 (8)	284 (8)
Amputation	40 (10)	6 (14)	239 (8)	8 (5)	293 (8)
None of the above	379 (90)	38 (86)	2,434 (80)	153 (86)	3,004 (82)
Discharge status					
Alive	414 (99)	44 (100)	2,893 (96)	174 (98)	3,525 (96)
Dead	5 (1)	0	135 (4)	3 (2)	143 (4)

^aInterquartile range (IQR); ^bAnti-personnel mine; ^cBombs, shells, missiles, and other explosives

Females were more likely to be injured by indiscriminate weapons than men and had more extensive surgical treatment needs (*Paper I*)

A total of 3,028 patients (378 females) were treated for weapon-related injuries at ICRC hospital in Peshawar from February 2009 to May 2012. 12.4% of the treated patients were females. This contrasts sharply with reports from other armed conflicts where females have constituted up to 43% of the treated patients.^{153, 154} The observed difference may be explained by the fact that ICRC facilities are devoted to only treating patients with weapon-related injuries, whereas other health care facilities in conflict areas may also treat patients with conditions that are not weapon-related. This notion is supported by a report, published in 2016, from the Médecines sans Frontières (MSF) trauma center in Kunduz, Afghanistan, that operated in a similar context as the ICRC hospital in Peshawar. At this center, a comparable proportion of the treated patients were females.¹⁵⁵ It could also be explained by differences in demographics and cultural structures.

In our material, females were injured by fragments from mortars and bombs significantly more often than males. Most likely, these injuries were sustained in public places or domestic settings. Recent reports from Syria reveal a similar pattern of injuries among women and children, with the highest death tolls caused by fragments emanating from air bombardments and ground explosives.¹⁵⁶ These weapons are indiscriminate by nature, affecting civilians to a proportionally greater extent than combatants, as shown by data from Kabul as far back in time as 30 years and also from Iraq between 2003 and 2008.^{157, 158}

Furthermore, females arrived sooner after the injury and had more altered vital parameters than the males. Females required more surgical procedures per person and were given more blood transfusions. They were also subjected to more surgical procedures involving the skull, thorax, and abdomen. This is more or less the opposite of what Tounsi *et al.* reported in 2019, from the MSF Trauma Centre in Kunduz, Afghanistan.¹⁵⁴ They found that females arrived later and were less likely to undergo surgery compared to males. However, less than 10% of the females treated at MSF's Trauma Center in Kunduz had weapon-related injuries, whereas ICRC facilities, as mentioned earlier, only treated weapon-related injuries.¹⁵⁴ Comparisons between surgical treatment needs for females in these facilities are therefore difficult to do. Furthermore, the difference in surgical treatment needs for females and males observed in both Tounsi's and our study could be attributed to survivorship bias. Supporting this notion is that males in our material reached the hospital significantly later and with less severe injuries than females. This is consistent with a report from Coupland from the same area where he suggested that females injured at a greater distance from the ICRC hospital with less severe injuries were never transported to the hospital.⁶⁹ This could be due to limited transportation resources and that men were given priority. Additionally, the greater alteration of vital parameters found in females indicates that the females represent a different cohort of weapon-wounded than the cohort of males. Most likely, the female cohort comes from areas closer to the hospital than the male cohort. Given the shorter transportation time to the hospital, they were more likely to survive more severe injuries than males.

In low-resource conflict settings the risk of amputation was unchanged over time (*Paper II*)

Consecutive data from 535 patients treated in 2009 – 2012 and 463 patients treated in 1992 – 1995 were analysed. After adjusting for confounders, particularly the degree of tissue damage, no difference in amputation-risk could be detected between 1992 – 1995 and 2009 – 2012, nor were there any statistically significant differences in the use of DBR/COD, length of hospital stay, or in-hospital mortality. However, the use of external fixation, SSG, and blood transfusion had declined in 2009 – 2012. There are several plausible explanations for the observed differences in surgical procedures between the two periods. Surgeons deployed by the ICRC during both periods were predominantly general surgeons from high-resource countries (personal communication with the ICRC health unit). Their familiarity with basic orthopaedic, plastic, and vascular surgery may have varied. This is especially true for the later period as current trends in surgical training in high-resource countries have favoured subspecialisation with specific rather than general skill sets.¹⁵⁹ In a recent survey among ICRC health care staff on self-perceived training needs, 31% requested additional training in fracture surgery, 20% in reconstructive surgery and 19% in vascular surgery.²⁷ This, together with our findings, indicates that the pre-deployment preparation and international team composition should be adjusted to address context varying injuries better. The decreased use of external fixation in 2009 – 2012 could also be due to more restrictive use of this technique, based on changes in ICRC treatment practices between the periods (personal communication with former ICRC coordinator of surgical programs Åsa Molde). The frequency of amputations in this study remained the same over time, whereas it has gradually decreased in civilian high-resource settings.^{160, 161} This discrepancy in treatment routines between high- and low-resource settings is not unusual and is also seen for other types of trauma.²⁶ Availability of equipment, medicines, and familiarity with reconstructive techniques including flaps, will affect how the surgeons in the field choose to deal with complex limb injuries.

RCWS predicted surgical resource consumption better than KTS and RTS (Paper III)

When comparing RCWS with KTS and RTS, commonly used trauma scores in non-conflict settings, RCWS had a better ability to predict surgical resource consumption. This is consistent with previous studies that have demonstrated an association between RCWS and the need for surgery, in-hospital amputation, and blood transfusions.^{65, 162, 163} Furthermore, in a study from our group, RCWS correlated with the number of surgeries, blood transfusions, and LOS in paediatric patients with weapon-related extremity wounds.¹⁶⁴ The better predictive ability for RCWS, compared to KTS and RTS, might not be surprising since both the former scores were designed to predict mortality following trauma.^{74, 76} However, to our knowledge, no instrument or trauma score has yet been validated to measure surgical resource use in conflict settings. KTS and RTS were chosen as they are commonly used trauma scores and have been shown to correlate with resource-related outcomes in trauma settings.^{72-74, 165, 166} Furthermore, RCWS, KTS, and RTS are similar in the aspect that they can be assessed without the need of advanced diagnostic equipment or calculations. A source of error is that we had a large number of patients with missing vital parameters in our dataset, potentially affecting the predictive abilities for KTS and RTS. To address this issue, we performed a logistic regression analysis using imputed data where data was missing, e.g., for missing vital parameters, without any substantial changes in KTS and RTS predictive ability for surgical resource consumption.

Consistent with other studies from armed conflicts, a low in-hospital mortality was observed at both hospitals.^{119, 155} In the logistic regression analysis, none of the components of RCWS, KTS, and RTS were found to be significant predictors for in-hospital mortality. However, when performing the logistic regression analysis with imputed data, RCWS wound types threatening life and threatening life or limb and systolic blood pressure 76 – 89 mmHg were found to be significant predictors for in-hospital mortality, rendering significantly better predictive ability for RCWS than KTS and RTS. The grade component of RCWS has previously been found to correlate with mortality in patients with conflict-related abdominal wounds with penetration of the peritoneum.¹⁶⁷ Otherwise, RCWS predictive ability for mortality is poorly examined. In contrast to the findings in this study, KTS and RTS have demonstrated good predictive ability for mortality following weapon-related injuries in non-conflict settings.^{168, 169} The inferior performance of KTS and RTS in the present study could be explained by survivorship bias. The low in-hospital mortality and that more than 90% of the patients in our material arrived more than six hours after the injury occurred supports this assumption. The conclusion that RCWS has better predictive ability for in-hospital mortality than KTS and RTS in conflicts must therefore be made with caution.

Honey may be non-inferior to gentamicin in reducing *S. aureus* colonization, but not in reducing wound size (*Paper IV*)

Despite the established antimicrobial properties of honey in *in vitro* models, similar data from more complex experimental models and humans are scarce.^{103, 106} In a study by Medeiros *et al.*, a significant reduction of the bacterial count could be demonstrated with *Melipona scutellaris* honey treatment compared to saline treatment of methicillin-resistant *S. aureus* (MRSA) infected wounds in rats.¹⁷⁰ In this study, inoculation was done by applying a gauze with 5×10^7 colony forming units (CFU) of MRSA (ATCC 43300), and then wounds were sutured closed for 24 hours. Wounds were then opened, the cavity drained, and treatment was initiated. The bacterial count was assessed only by quantitative cultures. Given the method of inoculation and short period before initiation of treatment in Medeiros study there is reason to suspect that the infection of MRSA was superficial and therefore accessible to topical treatment. To better resemble actual clinical settings in armed conflicts, we chose to inoculate wounds with *S. aureus* by tunneling through adjacent healthy tissue, wounds were not closed, and more importantly, treatment was initiated after 48 hours. The porcine model was chosen as it is a pragmatic approach to simulate the pathophysiology of infected traumatic wounds seen in humans without the interference of confounding factors such as comorbidity, nutritional state, time since injury and mechanism of injury that otherwise could have influenced the results.^{146, 171, 172} In our model, we did not observe any reduction in the bacterial count in the wounds treated with topical Manuka honey for the duration of the study. This is most likely due to the inability of honey to reach bacteria that had infiltrated in the tissue. Tissue infiltration of *S. aureus* was confirmed in the immunohistochemical analysis, as we could identify growth of bacteria in the wound tissue and not only on the surface of the wound. For the wounds treated with IM gentamicin a reduction in bacterial count was observed after two days of treatment, which included the first day of treatment when gentamicin was administered twice compared to once daily for the remaining treatment. This reduction in bacterial count was not sustained for the duration of the study, conceivably attributed to the inability of gentamicin to reach bacteria in devitalized tissue, dosage of antibiotics was too low, or a combination thereof.

Consistent with the lack of reduction in bacterial count, we did not observe a reduction in wound surface area with topical honey treatment. Conversely, we did not observe an increase in wound surface area, which would be expected if the Manuka honey did not have any effect on the bacterial activity. Manuka honey could therefore have exerted an antimicrobial effect on the bacteria colonizing the surface of the wound, but not on the bacteria that had infiltrated the tissue. For wounds treated with intramuscular gentamicin, we observed a reduction in wound surface area and signs of reepithelization. This was probably because gentamicin inhibited growth of *S. aureus* in surrounding tissue and thereby enabled wound repair. Our negative findings regarding the efficacy of Manuka honey on healing of infected wounds are in line with the conclusion of the Cochrane review from 2015, as only one study was identified that could provide moderate evidence for the efficacy of honey as treatment for infected wounds.^{106, 173} In this randomized controlled trial, by Al-Waili *et al.*, severely infected wounds following caesarian section or total abdominal hysterectomies were treated with either topical honey or antiseptic washes. Wounds treated with honey dressings exhibited a shorter time to eradication of bacterial infection, wound healing, and reduced scar formation.¹⁷³ However, both groups were treated with systemic antibiotics, and studies have indicated that the chosen antiseptic may negatively affect wound healing.^{174, 175} It is therefore unclear

if Al-Waili *et al.* could demonstrate any additional benefit with topical honey compared to systemic antibiotics only.

Our findings of unchanged wound size and *S. aureus* count in wound tissue indicate that the use of topical Manuka honey may be of benefit to prevent further progression of a wound infection and subsequent complications. This notion is especially interesting in settings where antibiotics and surgical care are not immediately available, such as in armed conflicts. Manuka honey dressings could be used at the site of injury, at trauma stabilization points, and during transports to inhibit further progression of a wound infection until definite care can be undertaken. Manuka honey may also constitute a valuable adjunct to antibiotics and surgical debridement. *In vitro* and *ex vivo* studies have demonstrated increased antimicrobial activity when using antibiotics combined with Manuka honey against *S. aureus*, *P. aeruginosa*, and MRSA.¹⁷⁶⁻¹⁷⁸ Furthermore, a study by Jenkins and Cooper showed that subinhibitory concentrations of Manuka honey made MRSA susceptible to oxacillin again, reversing the resistance.¹⁷⁹ Essential to this synergistic effect of antibiotics and Manuka honey is Manuka honey's ability to interrupt biofilms produced by bacteria, thereby increasing the bacteria's susceptibility to antibiotics.^{180, 181} Honey's antimicrobial and antibiofilm activity could also be of benefit in the most fundamental part of the treatment of infected wounds in armed conflict, the surgical debridement. Applying honey dressings at the end of the debridement could inhibit recolonization of pathogenic bacteria on the wound surface and subsequently hinder redevelopment of devitalized tissue. This may possibly result in a diminished need for repeated debridements and a shorter time to achieve wound closure. However, this needs to be extensively evaluated in *in vivo* studies before honey can become relevant in the treatment of infected wounds in armed conflicts.

METHODOLOGICAL CONSIDERATIONS

Research on Routinely Collected Data (*Papers I – III*)

Strengths

The overall aim of this thesis was to explore the relationship between injury mechanism, injury patterns, and surgical resources consumption to identify predictive factors and treatment practices that can improve how surgical resources are used in armed conflicts. Using routinely collected clinical data to study this relationship inherently poses limitations on study design and subsequently the strength of the results. Ideal would have been to do a multi-site prospective observational study. However, conducting prospective studies in settings where staffing, material resources, work conditions, and infrastructure are strained poses limitations on what can be expected in terms of extent and consistency of data collection. Furthermore, research on routine data is essential for generating a well-founded hypothesis that would justify implementing a study that could provide strong evidence for the suggested hypothesis. The retrospective cohort design used, with already collected data, allowed for a relatively quick and straightforward data collection without study subjects being lost during the study. Since we included all eligible patients at the study locations during the given periods, the risk for selection bias should be reduced. Recall bias should not be a problem as the data were collected when the patients were treated. Additionally, observer bias is less of a problem than it would be in a prospective cohort study.

Limitations

The data collected meets the intended needs, and we have good control of how it was collected. Nevertheless, the cohort retrospective study design does not allow for the same possibilities of matching the study subjects regarding confounding factors such as age, gender, time since injury, training of medical staff, and fluctuations in access to drugs, blood products, and disposable material as a prospective study would. The predominance of males in the material used in this thesis could indicate a selection bias where many of the patients were combatants and not civilians. It could also be a result of how the society in the study area is structured. In Muslim countries, it may be anticipated that men spend more time outside of the home and thereby runs a greater risk of being exposed to the consequences of an armed conflict. In *Paper I* the issue of selection bias was addressed by assessing injury mechanism and surgical treatment needs in females and children under 16 years, as a strictly civilian group. Even so, I cannot claim that the data examined represent how the mechanism of injury, injury patterns, and treatment needs were for the whole population affected by the conflicts included in this thesis. To explore this aim, a different study design with data from several sources would be needed. Survivorship bias, especially regarding the relationship between trauma scores and surgical resource consumption examined in *Paper III*, is present, and caution must therefore be taken when applying the results from this thesis for prehospital care or surgical care with other capabilities than studied hospital sites. Finally, there is a relatively high proportion of missing data for some variables, especially for vital parameters analysed in *Paper III*. This data is most likely not missing at random. The reasons could be related to lack of stethoscopes, blood pressure cuffs, skills, motivation, or documentation. I have tried to address this issue by also analysing a dataset with imputed values for missing data with only minor variations in results compared to the analysis of patients with complete data.

Internal Validity

Since I cannot validate the entries in the databases, I must assume that the internal validity of the results could be affected. Given the limitations of routinely collected clinical data, there could be a problem with hidden bias as there might be confounders, unknown to me, that have a significant influence on the observed effects. Hence, the trouble with differentiating between cause and effect is present with the chosen study design, which I have tried to take into consideration when interpreting the data.

External Validity

A factor that strengthens the external validity is that all patients studied were treated according to the same treatment protocols that all ICRC hospitals adhere to. Furthermore, ICRC hospitals in different conflicts have similar setup and capabilities.¹¹⁹ A potential threat to the external validity is variations in how armed conflict is conducted in different settings. Weapons used, infrastructure, affected population, and differences in injury patterns can all affect the external validity of the results in this thesis. Therefore, I cannot claim that the results can be applied globally to all treatment facilities in present and future conflicts.

The Porcine Wound Model (*Paper IV*)

Strength

The porcine model used in *Paper IV* is a well-established model for human wound healing.^{146, 172} The skin of the pig has been demonstrated to have similar cellular composition, morphology, and immunological properties to human skin.^{182, 183} The method of bacterial inoculation and time to initiation of treatment that was chosen allowed for infiltration of bacteria into the wound tissue. The presence of bacteria in the wound tissue was also verified through histological and immunohistochemical assays. Moreover, the venous blood analyses demonstrated that both animals had a systemic response consistent with a severe infection. Therefore, the selected study design was well suited to provide results that could be useful in the treatment of weapon-caused infected wounds in humans.

Limitations

The study was designed as a non-inferiority study regarding the efficacy of topically applied honey compared to intramuscular gentamicin as a treatment for infected wounds. Thus, the study design implies that gentamicin would be effective and that we would observe a decrease in the bacterial count in the wound with gentamicin. However, we could only demonstrate a decrease in the bacterial count during the first two days of treatment when gentamicin was administered twice, but this was not sustained for the duration of the treatment. Concerns must therefore be raised regarding the choice and dosage of antibiotics. Furthermore, in the pig that received topically applied honey treatment 30 wounds were created, compared to 20 wounds in the pig that received gentamicin. This corresponds to approx. 0.24% and 0.16% of total surface area, for pig treated with topical honey and intramuscular gentamicin respectively.¹⁸⁴ The reason for the difference in number of wounds was that I initially intended to have wounds that did not receive any treatment at all. However, at the start of the treatment, we decided to apply topical honey to all 30 wounds to ensure adequate data collection, even if wounds were excluded due to leakage of honey. Most likely, the minor difference in wound area of total surface area in the two pigs did not have an impact on the primary outcome of comparison of reduction in the bacterial count for the two treatments. Nonetheless, it cannot be excluded that the difference in wound area for the different treatments could have affected the comparison of our secondary outcomes: mean reduction of wound area per wound and inflammatory response.

Internal Validity

The porcine model was chosen as it allowed us to study a cause-and-effect relationship between treatments and outcomes, in a complex model with biological properties relevant to humans, with a minimum of confounding factors that otherwise could have influenced this relationship. Other than problems stated in the general weakness section, I have not been able to identify any other systematic errors that could have had a substantial impact on the cause-and-effect relationship that was studied. The internal validity of the study is therefore considered robust.

External Validity

The underlying intention of the study design was to obtain results that could have implications on how wounds are treated in clinical settings. The chosen study design does not take into consideration that wounds in conflict settings frequently are contaminated with foreign material and have an extensive amount of devitalized tissue due to the high energy transfer at the time of injury.^{68, 90} Additionally, wound healing depends on several factors such as comorbidities, nutritional state, time since injury, and concurrent injuries, which were outside the scope of this study. And maybe most important, the best treatment for infected wounds is surgery, as clearly emphasized in the ICRC War Surgery book.⁶⁸ However, given the lack of existing data from more complex *in vivo* models, I found it essential to first quantitatively assess the efficacy of honey without any confounding factors, such as surgical debridement or antibiotics. The applicability of the results from this study to human clinical settings is therefore limited.

ETHICAL CONSIDERATIONS

For this thesis data were collected both from human and animal research subjects. Inherent to both these sources of data are specific ethical concerns that needs to be addressed when planning and implementing the studies.

Human Research Subjects (*Papers I – III*)

Most of the data for *Papers I – III* is derived from an essentially prospective database created by Zaher Osman (co-author of *Papers I – III*) from the ICRC hospital in Peshawar, Pakistan. The intended use for the database was not clearly defined when it was created. Conceivably it was created as an instrument for quality control. No information was given to the patients included in the database, nor was consent sought. Over time, an idea developed within the ICRC that it might be possible to do research on the database. At the start of this research project, the question of an ethical review was raised. The research group discussed this at length as it was unclear if there was a need for an ethical review since the research project we were planning was more from a quality control aspect, and all analysis would be done on a cohort level. It was decided that we should go through with an application for ethical review to avoid any uncertainties when submitting the scientific reports. An ethical review at this stage could also give us valuable input for future similar research projects. Where would the ethical review take place? In Linköping, where the storage and analysis of data would take place? Or in Geneva, where the headquarter of ICRC, who owns the data, is located? The most logical would be to have the ethical review done in Pakistan, where the research subjects were treated, and the data were collected. Two concerns immediately emerged with having the review done in a conflict setting. Firstly, academic institutions in areas with ongoing armed conflicts are often non- or malfunction, making local ethical approvals difficult to obtain. Secondly, based on colleagues' experiences, the reviews sometimes focus more on the political implications of the study rather than the ethical issues that the study could present. In this specific setting, this is sensitive because of the ongoing border conflict between Afghanistan and Pakistan. We decided, despite this, to pursue an ethical review in Pakistan. An ICRC representative was asked to determine the correct procedure and where to submit the ethical review application. In his inquiries, he was told by a high-level governmental official that if we submitted an application in Pakistan, no ethical review board would ever approve it. This was not because of any ethical issues with the study but for the political implications the study could have. It could be seen as very problematic if it became known that Afghans and potentially members of the Islamic State of Iraq and the Levant (ISIL), Talibans, or other terrorist groups had received medical care within the borders of Pakistan. With this at hand, the following ethical questions surfaced:

- Is it acceptable to follow through with the purposed study without a local ethical review?
- Who would safeguard the research subjects' interest?
- If the study would cause harm that we have not foreseen, what are our responsibilities?
- How can we inform the affected population of the findings of the study?
- How can we make sure that we live up to the Helsinki declaration?¹⁸⁵

An easy solution to the ethical questions raised would be to restrict the ethical analysis to just the study at hand and not its possible ramifications:

1. Autonomy: as the study will be retrospective, and the patients cannot be traced, informed consent is therefore not applicable. Confidentiality is guaranteed, as the data will be anonymized.
2. Beneficence: there is no benefit to the patients treated in the past, but there is a potential benefit for future patients in the same situation.
3. Non-maleficence: there is no harm to the patients, as data will be anonymized.
4. Justification: there is an obligation to use clinical material to improve care for future patients in similar contexts.

Therefore, the conclusion would be that there are no major ethical dilemmas with the proposed study. We should therefore go ahead with the study without a local ethical review. On the other hand, if we in our ethical analysis take into consideration the potential ramifications the study could have for the research subjects and the population living in the border area, several ethical problems arise:

1. Autonomy: when patients were admitted to the studied ICRC hospitals, they should have been given information about the existence of the database. Patients could then have consented or chosen not to be included in the database.
2. Beneficence: there is no benefit for the patients treated in the past, but there is a potential benefit for future patients in the same situation. This holds true even if we take into consideration the potentially negative political implications the study could have.
3. Non-maleficence: even though the data is anonymized, there are potential threats for Afghan patients that have been treated at the ICRC hospital and lives in the border region or remain in Pakistan. The information that Afghan citizens have been given free health care within Pakistan could cause ethnic motivated attacks and even further intensify the border conflict.
4. Justification: it is hard to justify the proposed study if individuals could come to harm, even if there is a gain for patients in the future.

Therefore, the study could pose problems for the research subjects, the local population and perhaps for future research projects in similar contexts. We therefore concluded that an ethical review was needed. Since an ethical review in Pakistan was not feasible, the only realistic solution was to do an internal ethical review within the ICRC and an ethical review for exploring the data in Sweden. Furthermore, consideration had to be taken to the politically sensitive context for the study when preparing the manuscripts. With these measures, I think we accomplished to live up to the Helsinki declaration's intentions, even without a local ethical review.¹⁸⁵

Animal Research Subjects (*Paper IV*)

To evaluate the efficacy of honey as a treatment for infected wounds (*Paper IV*), we decided to use the porcine model and not human research subjects. This was primarily because we could identify that there was an apparent lack of data regarding the safety and efficacy of honey in the treatment of infected wounds in humans. Furthermore, the porcine model would allow us to quantitatively assess the efficacy of honey without confounding factors, such as comorbidities, concurrent injuries, surgical debridement, and antibiotics. This would not have been possible in human research subjects to the same extent. There are specific ethical issues that need to be considered when conducting experiments on animals. A well-known approach to examine these issues is by using the principles of the 3Rs (Replacement, Reduction, and Refinement) established by Russell and Burch in 1959:¹⁸⁶

Replacement

As *in vitro* models have demonstrated the antimicrobial effect of honey, arguments could be made that such a model could have been used to answer the research question.^{100, 101} Thus, avoiding the use of animals. However, current *in vitro* models are not complex enough to have any real resemblance to an infected wound in humans. Trying to answer the research question with an *in vitro* model would therefore not be sufficient. The porcine model was chosen as it is a well-established wound model and has been demonstrated to be highly transferable to humans.¹⁴⁶

Reduction

Prior to initiation of the experiment, power calculations with several different outcome models were performed to establish the lowest number of animals needed to answer the research question with good reliability.

Refinement

To minimize pain, suffering, and distress for the pigs, the experiment was performed under ethical approval from the regional animal ethics board and in strict accordance with the guidelines postulated by the Medical Faculty at Linköping University. The pigs were socially housed and acclimatised for more than 72 hours before start of the experiment. They were kept in boxes measuring 2x2.5m² with a light/dark cycle of 12h/12h with an ambient temperature of 18 – 20°C. The pig's wellbeing and skin temperature were monitored at least three times daily. Prior to any procedure, the animals were sedated, and at the time of wound creation, inoculation, and termination, general anesthesia was administered. Signs of postoperative pain were actively treated with analgesia.

CONCLUSIONS

- I. Females treated at an ICRC hospital in northwestern Pakistan were markedly affected by indiscriminate weapons. Their surgical treatment needs were greater than those of males treated at the same hospitals.
- II. In resource-constrained conflict settings, the risk of amputation appears unchanged over time, while the use of external fixation and split skin grafts was less common in 2009 – 2012 than in 1992 – 1995.
- III. RCWS appears to predict high surgical resource consumption better than, and in-hospital mortality at least equal to, Kampala Trauma Score (KTS) and Revised Trauma Score (RTS).
- IV. Numbers of *S. aureus* in the tissue was not significantly different following treatment with topically applied Manuka honey compared to treatment with intramuscular gentamicin. Wound area was unchanged with topical Manuka honey and decreased with intramuscular gentamicin. Topically applied Manuka honey could therefore be non-inferior to intramuscular gentamicin in reducing *S. aureus* colonization on the wound's surface, but not in reducing wound size. The use of Manuka honey dressings prior to definite care may therefore be of value.

CLINICAL IMPLICATIONS

In this thesis, aspects of surgical care in armed conflicts have been explored. Even though the generalisability of the findings is limited, I still think there are some merits to discussing their clinical implications in a broader sense. The findings in this thesis do not have any substantial consequences for the daily clinical work of surgeons. This was, however, never my intention with this thesis, but more so an effort to understand what the surgical treatment needs are in armed conflicts and how they can be met.

The findings in *Paper I* regarding injury mechanism and surgical treatment needs in a strictly defined civilian cohort adds to the body of knowledge needed for health care providers to meet civilian's treatment needs better. In a broader sense, the findings emphasize the importance for health care providers to be sensitive to the cultural context, demographics and weapons used when providing surgical care in an armed conflict.

In *Paper II*, the risk of amputation appears to be unchanged over time, while the use of external fixation and split skin grafts seems to have decreased. These results contrast with the improved limb salvage results seen during the same period in high-resource settings.^{160, 161} It probably reflects the significant challenges of providing limb-preserving techniques in a low-resource conflict setting. Adjusting pre-deployment preparation, team composition, and adopting limb-preserving techniques from civilian trauma and military settings could be strategies to mitigate these challenges.

In *Paper III*, RCWS was able to predict the used definition of surgical resource consumption. RCWS could consequently be used to facilitate that the surgical resources available meet the actual treatment needs. The systematic use of RCWS could also enable quality audits and research in conflict settings. The inclusion of RCWS in the minimum data set recommended by Stanford humanitarian surgical response in conflict working group (Table 3) should therefore be considered.⁸²

In *Paper IV*, topically applied honey was evaluated as a solitary treatment for infected wounds. The obtained findings, coupled with the current lack of other scientific evidence supporting its use, warrants further investigations before honey can be recommended as treatment for infected wounds in armed conflicts.

FUTURE PERSPECTIVE

Future Clinical Perspective

Successful surgical treatment outcomes in trauma and military settings are highly dependent on if the patient has received adequate prehospital care and prompt transportation to the hospital.^{187, 188} For most of the patients included in this thesis this was not the case, which most likely has contributed to the selection bias discussed previously. In this context, it is of value to examine how prehospital care and transportation were organized for civilians during the Battle of Mosul. In response to an evident lack of trauma care for civilians in the Battle of Mosul (October 2016 – July 2017), the WHO developed a response that was heavily inspired by trauma pathways used by military organizations with Trauma Stabilizations Points (TSP) 10 – 15 minutes from the battle lines, field hospitals approximately an hour away and a tertiary level of care for more complex injuries. This novel approach to trauma care for civilians is interesting in several aspects: It was the first time the WHO took on a directly coordinating role. Several actors were involved at all levels of the pathway: NGOs, United Nations (UN) agencies, local health care providers, military, and private companies. Criticism has been raised that the trauma care system in Mosul sets a dangerous precedent where NGOs takes on responsibilities that, according to the Geneva Conventions, falls under the warring parties responsibility.¹⁸⁹ Furthermore, it is unclear if this military approach to civilian trauma care resulted in any real benefit for the patients as WHO's trauma system lacked many of the elements that in military systems have been demonstrated to be major contributors to mortality reduction, such as forward blood products availability, short prehospital transportation time and the possibility of air evacuation.¹⁸⁷ Nonetheless, the trauma system WHO implemented in the Battle of Mosul provides indications for how the prehospital care for civilians in armed conflicts may be organized in the future.

Future Research Perspective

In a lighthearted systematic literature review published in BMJ, by professor Anthony David, a majority of the included articles determined that there were more questions than answers as a result of their studies.¹⁹⁰ This also holds true for this thesis. The following research topics are some that would be relevant to explore further:

- Development of a universal database for injured in armed conflicts
The generalizability of the findings in this thesis would have improved if data from different care providers and conflicts settings were available. The WHO is currently discussing the possibilities of establishing a universal database for conflict settings.^{11, 66} However, before any implementation, several ethical, technical, and validity issues need to be addressed. An interdisciplinary research approach to address these issues would be preferable.
- Determining the interobserver variability for RCWS
How much variation there are between different surgeons in their assessment of a wound RCWS is unknown. A reasonable interobserver variability needs to be established before assessing RCWS predictive abilities in prospective trials or advocating for its global implementation.
- How to define surgical resource consumption
The definition used in *Paper III* was based on the ICRC definition of surgical workload. Both quantitative and qualitative research is needed to establish a definition that can be applied outside the context of ICRC.
- Prospective studies on RCWS predictive abilities
In *Paper III* we explored the predictive abilities for RCWS for surgical resource consumption and in-hospital mortality with encouraging results. However, the use of retrospective data and an unestablished outcome measure poses limitations on the generalizability of the findings. Therefore, there are good reasons to investigate RCWS predictive abilities for context-relevant outcomes using a prospective multisite study design.
- The role of honey in wound treatment
The findings in *Paper IV* indicated that honey may have the potential of inhibiting further progression of a wound infection. Furthermore, topically applied honey could constitute a valuable adjunct to antibiotics and surgical debridements, which is fundamental in the treatment of severely infected wounds in armed conflicts.⁶⁸ All of these are subjects worth exploring further. Additionally, it would be of value to assess the use of honey as a treatment for neglected wounds in low-resource settings. Small studies and case series from high-resource settings have demonstrated beneficial effects of honey dressings in the treatment of leg ulcers.^{191, 192}

PERSONAL REFLECTIONS

During medical school, somebody gave me the advice that if you decide to go into research, it should be about something you care about. This advice fell on deaf ears at the time, as I was not even sure I would go into research. However, my first fieldwork with MSF in Congo-Brazzaville, 2005 – 2006, was an eye-opener for me regarding the challenges of providing surgical care in resource-constrained conflict settings. Moreover, I was astonished by how much one can do with limited resources and what impact minor surgical procedures can have on the livelihood of whole families. The question that captivated me the most was how do you make the most of the resources and skills you have? I'm grateful that I was given the opportunity to explore issues relating to this question, guided by three supervisors with extensive clinical and scientific experience in the field.

Lessons learned

When starting this Ph.D. project, I thought the main obstacle would be to find time to do the actual research. However, with an understanding family and an accommodating employer, this turned out not to be an issue. Instead, the most challenging hurdle to pass turned out to be getting funding. After initial successes with my funding applications, I was out of luck for the last years of the work with this thesis. I had to spend, in my opinion, an unproportioned amount of time with funding applications and other administrative tasks, wondering when I would get any research done. However, as I'm sitting here completing this thesis, I must admit that I am thankful to my supervisors for guiding me through these "boring" but essential aspects of the postgraduate education. Additionally, I must admit my co-supervisor Johan von Schreeb was right. He told me during one of the first times we met that somehow funding always seems to work out in the end, which was also the case for me.

Research is easy, doing high-quality research is another story altogether. During the work with this thesis, I have become increasingly aware of how important it is to do the legwork before starting the data collection. The somewhat lacking legwork in parts of this thesis is maybe most evident in *Paper III*. The definition I used for high surgical resource consumption would have been more robust if I had also used qualitative methods in developing this definition. Delphi panel methodology with surgeons with experience working in resource-constrained conflict settings could have been one approach to reach a more well-grounded definition.¹⁹³ The positive aspect of the lack of legwork in *Paper III* is that I have become aware of qualitative research.

Manuscript writing and subsequent submission can be an excruciating process. The reviewer's feedback sometimes tends to hit you where you hurt the most. However, when editing your manuscript for the tenth time and realizing that the reviewer's feedback actually is improving the manuscript is an important insight. For me, this process could have been a bit less excruciating if I, from the onset, had followed the ARRIVE and STROBE guidelines more closely.^{129, 149}

The most rewarding experience with doing this Ph.D. project has been interacting with colleagues from all over the world, who also are passionate about improving surgical care for victims of armed conflicts.

FUNDING

This research project was investigator-initiated. No funding body has had any influence over study design, results, interpretation of results or composing of reports.

The studies in this thesis were funded by:

- Elsa and Sigurd Golje Foundation
- Linköping Society of Medicine
- RALF and ALF grants, Region Östergötland
- Kamprad Family Foundation

ACKNOWLEDGEMENTS

“No man is an island entire of itself.” (John Donne, 1572 – 1631, English scholar and priest).

This thesis would never have been brought to completion without the tireless support and concern of so many people. I would therefore like to express my immeasurable gratitude to the following persons:

Peter Andersson, main supervisor, for creating a generous and open atmosphere where insights as well as mistakes are encouraged. Always reminding me to put my family first. I could not have asked for a better supervisor.

Andreas Wladis, co-supervisor, for pushing me forward and excellent feedback on everything from study design, oral presentations to manuscripts.

Johan von Schreeb, co-supervisor, for the seed that became this thesis, asking the tough questions and for constantly reminding me that research is a process that requires sitting on your butt doing the groundwork and that a well-grounded hypothesis is more important than the results.

Zaher Osman, co-author *Papers I – III*, for having the foresight in creating the ICRC injury database that made this thesis possible.

Johan Junker, principal investigator *Paper IV*, for your no-nonsense approach to research, opened and sharp mind with just the right amount of skepticism.

Kaspar Holmgren, co-author *Paper II*, for doing a meticulous legwork for *Paper II*.

Maria Lampi, co-author *Paper IV*, for your hard work, positive and relaxed attitude.

Lars Valter, my go-to statistician, for solid statistical advice and great talks on everything from speaker placements to eloping cats.

Frederike Haverkamp and **Edward Tan**, Dutch research collaborators, for our stimulating and productive collaboration. Looking forward to keeping this going for many years. Who knows, maybe we will find science?

Jonathan Rakar, **Annika Starkenberg** and **Krisitina Briheim** for welcoming me into the laboratory and with great enthusiasm teaching and assisting me in the laboratory work for *Paper IV*.

Danne Linghammar, director of animal studies, and **Anders Sandberg**, veterinarian, for considerate monitoring, animal care and technical assistance during the animal experimentation for *Paper IV*.

Alexander Larsson and **Elvira Lindholm**, fellow Ph.D.-candidates, and slide reviewers for *Paper IV*, for willingly assisting me without any questions asked.

Andreas Älgå, fellow researcher, for being a great sounding board and researcher.

Robin Coupland and **Harald Veen**, former ICRC Chief Surgeons, for opening the doors to the ICRC and thereby making this thesis possible.

The patients and **animals** who made this thesis possible.

Susanna Lönnqvist, project coordinator, for enthusiastic support and meticulous proofreading.

Ann Cherian and **Peter Cox**, colleagues, for speedy and constructive language proofreading.

Hans Krook, **Jessica Frisk** and **Lars Lönn**, former and present heads of the Surgical Department at Vrinnevi hospital, for recruiting me and your unwavering support in this pursuit.

Staffan Haapaniemi, **Jakob Hager**, **Jonas Widell**, **Vidar Hjertberg**, **Karin Almlöv** and **Shiraz Qadar**, colleagues at the Colorectal section, for being the best colleagues I could ask for. This thesis would never have been completed without you picking up my slack.

Gunnar Arbman, senior surgical colleague, for being such an excellent role model.

Kalev Teder, senior surgical colleague, for my first inguinal hernia repair as an intern.

My colleagues at the Surgical Department for the awesome surgical care you provide every day. Love working with every one of you.

The PD-section for its relentless pursuit of perfection.

Lotta, **Hampus**, **Mårten** and **Disa**, my family, for constantly reminding me that there are more important things in life than this thesis. I'm eternally grateful.

Helena Iacobaeus, **Olle Muhrbeck**, **Anders Landin** and **Eva Oddby**, my parents, for your unyielding support no matter what path I have chosen.

Martina Iacobaeus, **Pontus Muhrbeck**, **Torsten Muhrbeck**, **Hugo Muhrbeck**, **Olivia Muhrbeck**, **Paul Muhrbeck** and **Julia Muhrbeck**, my siblings, for being as loving and mean as siblings should be.

Torsten Jonsson, my father-in-law, for being the loving and supporting father-in-law and grandfather that you are.

SAMMANFATTNING PÅ SVENSKA

Väpnade konflikter leder till kollaps av existerande hälso-och sjukvårdssystem, vilket snabbt begränsar eller omöjliggör kirurgisk vård för patienter med potentiellt livshotande skador. Dessutom är skador i väpnade konflikter ofta mer komplexa och resurskrävande än skador som förekommer i fredstid. För vårdgivare behövs därför kunskap om vad som påverkar det kirurgiska resursbehovet i konflikter, men även metoder för att kunna följa och förutsäga det samma. Internationella Rödakorskommittén (ICRC) har sedan 1990-talet använt ett system för sårklassificering (RCWS) för att hjälpa kirurger att bedöma och prioritera penetrerande sårskador. Det är okänt i vilken utsträckning RCWS eller andra klassificeringssystem som används i civil traumasjukvård kan användas för att förutsäga kirurgiskt resursbehov och dödlighet i resursknappa konfliktmiljöer.

En betydande del av kirurgiska resurser i konflikter används för behandling av infekterade sår. Många vårdgivare förespråkar användande av honung som del i denna behandling. Detta på grund av honungs bakteriedödande och uttorkande egenskaper. Det saknas dock vetenskapligt stöd för denna uppfattning. Om honung kan påvisas vara effektivt som behandling för infekterade sår skulle behovet av antibiotika och upprepade kirurgiska behandlingar kunna minskas. Detta skulle möjliggöra mer effektivt användande av kirurgiska resurser.

Denna avhandling syftar till att öka kunskapen kring hur kirurgiska resurser används i resursknappa konfliktmiljöer. Detta med målsättningen att identifiera metoder för att förutsäga och mäta det kirurgiska resursbehovet och göra användandet av det samma mera effektivt.

I tre av de studier som ingår i avhandlingen har rutinmässigt insamlade journaluppgifter från patienter som behandlats vid tre ICRC sjukhus använts. Patienterna hade vapen-orsakade skador från konflikten vid gränsen mellan Afghanistan och Pakistan eller konflikten i Kivu-regionen i Demokratiska republiken Kongo (DRC). I den första studien undersöktes förhållandet mellan kön, skademekanism, uppkomna skador och erhållen kirurgisk behandling för patienter som behandlats vid ICRC sjukhuset i Peshawar, Pakistan. I den andra studien undersöktes hur behandlingen av vapen-orsakade extremitetsskador skilde sig mellan perioderna 1992 – 1995 och 2009 – 2012 vid ICRC sjukhus i Quetta och Peshawar, Pakistan. I den tredje studien prövades hur väl RCWS samt poängssystem som används i civil traumasjukvård kunde förutsäga det kirurgiska behandlingsbehovet hos patienter vid ICRC sjukhus i Peshawar, Pakistan, och Goma, DRC. I den sista studien jämfördes honung och antibiotika som behandling av infekterade sår i en försöksdjursmodell. De båda behandlingarna utvärderades och jämfördes genom bakterieodlingar, mätningar av säryta, histologiska och immunhistokemiska analyser samt inflammatoriskt svar.

På de studerade ICRC sjukhusen var de flesta patienterna män i tjugoårsåldern med skador på armar eller ben. Skador från urskillningslösa vapen, som granater, missiler och bomber, var mer frekvent förekommande i gränskonflikten mellan Afghanistan och Pakistan, medan skottskador var vanligare i Kivu-konflikten. Behandling av mjukdelsskador var den vanligaste typen av kirurgi. Mindre än 20% av patienterna genomgick större operationer och dödligheten under sjukhusvistelsen var mindre än 5%. Kvinnor skadades i större omfattning av urskillningslösa vapen, hade allvarligare skador, fick oftare blodtransfusioner och behandlades med mera omfattande

kirurgi än männen. Trots att skador på armar och ben är vanligt förekommande i väpnade konflikter så har kirurgisk behandling som gör att amputation kan undvikas inte minskat under de senaste årtiondena. RCWS var bättre på att förutsäga kirurgisk resursförbrukning än de poängsystem som används i civil traumasjukvård. Slutligen visade den sista studien i avhandlingen att varken honung eller antibiotika minskar antalet bakterier i sår efter avslutad behandling. Däremot minskade sårens storlek med antibiotika, men var oförändrade med honung. Möjligen skulle därför honung kunna vara effektivt för att förhindra bakterieväxt på sårytan. Det finns därför anledning att undersöka om honung kan vara ett värdefullt tillägg till antibiotika och kirurgisk behandling. Detta skulle kunna förbättra användandet av de begränsade kirurgiska resurser som finns tillgängliga i väpnade konflikter.

REFERENCES

1. O'Neill KM, Greenberg SL, Cherian M, et al. Bellwether procedures for monitoring and planning essential surgical care in low- and middle-income countries: caesarean delivery, laparotomy, and treatment of open fractures. *World J Surg* 2016; **40**(11): 2611-9.
2. UCDP. <http://www.ucdp.uu.se> (accessed 15 October 2019).
3. ICRC. What is International humanitarian law? 2004. https://www.icrc.org/en/doc/assets/files/other/what_is_ihl.pdf (accessed 9 May 2021).
4. Gennarelli TA, Wodzin E. Abbreviated injury scale 2005: update 2008. Barrington, Ill.: Association for the Advancement of Automotive Medicine; 2008.
5. Arreguin-Toft IM. How the weak win wars : a theory of asymmetric conflict. Cambridge: Cambridge University Press; 2005.
6. Stuhmiller JH. Blast injury: translating research into operational medicine. In: Friedl KE, Santee WR, eds. Military quantitative physiology: problems and concepts in military operational medicine. Falls Church, Virginia: Office of the Surgeon General; 2008: 267-302.
7. ICRC. War & law. <https://www.icrc.org/en/war-and-law> (accessed 9 May 2021).
8. Goldstuck ND. Healthcare in low-resource settings: the individual perspective. *Healthc Low Resours Settings* 2014; **2**(2).
9. WHO. Innovative care for chronic conditions: building blocks for action. 2002. <https://www.who.int/chp/knowledge/publications/iccreport/en/> (accessed 14 May 2021).
10. Bhangu A, Singh P, Lundy J, Bowley DM. Systemic review and meta-analysis of randomized clinical trials comparing primary vs delayed primary skin closure in contaminated and dirty abdominal incisions. *JAMA Surgery* 2013; **148**(8): 779-86.
11. WHO. Emergency and essential care. <https://www.who.int/surgery/en/> (accessed 9 May 2021).
12. Biology online dictionary. 2021. <https://www.biologyonline.com/dictionary/i> (accessed 1 May 2021).
13. McCord C, Ozgediz D, Beard J, Debas H. General surgical emergencies; definitions of levels of hospital care. In: Debas H, Donkor P, Gawande A, Jamison D, Kruk M, Mock C, eds. Essential surgery: disease control priorities. Washington (DC), US: The International Bank for Reconstruction and Development / The World Bank; 2015.
14. Ransom H, Olsson JM. Allocation of health care resources: principles for decision-making. *Pediatr Rev* 2017; **38**(7): 320-9.
15. Sorokin PA. Man and society in calamity; the effects of war, revolution, famine, pestilence upon human mind, behavior, social organization, and cultural life: New York, Greenwood Press; 1968.
16. Freeman RE. Strategic management : a stakeholder approach. Boston: Pitman; 1984.
17. Harper D. Online etymology dictionary. https://www.etymonline.com/word/war#etymonline_v_44585 (accessed 5 May 2021).
18. Mark JJ. War in ancient times. September 2, 2021. <https://www.worldhistory.org/war/> (accessed 6 May 2021).
19. Murray CJ, King G, Lopez AD, et al. Armed conflict as a public health problem. *BMJ* 2002; **324**(7333): 346-9.
20. GBD 2015 Mortality and Causes of Death Collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980-2015: a systematic analysis for the global burden of disease study 2015. *Lancet* 2016; **388**(10053): 1459-544.

21. Jawad M, Hone T, Vamos EP, et al. Estimating indirect mortality impacts of armed conflict in civilian populations: panel regression analyses of 193 countries, 1990 – 2017. *BMC Med* 2020; **18**(1): 266.
22. Leaning J, Guha-Sapir D. Natural disasters, armed conflict, and public health. *N Engl J Med* 2013; **369**(19): 1836-42.
23. Lunze FI, Offergeld C, Eichhorn T, et al. Trauma in conflict and postconflict settings: contributions to health systems strengthening. *Minerva Chir* 2013; **68**(3): 275-80.
24. Atiyeh BS, Gunn SW, Hayek SN. Military and civilian burn injuries during armed conflicts. *Ann Burns Fire Disasters* 2007; **20**(4): 203-15.
25. Connolly M, Ibrahim ZR, Johnson ON. Changing paradigms in lower extremity reconstruction in war-related injuries. *Mil Med Res* 2016; **3**: 9.
26. Hofman K, Primack A, Keusch G, Hrynkow S. Addressing the growing burden of trauma and injury in low- and middle-income countries. *Am J Public Health* 2005; **95**(1): 13-7.
27. Haverkamp FJC, Veen H, Hoencamp R, et al. Prepared for mission? A survey of medical personnel training needs within the International Committee of the Red Cross. *World J Surg* 2018; **42**(11): 3493-500.
28. Djalali A, Ingrassia PL, Corte FD, et al. Identifying deficiencies in national and foreign medical team responses through expert opinion surveys: implications for education and training. *Prehosp Disaster Med* 2014; **29**(4): 364-8.
29. Chu K, Stokes C, Trelles M, Ford N. Improving effective surgical delivery in humanitarian disasters: lessons from Haiti. *PLoS Med* 2011; **8**(4): e1001025.
30. Chu KM, Trelles M, Ford NP. Quality of care in humanitarian surgery. *World J Surg* 2011; **35**(6): 1169-72.
31. Van Way C. War and trauma: a history of military medicine - part I. *Mo Med* 2016; **113**(4): 260-3.
32. Van Way C. War and trauma: a history of military medicine - part II. *Mo Med* 2016; **113**(5): 336-40.
33. Manring MM, Hawk A, Calhoun JH, Andersen RC. Treatment of war wounds: a historical review. *Clin Orthop Relat Res* 2009; **467**(8): 2168-91.
34. Gawande A. Casualties of war – military care for the wounded from Iraq and Afghanistan. *N Engl J Med* 2004; **351**(24): 2471-5.
35. Blidstein G. Tikkun Olam : social responsibility in Jewish thought and law. Northvale, NJ: Jason Aronson; 1997.
36. Islamic Relief Worldwide. <https://www.islamic-relief.org/zakat/> (accessed 9 May 2021).
37. Ellis DI, Nakayama DK, Fitzgerald TN. Missions, humanitarianism, and the evolution of modern global Surgery. *Am Surg* 2020; **87**(5): 681-5.
38. Doyle GW. Builders of the Chinese church : pioneer Protestant missionaries and Chinese church leaders. Eugene, Oregon: Pickwick Publications; 2015.
39. Götz N. The Good plumpuddings' belief: British voluntary aid to Sweden during the Napoleonic wars. *Int Hist Rev* 2015; **37**(3): 519-39.
40. Fee E, Garofalo ME. Florence Nightingale and the Crimean War. *Am J Public Health* 2010; **100**(9): 1591.
41. ICRC. History of the ICRC. <https://www.icrc.org/en/who-we-are/history> (accessed 9 May 2021).
42. Boissier P. From Solferino to Tsushima: history of the International Committee of the Red Cross. Geneva: Henry Dunant Institute; 1985.
43. ICRC. The fundamental principles of the Red Cross and Red Crescent. https://www.icrc.org/en/doc/assets/files/other/icrc_002_0513.pdf (accessed 9 May 2021).
44. ICRC. The ICRC's mandate and mission. <https://www.icrc.org/en/mandate-and-mission> (accessed 9 May 2021).

45. UN. What are humanitarian principles? https://www.unocha.org/sites/dms/Documents/OOM-humanitarianprinciples_eng_June12.pdf (accessed 9 May 2021).
46. IFRIC. Who we are. <https://media.ifrc.org/ifrc/ifrc100/> (accessed 9 May 2021).
47. Reid DA, Gilbo PF. Beyond conflict : the International Federation of Red Cross and Red Crescent Societies 1919-1994. Geneva: International Federation of Red Cross and Red Crescent Societies; 1997.
48. Alexander A. A short history of international humanitarian law. *Eur J Int Law* 2015; **26**(1): 109-38.
49. Gardam J. Necessity, Proportionality and the use of force by states. Cambridge: Cambridge University Press; 2004.
50. Jr RLF. Enforcement: the difference between the law of war and the Geneva conventions. *Ga J Intl & Comp L* 1983; **13**(303).
51. Chelimo GC. Defining armed conflict in international humanitarian law. *Inquiries Journal/Student Pulse*, 2011. <http://www.inquiriesjournal.com/a?id=1697> (accessed 1 May 2021).
52. The essence of armed conflict. *Armed Confl Surv* 2018; **4**(1): 15-21.
53. Paulus Andreas VM. Asymmetrical war and the notion of armed conflict – a tentative conceptualization. *Int Rev Red Cross* 2009; **91**(873): 95-125.
54. UN. Indiscriminate attacks and indiscriminate weapons in international humanitarian law. New York, US: United Nations Human Rights Office of the High Commissioner; 2016.
55. Meara JG, Leather AJ, Hagander L, et al. Global Surgery 2030: evidence and solutions for achieving health, welfare, and economic development. *Am J Obstet Gynecol* 2015; **213**(3): 338-40.
56. WHO. Resolution A68/15: Strengthening emergency and essential surgical care and anaesthesia as a component of universal health coverage. Geneva, Switzerland: World Health Organization; 2015.
57. UN. Resolution A70/1: Transforming our world: the 2030 Agenda for Sustainable Development. New York: United Nations; 2015.
58. Geiß R. Armed violence in fragile states: low-intensity conflicts, spillover conflicts, and sporadic law enforcement operations by third parties. *Int Rev Red Cross* 2009; **91**(873): 127-142.
59. Alkire BC, Raykar NP, Shrimel MG, et al. Global access to surgical care: a modelling study. *Lancet Glob Health* 2015; **3**(6): e316-23.
60. Murray CJ, Ezzati M, Flaxman AD, et al. GBD 2010: design, definitions, and metrics. *Lancet* 2012; **380**(9859): 2063-6.
61. Backstrom F, Backstrom D, Sadi L, et al. Surgical needs at the end of the battle of Mosul: results from Mosul General Hospital. *World J Surg* 2020; **44**(3): 673-9.
62. von Schreeb J, Riddez L, Samnegard H, Rosling H. Foreign field hospitals in the recent sudden-onset disasters in Iran, Haiti, Indonesia, and Pakistan. *Prehosp Disaster Med* 2008; **23**(2): 144-51.
63. Jeffrey SJ. Antipersonnel mines: who are the victims? *J Accid Emerg Med* 1996; **13**(5): 343-6.
64. Rautio J, Paavolainen P. Afghan war wounded: experience with 200 cases. *J Trauma* 1988; **28**(4): 523-5.
65. Hinsley DE, Rosell PA, Rowlands TK, Clasper JC. Penetrating missile injuries during asymmetric warfare in the 2003 Gulf conflict. *Br J Surg* 2005; **92**(5): 637-42.
66. Wild H, Stewart BT, LeBoa C, et al. Epidemiology of injuries sustained by civilians and local combatants in contemporary armed conflict: an appeal for a shared trauma registry among humanitarian actors. *World J Surg* 2020; **44**(6): 1863-73.
67. Gertsch P. Assessment of hospital workload in war surgery. *Br J Surg* 1987; **74**(9): 831-3.

68. Giannou C, Baldan M, Comité international de la Croix-Rouge. War surgery : working with limited resources in armed conflict and other situations of violence. Geneva: ICRC; 2019.
69. Coupland RM, Korver A. Injuries from antipersonnel mines: the experience of the International Committee of the Red Cross. *BMJ* 1991; **303**(6816): 1509-12.
70. Ramalingam T. Extremity injuries remain a high surgical workload in a conflict zone: experiences of a British Field Hospital in Iraq, 2003. *J R Army Med Corps* 2004; **150**(3): 187-90.
71. Azad AD, Kong VY, Clarke DL, et al. Use of vital signs in predicting surgical intervention in a South African population: A cross-sectional study. *Int J Surg* 2020; **79**(7): 300-4.
72. Emerman CL, Shade B, Kubincanek J. A comparison of EMT judgment and prehospital trauma triage instruments. *J Trauma* 1991; **31**(10): 1369-75.
73. Raux M, Sartorius D, Le Manach Y, et al. What do prehospital trauma scores predict besides mortality? *J Trauma* 2011; **71**(3): 754-9.
74. Kobusingye OC, Lett RR. Hospital-based trauma registries in Uganda. *J Trauma* 2000; **48**(3): 498-502.
75. Sartorius D, Le Manach Y, David JS, et al. Mechanism, glasgow coma scale, age, and arterial pressure (MGAP): a new simple prehospital triage score to predict mortality in trauma patients. *Crit Care Med* 2010; **38**(3): 831-7.
76. Champion HR, Sacco WJ, Copes WS, et al. A revision of the Trauma Score. *J Trauma* 1989; **29**(5): 623-9.
77. Baker SP, O'Neill B, Haddon W, Jr., Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *J Trauma* 1974; **14**(3): 187-96.
78. Boyd CR, Tolson MA, Copes WS. Evaluating trauma care: the TRISS method. Trauma Score and the Injury Severity Score. *J Trauma* 1987; **27**(4): 370-8.
79. Soreide K. Epidemiology of major trauma. *Br J Surg* 2009; **96**(7): 697-8.
80. Coupland RM. The Red Cross classification of war wounds: the E.X.C.F.V.M. scoring system. *World J Surg* 1992; **16**(5): 910-7.
81. WHO. Emergency and Trauma Care. <https://www.who.int/emergencycare/gaci/activities/en/> (accessed 7 May 2021).
82. Wren SM, Wild HB, Gurney J, et al. A consensus framework for the humanitarian surgical response to armed conflict in 21st century warfare. *JAMA Surg* 2019; **155**(2): 114-121.
83. Haider AH, Saleem T, Leow JJ, et al. Influence of the National Trauma Data Bank on the study of trauma outcomes: is it time to set research best practices to further enhance its impact? *J Am Coll Surg* 2012; **214**(5): 756-68.
84. WHO. WHO/ICRC technical meeting for global consensus on triage 11–12 January 2017. <https://www.humanitarianresponse.info/en/operations/stima/document/guidance-triage>. (accessed 6 May 2021).
85. Weeks SR, Stevens KA, Haider AH, et al. A modified Kampala trauma score (KTS) effectively predicts mortality in trauma patients. *Injury* 2016; **47**(1): 125-9.
86. Leaper DJ, Harding KG. Wounds : biology and management. Oxford: Oxford University Press; 1998.
87. Robson MC, Lea CE, Dalton JB, Hegggers JP. Quantitative bacteriology and delayed wound closure. *Surg Forum* 1968; **19**: 501-2.
88. Bendy RH, Jr., Nuccio PA, Wolfe E, et al. Relationship of quantitative wound bacterial counts to healing of decubiti: effect of topical gentamicin. *Antimicrob Agents Chemother* 1964; **10**: 147-55.

89. Sahli ZT, Bizri AR, Abu-Sittah GS. Microbiology and risk factors associated with war-related wound infections in the Middle East. *Epidemiol Infect* 2016; **144**(13): 2848-57.
90. Eardley WG, Brown KV, Bonner TJ, et al. Infection in conflict wounded. *Philos Trans R Soc Lond B Biol Sci* 2011; **366**(1562): 204-18.
91. Alga A, Wong S, Shoaib M, et al. Infection with high proportion of multidrug-resistant bacteria in conflict-related injuries is associated with poor outcomes and excess resource consumption: a cohort study of Syrian patients treated in Jordan. *BMC Infect Dis* 2018; **18**(1): 233.
92. Fares Y, El-Zaatari M, Fares J, et al. Trauma-related infections due to cluster munitions. *J Infect Public Health* 2013; **6**(6): 482-6.
93. Bowyer G. Debridement of extremity war wounds. *J Am Acad Orthop Surg* 2006; **14**(10): 52-6.
94. Whelan TJ, Jr. Surgical lessons learned and relearned in Vietnam. *Surg Annu* 1975; **7**: 1-23.
95. Burkhalter WE, Butler B, Metz W, Omer G. Experiences with delayed primary closure of war wounds of the hand in Viet Nam. *J Bone Joint Surg Am* 1968; **50**(5): 945-54.
96. Saikaly SK, Khachemoune A. Honey and Wound Healing: An Update. *Am J Clin Dermatol* 2017; **18**(2): 237-51.
97. Blair SE, Carter DA. The potential for honey in the management of wounds and infection. *Aus Infect Control* 2005; **10**(1): 24-31.
98. Biglari B, Swing T, Büchler A, et al. Medical honey in professional wound care. *Expert Rev Dermatol* 2013; **8**(1): 51-6.
99. Administration USFaD. 510(k) premarket notification. July 12, 2007. http://www.accessdata.fda.gov/cdrh_docs/pdf5/K053095.pdf (accessed 26 January 2020).
100. Johnston M, McBride M, Dahiya D, et al. Antibacterial activity of Manuka honey and its components: an overview. *AIMS Microbiol* 2018; **4**(4): 655-64.
101. Irish J, Blair S, Carter DA. The antibacterial activity of honey derived from Australian flora. *PLoS One* 2011; **6**(3): e18229.
102. Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *J R Soc Med* 1999; **92**(6): 283-5.
103. Hixon KR, Klein RC, Eberlin CT, et al. A critical review and perspective of honey in tissue engineering and clinical wound healing. *Adv Wound Care* 2019; **8**(8): 403-15.
104. WHO. Global action plan on antimicrobial resistance. 2017. <https://www.who.int/antimicrobial-resistance/global-action-plan/research-development/en/> (accessed 24 January 2021).
105. Mulani MS, Kamble EE, Kumkar SN, et al. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Front Microbiol* 2019; **10**: 539.
106. Jull AB, Cullum N, Dumville JC, et al. Honey as a topical treatment for wounds. *Cochrane Database Syst Rev* 2015; (3): CD005083.
107. World Bank. World development indicators. 2012. <https://data.worldbank.org> (accessed 3 October 2020).
108. WHO. Global health expenditure database. 2012. <https://apps.who.int/nha/database/Select/Indicators/en> (accessed 1 December 2020).
109. Kaura V. The Durand line: a British legacy plaguing Afghan-Pakistani relations. 2017. <https://www.mei.edu/publications/durand-line-british-legacy-plaguing-afghan-pakistani-relations> (accessed 16 May 2021).
110. Christina Wagner AK. The changing character of the Durand line. *Int Asianforum* 2013; **44**(1-2): 71-84.
111. Qassem AS, Durand HM. Pak-Afghan relations: the Durand line issue. *Policy Perspect* 2008; **5**(2): 87-102.

112. Khan A, Wagner C. The changing character of the Durand Line. *Strategic Studies* 2013; **33**(2): 19-32.
113. Lyall G. Rebellion and conflict minerals in North Kivu. *Confl Trends* 2017; **2017**(1): 13-9.
114. Watch HR. Democratic Republic of Congo civilians attacked in North Kivu. New York, NY, US, 2005.
115. Autesserre S. The Trouble with the Congo: local violence and the failure of international peacebuilding. Cambridge: Cambridge University Press; 2010.
116. Rakisits C. Child Soldiers in the east of the Democratic Republic of the Congo. *Refug Surv Q* 2009; **27**(4): 108-22.
117. Peterman A, Palermo T, Bredenkamp C. Estimates and determinants of sexual violence against women in the Democratic Republic of Congo. *Am J Public Health* 2011; **101**(6): 1060-7.
118. Chu K, Havet P, Ford N, Trelles M. Surgical care for the direct and indirect victims of violence in the eastern Democratic Republic of Congo. *Confl Health* 2010; **4**: 6.
119. Haverkamp FJC, van Gennip L, Muhrbeck M, et al. Global surgery for paediatric casualties in armed conflict. *World J Emerg Surg* 2019; **14**: 55.
120. Andersson P, Muhrbeck M, Veen H, et al. Hospital workload for weapon-wounded females treated by the International Committee of the Red Cross: more work needed than for males. *World J Surg* 2018; **42**(1): 93-8.
121. Bilukha OO, Brennan M, Woodruff BA. Death and injury from landmines and unexploded ordnance in Afghanistan. *JAMA* 2003; **290**(5): 650-3.
122. Meddings DR. Civilians and war: a review and historical overview of the involvement of non-combatant populations in conflict situations. *Med Confl Surviv* 2001; **17**(1): 6-16.
123. Gustilo RB, Anderson JT. Prevention of infection in the treatment of one thousand and twenty-five open fractures of long bones: retrospective and prospective analyses. *J Bone Joint Surg Am* 1976; **58**(4): 453-8.
124. Gustilo RB, Mendoza RM, Williams DN. Problems in the management of type III (severe) open fractures: a new classification of type III open fractures. *J Trauma* 1984; **24**(8): 742-6.
125. Dickens CJF, Kilcoyne CKG, Kluk CMW, et al. Risk factors for infection and amputation following open, combat-related calcaneal fractures. *JBJS* 2013; **95**(5): e24.
126. Eshaya-Chauvin B, Coupland RM. Transfusion requirements for the management of war injured: the experience of the International Committee of the Red Cross. *Br J Anaesth* 1992; **68**(2): 221-3.
127. Korver AJ. Injuries of the lower limbs caused by antipersonnel mines: the experience of the International Committee of the Red Cross. *Injury* 1996; **27**(7): 477-9.
128. Wagner Z, Heft-Neal S, Wise PH, et al. Women and children living in areas of armed conflict in Africa: a geospatial analysis of mortality and orphanhood. *Lancet Glob Health* 2019; **7**(12): e1622-e31.
129. Ghaferi AA, Schwartz TA, Pawlik TM. STROBE: reporting guidelines for observational studies. *JAMA Surgery* 2021; **156**(6): 577-8.
130. Kim HY. Statistical notes for clinical researchers: Chi-squared test and Fisher's exact test. *Restor Dent Endod* 2017; **42**(2): 152-5.
131. Davidovic LB, Cinara IS, Ille T, et al. Civil and war peripheral arterial trauma: review of risk factors associated with limb loss. *Vascular* 2005; **13**(3): 141-7.
132. Lerner A, Soudry M. Armed conflict injuries to the extremities : a treatment manual. Heidelberg ; New York: Springer; 2011.
133. Roy N, Gerdin M, Schneider E, et al. Validation of international trauma scoring systems in urban trauma centres in India. *Injury* 2016; **47**(11): 2459-64.

134. Tamim H, Al Hazzouri AZ, Mahfoud Z, et al. The injury severity score or the new injury severity score for predicting mortality, intensive care unit admission and length of hospital stay: experience from a university hospital in a developing country. *Injury* 2008; **39**(1): 115-20.
135. Weeks SR, Juillard CJ, Monono ME, et al. Is the Kampala trauma score an effective predictor of mortality in low-resource settings? A comparison of multiple trauma severity scores. *World J Surg* 2014; **38**(8): 1905-11.
136. Halligan S, Altman DG, Mallett S. Disadvantages of using the area under the receiver operating characteristic curve to assess imaging tests: a discussion and proposal for an alternative approach. *Eur Radiol* 2015; **25**(4): 932-9.
137. Forrester JD, Forrester JA, Basimouneye JP, et al. Sex disparities among persons receiving operative care during armed conflicts. *Surgery* 2017; **162**(2): 366-76.
138. Kotwal RS, Howard JT, Orman JA, et al. The effect of a golden hour policy on the morbidity and mortality of combat casualties. *JAMA Surg* 2016; **151**(1): 15-24.
139. Petersen K, Riddle MS, Danko JR, et al. Trauma-related infections in battlefield casualties from Iraq. *Ann Surg* 2007; **245**(5): 803-11.
140. Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest* 2003; **111**(9): 1265-73.
141. Girma A, Seo W, She RC. Antibacterial activity of varying UMF-graded Manuka honeys. *PLoS One* 2019; **14**(10): e0224495.
142. Patton T, Barrett J, Brennan J, Moran N. Use of a spectrophotometric bioassay for determination of microbial sensitivity to manuka honey. *J Microbiol Methods* 2006; **64**(1): 84-95.
143. Nolan VC, Harrison J, Wright JEE, Cox JAG. Clinical Significance of Manuka and medical-grade honey for antibiotic-resistant infections: a systematic review. *Antibiotics* 2020; **9**(11): 766.
144. MSF. Clinical guidelines: diagnostic and treatment manual. Paris: Médecins Sans Frontières; 2019.
145. Tam VH, Kabbara S, Vo G, et al. Comparative pharmacodynamics of gentamicin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2006; **50**(8): 2626-31.
146. Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. *Wound Repair Regen* 2001; **9**(2): 66-76.
147. Junker JPE, Lee CCY, Samaan S, et al. Topical delivery of ultrahigh concentrations of gentamicin is highly effective in reducing bacterial levels in infected porcine full-thickness wounds. *Plast Reconstr Surg* 2015; **135**(1): 151-9.
148. Daly LT, Tsai DM, Singh M, et al. Topical minocycline effectively decontaminates and reduces inflammation in infected porcine wounds. *Plast Reconstr Surg* 2016; **138**(5): 856e-68e.
149. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. *PLoS Biol* 2020; **18**(7): e3000410.
150. Forrester JD, August A, Cai LZ, et al. The golden hour after injury among civilians caught in conflict zones. *Disaster Med Public Health Prep* 2019; **13**(5-6): 1074-82.
151. UN. Convention on the prohibition of the use, stockpiling, production and transfer of anti-personnel mines and on their destruction. 1997. <https://geneva-s3.unoda.org/static-unoda-site/pages/templates/anti-personnel-landmines-convention/APILC%2BEnglish.pdf> (accessed 8 May 2021).
152. Trunkey DD. Trauma. Accidental and intentional injuries account for more years of life lost in the U.S. than cancer and heart disease. Among the prescribed remedies are improved preventive efforts, speedier surgery and further research. *Sci Am* 1983; **249**(2): 28-35.

153. Trelles M, Dominguez L, Tayler-Smith K, et al. Providing surgery in a war-torn context: the Medecins Sans Frontieres experience in Syria. *Confl Health* 2015; **9**: 36.
154. Tounsi LL, Daebes HL, Gerdin Warnberg M, et al. Association between gender, surgery and mortality for patients treated at Medecins Sans Frontieres Trauma Centre in Kunduz, Afghanistan. *World J Surg* 2019; **43**(9): 2123-30.
155. Trelles M, Stewart BT, Hemat H, et al. Averted health burden over 4 years at Médecins Sans Frontières (MSF) Trauma Centre in Kunduz, Afghanistan, prior to its closure in 2015. *Surgery* 2016; **160**(5): 1414-21.
156. Guha-Sapir D, Rodriguez-Llanes JM, Hicks MH, et al. Civilian deaths from weapons used in the Syrian conflict. *BMJ* 2015; **351**: h4736.
157. Hicks MH, Dardagan H, Bagnall PM, et al. Casualties in civilians and coalition soldiers from suicide bombings in Iraq, 2003-10: a descriptive study. *Lancet* 2011; **378**(9794): 906-14.
158. Coupland RM, Samnegaard HO. Effect of type and transfer of conventional weapons on civilian injuries: retrospective analysis of prospective data from Red Cross hospitals. *BMJ* 1999; **319**(7207): 410-2.
159. Stitzenberg KB, Sheldon GF. Progressive specialization within general surgery: adding to the complexity of workforce planning. *J Am Coll Surg* 2005; **201**(6): 925-32.
160. Dillingham TR, Pezzin LE, MacKenzie EJ. Limb amputation and limb deficiency: epidemiology and recent trends in the United States. *South Med J* 2002; **95**(8): 875-83.
161. Goodney PP, Tarulli M, Faerber AE, et al. Fifteen-year trends in lower limb amputation, revascularization, and preventive measures among medicare patients. *JAMA Surg* 2015; **150**(1): 84-6.
162. Coupland RM. Hand grenade injuries among civilians. *JAMA* 1993; **270**(5): 624-6.
163. Muhrbeck M, Holmgren K, Osman Z, et al. Trends in demographics and surgical treatment of weapon-related limb injuries over two decades in a resource-scarce setting. *World J Surg* 2019; **43**(11): 2681-8.
164. van Gennip L, Haverkamp FJC, Muhrbeck M, et al. Using the Red Cross wound classification to predict treatment needs in children with conflict-related limb injuries: a retrospective database study. *World J Emerg Surg* 2020; **15**(1): 52.
165. Haac B, Varela C, Geyer A, et al. The utility of the Kampala trauma score as a triage tool in a sub-Saharan African trauma cohort. *World J Surg* 2015; **39**(2): 356-62.
166. Gardner A, Forson PK, Oduro G, et al. Diagnostic accuracy of the Kampala Trauma Score using estimated Abbreviated Injury Scale scores and physician opinion. *Injury* 2017; **48**(1): 177-83.
167. Coupland R. Abdominal wounds in war. *Br J Surg* 1996; **83**(11): 1505-11.
168. Aspelund AL, Patel MQ, Kurland L, et al. Evaluating trauma scoring systems for patients presenting with gunshot injuries to a district-level urban public hospital in Cape Town, South Africa. *Afr J Emerg Med* 2019; **9**(4): 193-6.
169. Arikan AA, Selcuk E, Bayraktar FA. Predicting outcomes of penetrating cardiovascular injuries at a rural center by different scoring systems. *Braz J Cardiovasc Surg* 2020; **35**(2): 198-205.
170. Medeiros Vde F, Azevedo IM, Rego AC, et al. Antibacterial properties and healing effects of Melipona scutellaris honey in MRSA-infected wounds of rats. *Acta Cir Bras* 2016; **31**(5): 327-32.
171. Tsai DM, Tracy LE, Lee CC, et al. Full-thickness porcine burns infected with Staphylococcus aureus or Pseudomonas aeruginosa can be effectively treated with topical antibiotics. *Wound Repair Regen* 2016; **24**(2): 356-65.
172. Junker JP, Lee CC, Samaan S, et al. Topical delivery of ultrahigh concentrations of gentamicin is highly effective in reducing bacterial levels in infected porcine full-thickness wounds. *Plast Reconstr Surg* 2015; **135**(1): 151-9.

173. Al-Waili NS, Saloom KY. Effects of topical honey on post-operative wound infections due to gram positive and gram negative bacteria following caesarean sections and hysterectomies. *Eur J Med Res* 1999; **4**(3): 126-30.
174. Leaper DJ, Simpson RA. The effect of antiseptics and topical antimicrobials on wound healing. *J Antimicrob Chemother* 1986; **17**(2): 135-7.
175. Atiyeh BS, Dibo SA, Hayek SN. Wound cleansing, topical antiseptics and wound healing. *Int Wound J* 2009; **6**(6): 420-30.
176. Roberts AEL, Powell LC, Pritchard MF, et al. Anti-pseudomonad activity of Manuka honey and antibiotics in a specialized ex vivo model simulating cystic fibrosis lung infection. *Front Microbiol* 2019; **10**: 869.
177. Jenkins R, Cooper R. Improving antibiotic activity against wound pathogens with manuka honey in vitro. *PLoS One* 2012; **7**(9): e45600.
178. Liu MY, Cokcetin NN, Lu J, et al. Rifampicin-Manuka honey combinations are superior to other antibiotic-Manuka honey combinations in eradicating *Staphylococcus aureus* biofilms. *Front Microbiol* 2017; **8**: 2653.
179. Jenkins RE, Cooper R. Synergy between oxacillin and manuka honey sensitizes methicillin-resistant *Staphylococcus aureus* to oxacillin. *J Antimicrob Chemother* 2012; **67**(6): 1405-7.
180. Maddocks SE, Lopez MS, Rowlands RS, Cooper RA. Manuka honey inhibits the development of *Streptococcus pyogenes* biofilms and causes reduced expression of two fibronectin binding proteins. *Microbiology* 2012; **158**(3): 781-90.
181. Campeau ME, Patel R. Antibiofilm activity of Manuka honey in combination with antibiotics. *Int J Bacteriol* 2014; **2014**: 795281.
182. Vardaxis NJ, Brans TA, Boon ME, et al. Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. *J Anat* 1997; **190**: 601-11.
183. Lavker RM, Dong G, Zheng PS, Murphy GF. Hairless micropig skin. A novel model for studies of cutaneous biology. *Am J Pathol* 1991; **138**(3): 687-97.
184. Kelley KW, Curtis SE, Marzan GT, et al. Body surface area of female swine. *J Anim Sci* 1973; **36**(5): 927-30.
185. World Medical Association. Declaration of Helsinki. Ethical principles for medical research involving human subjects. *J Indian Med Assoc* 2009; **107**(6): 403-5.
186. Russell WMS, Burch RL. The principles of humane experimental technique. London,; Methuen; 1959.
187. Howard JT, Kotwal RS, Stern CA, et al. Use of combat casualty care data to assess the US Military Trauma System during the Afghanistan and Iraq conflicts, 2001 – 2017. *JAMA Surg* 2019; **154**(7): 600-8.
188. Murad MK, Larsen S, Husum H. Prehospital trauma care reduces mortality. Ten-year results from a time-cohort and trauma audit study in Iraq. *Scand J Trauma Resusc Emerg Med* 2012; **20**: 13.
189. Garber K, Kushner AL, Wren SM, et al. Applying trauma systems concepts to humanitarian battlefield care: a qualitative analysis of the Mosul trauma pathway. *Confl Health* 2020; **14**: 5.
190. David AS. Wanted—more answers than questions: literature review. *BMJ* 2001; **323**(7327): 1462-3.
191. Gethin G, Cowman S. Case series of use of Manuka honey in leg ulceration. *Int Wound J* 2005; **2**(1): 10-5.
192. Mayer A, Slezak V, Takac P, et al. Treatment of non-healing leg ulcers with honeydew honey. *J Tissue Viability* 2014; **23**(3): 94-7.
193. Hohmann E, Brand JC, Rossi MJ, Lubowitz JH. Expert opinion is necessary: Delphi panel methodology facilitates a scientific approach to consensus. *Arthroscopy* 2018; **34**(2): 349-51.

PAPERS I – IV

PAPER I

Hospital Workload for Weapon-Wounded Females Treated by the International Committee of the Red Cross: More Work Needed than for Males

Peter Andersson^{1,2,3} · Måns Muhrbeck⁴ · Harald Veen⁵ · Zaher Osman⁵ · Johan von Schreeb⁶

Published online: 9 August 2017

© The Author(s) 2017. This article is an open access publication

Abstract

Background Civilians constitute 33–51% of victims in armed conflicts. Several reports on civilian injuries exist, but few have focused on injuries afflicting females. We analyzed routinely collected data on weapon-related injuries from the International Committee of the Red Cross (ICRC) hospital in northwestern Pakistan in order to define injury patterns and types of surgical treatment for females.

Methods A total of 3028 patient files (376 females) from consecutively admitted patients to the ICRC-hospital in Peshawar from February 2009 to May 2012 were included. Information regarding injury-mechanism, time since injury, vital parameters at admission, type of injury, treatment and basic outcome was extracted from the files and analyzed. Comparisons between gender and age-groups were done by cross-table analyses or nonparametric tests.

Results Females were younger than males (20 vs. 25 years), arrived sooner after injury (24 vs. 48 h) ($p < 0.001$ for both) and were victims of bombs and missiles more frequently (64.4 vs. 54.6%) ($p < 0.001$). Vital parameters such as systolic blood pressure (110 vs. 113 mmHg) and pulse rate (100 vs. 86) were more affected at admission ($p < 0.001$ for both). Females were subjected to surgery (83.0 vs. 77.4%) ($p < 0.05$) and were given blood transfusions more often (18.8 vs. 13.6%) ($p < 0.01$). No differences in amputations or in-hospital mortality were found.

Conclusions Females treated at the ICRC-hospital in northwestern Pakistan are markedly affected by indiscriminate weapons such as bombs and missiles. Their average consumption of surgery is greater than for males, and this might be relevant in planning for staffing and facility needs in similar contexts.

Introduction

Civilians constitute 33–51% of victims in regions of armed conflict [1, 2]. Several reports on civilian injuries from such regions indicate that the highest proportions of

Presented orally at WADEM, Toronto 2017 and at the Swedish Surgical Society, Annual meeting, Malmö 2016.

✉ Peter Andersson
peter.andersson@regionostergotland.se

¹ Center for Teaching and Research in Disaster Medicine and Traumatology, University Hospital, 581 85 Linköping, Sweden

² Department of Surgery, University Hospital, 581 85 Linköping, Sweden

³ Department of Clinical and Experimental Medicine, Linköping University, 581 83 Linköping, Sweden

⁴ Department of Surgery, Vrinnevi Hospital, 603 79 Norrköping, Sweden

⁵ International Committee of the Red Cross, 19 Avenue de la Paix, 1202 Geneva, Switzerland

⁶ Department of Public Health Sciences, Karolinska Institutet, 171 65 Solna, Sweden

injuries of women and children are caused by indiscriminate weapons such as mortars and missiles fired from a distance [3, 4]. There are, however, few reports focusing on injuries afflicting females in regions of armed conflict. We have not identified any report that specifies the types of injuries or the workload that these injuries impose on needs at health care facilities. To fill this research gap we decided to analyze routinely collected surgical hospital data on weapon-related injuries from the International Committee of the Red Cross (ICRC). The ICRC is an organization working in conflict-areas independently running or otherwise supporting hospitals providing surgery with limited resources for free for the weapon-wounded. Since 1991 the ICRC administers a database where all weapon-wounded patients admitted to hospitals operated or supported by the organization are registered. At present there are more than 56,000 patients in the database for whom epidemiological and surgical treatment information on weapon-injuries inflicted in various regions of armed conflict is available.

Aim of the study

To define patterns of injury and type of surgical treatment for injured females admitted to the ICRC-hospital in northwestern Pakistan.

Patients and methods

A total of 3028 patient files (376 females) from patients consecutively admitted to the ICRC-hospital in Peshawar from February 2009 to May 2012 were included. The dates were the starting and ending dates for the ICRC-operated hospital which was located within the city. Only weapon-related injuries were treated in the hospital during the study-period. Patients' injuries were mainly generated from an ongoing asymmetric armed conflict characterized by the use of explosives, grenades, attacks by gunmen and remote-controlled aerial bombings on both sides of the Pakistani–Afghanistan border [5]. The distance between Peshawar and the Afghanistan border is 60 km. Injuries were also generated from attacks such as suicide bombings taking place within the city of Peshawar or in the surrounding area. Patients were not asked at admission whether they were civilians or combatants. The ICRC-hospital was, together with some small facilities run by non-governmental organizations, one of few hospitals in the area providing surgery for weapon-wounded also of afghan origin completely free of charge. Several public hospitals provided initial surgical care for free in the area, but these were mainly strictly located to Peshawar and were difficult to access for others than Pakistanis.

Information regarding sex, age, time since injury, vital parameters at admission including level of consciousness, mechanism of injury, type of treatment, body-region affected and outcome parameters such as discharge with or without follow-up as well as in-hospital mortality was prospectively entered into an Excel-database mainly by one of the authors (ZO).

We identified injury patterns from injury-mechanism and body-region affected as well as treatment practices for females in comparison to males treated at the hospital. In keeping with previous reports from ICRC patient-cohorts, patients younger than 16 years of age were classified as boys or girls and patients 16 years or older as men or women. Accordingly, all females together with all males aged less than 16 or more than 49 were classified as civilians [2, 6, 7]. During the study-period, 376 females (median age 20, range 0.3–84 years) were treated along with 2633 males (24, 1–80). Data regarding sex were missing for 19 patients.

Statistical methods

Statistical analysis was done using IBM SPSS Statistics software version 23 (IBM Corporation, Armonk, NY, USA). Types of surgical procedures and blood transfusions are given as percentages of patient numbers, i.e., if a patient had serial laparotomies or multiple transfusions only one such was counted. Consequently, repeat amputations on the same limb were counted as one and identified by the last level of amputation. Values are given as mean (SD) or median (IQR) when applicable. Comparisons between groups were done by cross-table analyses (χ^2 -test) or nonparametric tests (Mann–Whitney U) when appropriate. Significance level was set to $p < 0.05$ two-tailed test.

Results

Data from totally 3028 patient files were analyzed. The distribution of patients according to sex- and age-groups is shown in Fig. 1. Females constituted 12.4% of all patients and civilians 32.8%. Girls ($n = 141$) made up slightly more than one-third of all females, whereas boys ($n = 412$) constituted less than one-fifth of all males. Females were younger than males; median age 20 for females, 25 for males, interquartile ranges (IQR) 23 females, 14 males ($p < 0.001$) and arrived sooner after injury than males; median-time since injury 24 h for females and 48 h for males (IQR 88; 177) ($p < 0.001$). A total of 206 (54.8%) female patients reached hospital within 24 h compared to 1055 (40.1%) male patients. Vital parameters at admission such as systolic blood pressure, pulse rate and respiratory rate were more severely affected in females than in males ($p < 0.001$ for all). Level of consciousness was equally affected in females and males

(Table 1). Females were victims of bombs and missiles more frequently than males and more often sustained concomitant burns from subsequent fire, whereas males suffered from bullet-injuries to a greater extent (Table 2). Both sexes were equally affected by antipersonnel mines (APM). Table 3 shows the types of surgery needed for females and males, respectively, and also for age subgroups. The female group was subjected to any type of surgery of the skull, chest and abdomen more often than the male group ($p < 0.05$) as it was for any kind of surgery ($p < 0.05$) as well as for blood transfusions ($p < 0.01$). Girls, as a subgroup, were subjected to surgery of the skull, chest and abdomen to a greater extent than were boys ($p < 0.05$). However, the need for any type of surgery or blood transfusions did not differ between these two subgroups. No difference between sexes was found regarding the need for amputation or for in-hospital mortality.

Discussion

To our knowledge this is the first report focusing on injuries and surgery in females in the context of an asymmetric armed conflict such as the ongoing conflict in northwestern

Table 2 Comparison of mechanism of injury for all females and males treated at the ICRC's hospital for weapon-wounded in Peshawar, Pakistan, 2009–2012

Mechanism of injury	All females		All males		<i>p</i> Value
	<i>n</i> = 376		<i>n</i> = 2633		
Bomb or missile	242	(64.4)	1437	(54.6)	<i>p</i> < 0.001
Fire ^a	19	(5.0)	36	(1.4)	<i>p</i> < 0.001
Bullet	107	(28.5)	1012	(38.4)	<i>p</i> < 0.001
Antipersonnel mine	16	(4.3)	150	(5.7)	<i>p</i> = 0.25
Other	7	(1.9)	25	(0.9)	<i>p</i> < 0.01
Missing	4	(1.1)	9	(0.3)	–

Figures are numbers, percentages are given within brackets

^a Fire constitutes a subgroup within the group bomb or missile

Pakistan and bordering Afghanistan where a substantial inequality in military power exists between the warring parties [5]. In contrast with recent reports from other areas torn by armed conflict where females constitute up to 43% of the patients treated the percentage of females in this study was much smaller, 12.4% [8]. This difference may be explained by the fact that treatment at ICRC-facilities such as the hospital in Peshawar is strictly limited to weapon-related injuries. The low percentage of females treated corresponds quite well, however, with percentages reported from an Afghanistan trauma-center operating in a similar setting where 15% of patients treated were females [9]. Females were injured by fragments from mortars and bombs significantly more often than males. Most likely these injuries were sustained in public places or in domestic settings. Recent reports from Syria reveal a similar pattern of injuries among women and children with the highest death tolls caused by fragments emanating from air bombardments and ground explosives [1]. These weapons are indiscriminate by nature affecting civilians to a proportionally greater extent than combatants as shown by data from Kabul as far back in time as 25 years and also from Iraq between 2003 and 2008 [3, 10]. In the present study the data for females as representatives of the civilian group confirm these earlier findings. It might be claimed, however, that some of the difference between females and males might be due to a

Table 1 Vital parameters at hospital admission in females and males treated at ICRC's hospital for weapon-wounded in Peshawar, Pakistan, 2009–2012

Vital parameters	All females		Missing	All males		Missing	<i>p</i> Value
	<i>n</i> = 376			<i>n</i> = 2633			
Systolic blood pressure	110	(18.1)	10	113	(16.8)	60	<i>p</i> < 0.001
Pulse rate	100	(20.3)	182	86	(16.6)	1123	<i>p</i> < 0.001
Respiratory rate	22	(4.4)	182	21	(3.8)	1139	<i>p</i> < 0.001
Glasgow coma scale	14.6	(1.4)	177	14.7	(1.5)	1118	<i>p</i> = 0.14

Figures are mean (SD)

Table 3 Distribution of surgical procedures at the ICRC's hospital for weapon-wounded in Peshawar, Pakistan, 2009–2012

Type of procedure	Girls < 16 years <i>n</i> = 141	Women ≥ 16 years <i>n</i> = 235	All females <i>n</i> = 376	Boys < 16 years <i>n</i> = 412	Men 16–49 years <i>n</i> = 2021	Men > 49 years <i>n</i> = 200	All males <i>n</i> = 2633	<i>p</i> Value all females versus all males	<i>p</i> Value all girls versus all boys
Craniotomy	7 (5.0)	5 (2.1)	12 (3.2)	12 (2.9)	36 (1.8)	1 (0.5)	49 (1.9)	0.09	0.38
Thoracotomy	2 (1.4)	2 (0.9)	4 (1.1)	5 (1.2)	14 (0.7)	1 (0.5)	20 (0.8)	0.54	0.80
Chest-tube insertion	13 (9.2)	15 (6.4)	28 (7.4)	23 (5.6)	127 (6.3)	16 (8.0)	166 (6.3)	0.38	0.19
Laparotomy	14 (9.9)	26 (11.1)	40 (10.6)	26 (6.3)	196 (9.7)	8 (4.0)	230 (8.7)	0.23	0.21
Any type of surgery head/chest/abdomen	30 (21.3)	42 (17.9)	72 (19.1)	55 (13.3)	318 (15.7)	22 (11.0)	395 (15.0)	<0.05	<0.05
Arm-amputation	1 (0.7)	2 (0.9)	3 (0.8)	10 (2.4)	33 (1.6)	0 (0)	43 (1.6)	0.22	0.36
Above-knee amputation	5 (3.5)	9 (3.8)	14 (3.7)	12 (2.9)	52 (2.6)	11 (5.5)	75 (2.8)	0.35	0.93
Below-knee amputation	5 (3.5)	15 (6.4)	20 (5.3)	22 (5.3)	68 (3.4)	14 (7.0)	104 (3.9)	0.21	0.53
Any type of amputation	9 (6.4)	24 (10.2)	33 (8.8)	42 (10.2)	139 (6.9)	25 (12.5)	206 (7.8)	0.52	0.24
Any type of surgery soft-tissues ^a	93 (66.9)	174 (74.0)	267 (71.0)	276 (67.0)	1371 (67.8)	151 (75.5)	1798 (68.3)	0.29	0.90
Any type of surgery all kinds	111 (78.7)	201 (85.5)	312 (83.0)	315 (76.5)	1555 (76.9)	167 (83.5)	2037 (77.4)	<0.05	0.66
Any blood transfusion	26 (18.4)	44 (18.7)	70 (18.8)	73 (17.7)	253 (12.5)	28 (14.0)	354 (13.6)	<0.01	0.95
Mortality	10 (7.1)	14 (6.0)	24 (6.4)	20 (4.9)	89 (4.4)	12 (6.0)	121 (4.6)	0.13	0.43

Figures are numbers, percentages are given within brackets. Some patients have been subjected to several procedures but at different sites which results in more procedures than patients and a sum of percentages more than 100

^a Debridement, delayed primary closure, split skin graft or burn care

selection of less seriously injured males reaching hospital as indicated by their significantly later arrival after injury. Longer transportation distances might result in a greater proportion of pre-hospital deaths from fragmented munitions among males. Considering also the greater affection of vital parameters in females it seems most likely that females represent a different cohort of weapon-wounded treated in this setting than the cohort of males. Apparently the female cohort comes from areas closer to the health facility than does the male cohort, and also, consists of a larger proportion of children and of victims in worse condition upon arrival than the male cohort. The more affected vital parameters can to some part be explained by the larger proportion of children below 16 years of age among females but not completely since these differences, except for systolic blood pressure, persist also even after removal from the analysis of all data for children younger than 16 years of age (not shown). The differences in vital parameters, particularly blood pressure and respiratory rate, might seem to be negligible as seen from an individual clinical perspective, but on a group level their collective values signal a true poorer physical condition in females.

Injuries caused by APM were relatively uncommon among both females and males. The figure of 5.4% afflicted in the entire study-population contrasts sharply with figures reported from the beginning of the 1990s when 48% of all weapon-wounded from the same area in northwestern Pakistan and neighboring Afghanistan were injured as a result of mines [11]. This is likely a result of the global work against landmines by both governmental and non-governmental bodies that resulted in the Ottawa treaty endorsed by a majority of states in 1997 prohibiting their use [12]. In-hospital mortality, although slightly higher among females, did not differ significantly between sexes, and the total figure of 4.8% is in line with other studies from the same geographical area reporting rates of 2.5–7.0% [9, 13, 14]. Females consumed more hospital resources per person than did males since they were subjected to more surgical procedures involving the skull, thorax and abdomen, had more procedures as a whole, and given more blood transfusions. The explanation is probably that, as already mentioned, females represent a group of weapon-wounded coming from areas closer to the health facility and thereby are likely to survive more serious injuries than males during the time between being wounded and the time of arrival. Another explanation might be that females injured at a greater distance from the ICRC-hospital even with less severe injuries are never transferred to the hospital as already suggested by Coupland and Korver [6] in a report from the same area 1991. The reason for this might be that there were fewer transporters than needed and that males were given priority.

This study has limitations. It only covers the weapon-wounded females treated at one facility. Many weapon-

wounded patients and patients affected by terror-attacks are taken care of by public hospitals or other agencies operating in the area. Unfortunately the proportion of patients treated at the ICRC-hospital could not be determined since coordinated data on the total numbers of weapon-wounded in the area are lacking. Moreover, although data are prospectively collected, no particular study objective was identified prior to collection which might have affected quality of data. As the objective of this study was to describe the ICRC's experience of surgical treatment of the war wounded in northwestern Pakistan with focus on females, no patients were excluded. Therefore, a limited number of patients were included who had injuries older than three months. This most likely falsely affected mortality in a positive manner compared to other studies.

Conclusions

At the ICRC-hospital in Peshawar, Pakistan, where exclusively war-wounded patients were treated, females were in a minority. They were more likely than males to be injured by indiscriminate weapons such as bombs and missiles. Female patients arrived sooner after injury, in a worse clinical condition than males and, on average, had a higher requirement for blood and surgical services than males. This knowledge might be relevant in planning for staffing and facility needs in similar contexts.

Acknowledgements Lars Valter, Forum Östergötland, Linköping University, Sweden, for statistical advice. Dell Saulnier, Department of Public Health Sciences, Karolinska Institutet, Solna, Sweden, for help with transferring data into SPSS.

Funding was provided by Elsa and Sigurd Golje Foundation and Linköping Society of Medicine.

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare. ZO and HV have long-term contracts with the ICRC, and PA has had short-term contracts.

Ethical approval Exploration of data was approved by the Regional Ethical Review Board in Linköping, Sweden (Dnr. 2014/417-31).

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Guha-Sapir D, Rodriguez-Llanes JM, Hicks MH et al (2015) Civilian deaths from weapons used in the Syrian conflict. *BMJ* 351:h4736. doi:10.1136/bmj.h4736

2. Meddings DR (2001) Civilians and war: a review and historical overview of the involvement of non-combatant populations in conflict situations. *Med Confl Surviv* 17:6–16
3. Hicks MH, Dardagan H, Bagnall PM et al (2011) Casualties in civilians and coalition soldiers from suicide bombings in Iraq, 2003–2010: a descriptive study. *Lancet* 378:906–914
4. Michael M, Meddings DR, Ramez S et al (1999) Incidence of weapon injuries not related to interfactional combat in Afghanistan in 1996: prospective cohort study. *BMJ* 319:415–417
5. Arreguin-Toft I (2001) How the weak win wars: a theory of asymmetric conflict. *Int Secur* 26:93–128
6. Coupland R, Korver A (1991) Injuries from antipersonnel injuries: the experience of the International Committee of the Red Cross. *BMJ* 303:1509–1512
7. Bilukha OO, Brennan M, Woodruff BA (2003) Death and injury from landmines and unexploded ordnance in Afghanistan. *JAMA* 290:650–653
8. Trelles M, Dominguez L, Tayler-Smith K et al (2015) Providing surgery in a war-torn context: the Médecins Sans Frontières experience in Syria. *Confl Health* 15:36. doi:10.1186/s13031-015-0064-3
9. Trelles M, Stewart BT, Hemat H et al (2016) Averted health burden over 4 years at Médecins Sans Frontières (MSF) Trauma Centre in Kunduz, Afghanistan, prior to its closure in 2015. *Surgery* 160:1414–1421
10. Coupland RM, Samnegaard HO (1999) Effect of type and transfer of conventional weapons on civilian injuries: retrospective analysis of prospective data from Red Cross hospitals. *BMJ* 319:410–412
11. Jeffrey SJ (1996) Antipersonnel mines: who are the victims? *J Accid Emerg Med* 13:343–346
12. Available online: http://www.un.org/Depts/mine/UNDocs/ban_trty.htm
13. Rautio J, Paavolainen P (1998) Afghan war wounded: experience with 200 cases. *J Trauma* 28:523–525
14. Yasin MM, Nasreen G, Malik SA (2012) Injury pattern of suicide bomb attacks in Pakistan. *Eur J Trauma Emerg Surg* 38:119–127

PAPER II

Trends in Demographics and Surgical Treatment of Weapon-Related Limb Injuries Over Two Decades in a Resource-Scarce Setting

Måns Muhrbeck^{1,2} · Kaspar Holmgren³ · Zaher Osman⁴ · Johan von Schreeb⁵ ·
Andreas Wladis^{2,6} · Peter Andersson^{1,7}

© The Author(s) 2019

Abstract

Background In the past decades, surgical management of limb injuries in high-resource settings has improved. The possibility of limb salvage has increased. It is not known whether similar changes have transpired in resource-scarce conflict settings.

Methods Retrospective cohort study using routinely collected patient data from the International Committee of the Red Cross hospitals in Pakistan was conducted. Consecutive data from 2009 to 2012 (535 patients) and randomly selected data from 1992 to 1995 (463 patients) were used. Only patients with weapon-related limb injuries were included. Differences in surgical procedures were assessed with logistic regression to adjust for confounding factors.

Results Less injuries were related to mines in 2009–2012 than in 1992–1995 (3.7% vs. 20.3%, $p < 0.0001$), but injuries from bombs, shells and fragments were more frequent (38.5% vs. 19.4%, $p < 0.0001$) as were injuries with only a small degree of tissue damage (42.0% vs. 31.1%, $p = 0.0004$). In the logistic regression, the time period did not affect the risk of amputation, debridement, length of hospital stay or in-hospital mortality. The use of external fixation (OR 0.56, 95% CI 0.33–0.96, $p = 0.04$), split skin grafts (OR 0.31, 95% CI 0.21–0.45, $p < 0.0001$) and blood transfusion (OR 0.43, 95% CI 0.28–0.66, $p = 0.0001$) was less frequent in 2009–2012.

Conclusion In this resource-scarce conflict setting, the risk of amputation appears unchanged over time, while the use of external fixation and split skin grafts was less common in 2009–2012 than in 1992–1995. These results contrast with the improved limb salvage results seen in high-resource settings. It likely reflects the challenges of providing advanced limb-preserving techniques in a resource-scarce setting.

Introduction

Previous presentation of study: Preliminary results were presented orally at the Swedish Surgical Society, Surgical Society Annual Meeting, 21–25 August 2017, in Jönköping, Sweden, and an abstract has been presented orally at the 20th European Congress of Trauma & Emergency Surgery, 5–7 May 2019, in Prague, Czech Republic.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00268-019-05110-y>) contains supplementary material, which is available to authorized users.

✉ Måns Muhrbeck
mans.muhrbeck@liu.se

¹ Department of Surgery, and Department of Clinical and Experimental Medicine, Linköping University, 603 79 Norrköping, Sweden

Limb injuries account for a majority (50–75%) of survivable injuries in conflicts. Between 25 and 33% are combined with fractures, and the lower limbs are most commonly affected [1–5]. Amputation rates following major limb injuries in conflicts range between 7 and 19% [6–8]. In 2015, it was estimated that approximately 1.2

² Center for Teaching and Research in Disaster Medicine and Traumatology, University Hospital, Linköping, Sweden

³ Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

million people were amputated due to injuries globally [9]. Incidences vary considerably, ranging from 1.2 in Canada and up to 371 per 100,000 population in the Middle East and North Africa [9–11]. Over the last decades advancements in orthopaedic, plastic and vascular surgical techniques have made many severely injured limbs salvageable in high-resource settings [12, 13]. Data from the United States Healthcare Cost and Utilization Project showed a decrease in the amputation rates due to trauma by 51.5% between 1988 and 1996 [14]. Among Medicare patients, in the USA, a 45% decrease of lower limb amputations for all causes was observed between 1996 and 2011 [15]. It remains unclear whether similar improvements have been achieved in conflict-affected low- and middle-resource settings where constraints, such as logistical challenges and security issues, may limit implementations of advances in treatment practices [16]. These constraints in the delivery of and access to surgical care emphasize the importance of understanding what surgical resources are needed in a given context. Identifying trends in surgical treatment of limb injuries could therefore have implications on how surgical care is delivered in resource-scarce conflict settings. To fill this knowledge gap, we analysed routinely collected surgical hospital data from patients with weapon-related limb injuries treated at two of the International Committee of the Red Cross (ICRC) field hospitals, in Pakistan, during 1992–1995 and 2009–2012. The ICRC is a humanitarian organization providing medical assistance to victims of war and other situations of violence by independently running or otherwise supporting hospitals with limited resources where with the ICRC support surgery can be provided free for the weapon-wounded. The ICRC has since its first field mission in 1875 provided specialized surgical care to limb injured in conflicts [17].

Aim of study

To investigate trends in demographics and surgical practices of weapon-related limb injuries in a resource-scarce setting between 1992–1995 and 2009–2012 with a particular focus on amputation rates.

Patients and setting

Patients' injuries for both time periods were mainly caused during the ongoing armed conflict that was characterized by the use of explosives, grenades, attacks by gunmen and aerial bombings on both sides of the Pakistani–Afghanistan border [18]. In response to the conflict in Afghanistan, the ICRC opened its first hospital 60 kilometres from the Afghanistan border in Peshawar, north-western Pakistan, in 1981. This was followed by opening of another hospital in Quetta, 80 kilometres from the border and 840 km south of Peshawar, in 1983. Both hospitals closed in the 1990s, but due to intensification of the conflicts in the border area, the Peshawar hospital was reopened in 2009 and remained in operation until 2012 [19, 20]. Local citizens with weapon-related injuries were also treated at the hospitals and are included in the study. Those seeking care were not asked whether they were civilians or combatants. Both hospitals had access to rehabilitation centres for training and prosthesis fitting [19, 21]. Care at these ICRC's hospitals was free of charge.

Methods

This retrospective cohort study used routinely collected patient data. Inclusion criteria were all patients admitted to the ICRC's hospital in Quetta or Peshawar, during 2009–2012 and 1992–1995, respectively, with weapon-related penetrating injuries to the limbs. Exclusion criteria were if patients had: significant injury to other sites than limbs requiring medical attention other than a basic dressing, injuries older than seven days, traumatic amputations including partial or total avulsion of the limb or missing data on confounders (see statistical methods). Patients with the most significant injury located distal to the metacarpophalangeal joints of the hand or mid-tarsal joints of the foot were excluded because these commonly only required simpler surgical treatment, such as a single debridement (DBR) or change of dressing (COD). In case of multiple admissions, only the first one was included. Comparisons were made between the incidence of all of the following variables, as recorded for each of the two time periods: in-hospital amputations, external fixation, split skin graft (SSG), DBR or COD under general anaesthesia three times or more, blood transfusion, 21 days or more of hospital stay (LOS) and in-hospital mortality. DBR and COD done in the operation theatre were merged into one group in the analysis as both procedures required general anaesthesia.

The ICRC has since 1991 routinely used the Red Cross Wound Classification (RCWC) to categorize penetrating injuries. RCWC is based on entry and exit diameters of the

⁴ International Committee of the Red Cross, Geneva, Switzerland

⁵ Department of Public Health Sciences, Karolinska Institutet, Stockholm, Sweden

⁶ Department of Clinical and Experimental Medicine, Linköping University, Linköping, Sweden

⁷ International Medical Programme, Center for Teaching and Research in Disaster Medicine and Traumatology, University Hospital, Linköping, Sweden

wound, cavity, fracture severity, vital vessel injury and presence of metal fragments in the wound. For limb injuries, vital vessel injury is defined as injuries to vessels proximal to the knee or elbow. Depending on type (soft tissue, fracture, vital structure and vital structure with fracture) and grade (1–3), a penetrating wound is categorized into twelve different categories [22–24]. The grade directly translates into degree of tissue damage at injury [24]. Grade 1 was assigned small, grade 2 medium, and grade 3 extensive tissue damage. In this study, the grade for the main treated limb wound was registered for all patients to adjust for differences in degree of tissue damage between time periods. The presence of a second penetrating limb wound, other than one merely requiring basic dressing deemed as not affecting outcome, was dichotomously registered as existing or not.

Data for the 2009–2012 time period were collected from an anonymized database containing routinely collected data from all 3028 patients treated at the ICRC's hospital in Peshawar between 18 February 2009 and 9 May 2012. The data were entered, at the time, into an Excel database mainly by one of the authors (ZO), whose job primarily entailed maintaining the database at the ICRC's hospital in Peshawar.

Data from 1992 to 1995 were collected from paper-based patient files by two of the authors (MM and KH) from ICRC's archives in Satigny, Switzerland, between October and November 2016. Approximately 8 000 handwritten files are stored in these archives and include files from both ICRC's hospitals in Quetta and Peshawar. Files are stored in boxes in no particular order with 7–10 files in each box. Boxes that contained files from 1992 to 1995 were randomly selected from listings on Excel spreadsheets. Randomization was achieved by using built-in random functions in the Python programming language [25]. To ensure even distribution, the first 6 files from each box that met our inclusion criteria were selected. Considering the available data from the 2009–2012 database, there will be a minimum detectable difference of 6% at 80% statistical power between the time periods.

Statistical methods

Statistical analysis was made using SPSS Statistics software version 25 (IBM Corporation, Armonk, NY, USA). To adjust for the impact of patients who required extensive resources, the number of in-hospital amputations, external fixations, SSG and blood transfusions are given as proportion of patient numbers, i.e. if a patient had several in-hospital amputations, SSG, external fixations or blood transfusions, only one was counted.

Descriptive comparisons between the 2009–2012 and 1992–1995 periods were analysed using Fisher's exact test or Chi-square for categorical variables and Mann–Whitney *U*-test for continuous variables. Values are given as mean with standard deviation (SD) and median with interquartile range (IQR) where appropriate. To compare in-hospital amputations, use of external fixation, SSG and ≥ 3 DBR/COD, blood transfusion, ≥ 21 days LOS and in-hospital mortality, binary logistic regression analysis was performed to adjust for confounding factors between time periods. Gender, age (< 16 , 16–49 and > 49 yrs.), time since injury (< 24 , 24–48 and > 48 h), mean arterial pressure (< 65 and ≥ 65 mmHg) on admission, degree of tissue damage (small, medium and extensive), second penetrating wound (yes and no), vital vessel injury (yes and no) and time period (1992–1995 and 2009–2012) were identified as confounding factors as they had been shown to have significant influence in the univariate analysis or previously had been reported to influence the risk of outcomes, especially for in-hospital amputations [26, 27]. Forward Wald was used to calculate a final regression model. Differences in frequency of procedures between time periods are presented as odds ratios (OR) with 95% confidence intervals. *P*-values (two-tailed) less than 0.05 were considered significant for both the univariate and logistic regression analyses.

Results

A total of 535 patients treated in 2009–2012 and 463 treated in 1992–1995 were included in the final analysis (Fig. 1). There was a larger proportion of women than men and more patients under 16 years and over 49 years in 2009–2012 compared to 1992–1995 ($p < 0.01$ for both) (Table 1). More patients were treated for injuries related to bombs, shells and fragments ($p < 0.0001$) and fewer related to anti-personal mines (APM) in 2009–2012 than in 1992–1995 ($p < 0.0001$) (Table 2). There were no differences in time from injury to arrival at hospital (median 24 h, IQR 64, in 2009–2012 and 24 h, IQR 32, in 1992–1995).

Comparisons of injury patterns between the time periods are shown in Table 3. Large wounds with comminute fractures ($p < 0.0001$) were less frequent in 2009–2012 than 1992–1995, while simple wounds with or without fracture and fractures with vascular injuries were more frequent ($p < 0.01$ for both). Likewise, injuries resulting in a small degree of tissue damage were more frequent in 2009–2012 ($p = 0.0004$). A majority of injuries were to the lower limbs for both time periods (61.9%, in 2009–2012 and 64.4%, in 1992–1995, $p = 0.42$). There was no difference in the presence of a second penetrating limb wound

($p = 0.08$). The relationship between time period and surgical procedures, LOS and in-hospital mortality is shown in Table 4. The univariate analysis revealed that in 2009–2012 patients had fewer external fixations ($p = 0.008$), SSG ($p < 0.0001$), DBR/CODs ≥ 3 ($p = 0.02$), blood transfusions ($p < 0.0001$) and shorter LOS ($p = 0.007$) than in 1992–1995, but the risk of in-hospital amputations and mortality did not differ between the time periods. When adjusting for confounders by logistic regression, the time period did not prove to be related to the risk of in-hospital amputations, DBR/

Table 2 Mechanism of injury for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992–1995 and 2009–2012

Mechanism	1992–1995 Total = 463 n (%) of total	2009–2012 Total = 535 n (%) of total	Univariate analysis P value
Anti-personnel mine	94 (20.3)	20 (3.7)	< 0.0001
Bomb, shell, fragment	90 (19.4)	206 (38.5)	< 0.0001
Gunshot	273 (59.0)	306 (57.2)	0.57
Other	6 (1.3)	3 (0.6)	0.22

CODs ≥ 3 , LOS ≥ 21 days or in-hospital mortality. However, in the final regression model, the use of external fixations (OR 0.56, 95% CI 0.33–0.96, $p = 0.04$), SSG (OR 0.31, 95% CI 0.21–0.45, $p < 0.0001$) and blood transfusions (OR 0.43, 95% CI 0.28–0.66, $p = 0.0001$) was still less frequent in 2009–2012 than in 1992–1995. Complete regression models are shown in Supplement Tables 1–7.

Discussion

This is to our knowledge the first study focusing on trends in surgical treatment practices for limb injuries over a time period of two decades in a resource-scarce conflict setting. After adjusting for confounders, particularly the degree of tissue damage, no difference in amputation-risk could be detected between 1992–1995 and 2009–2012, nor were there any statistically significant differences in the use of DBR/COD, LOS or in-hospital mortality. However, the use of external fixation, SSG and blood transfusion had declined in 2009–2012. There are several plausible explanations for the observed differences in surgical procedures between the two time periods. Surgeons deployed by the ICRC during both time periods were predominantly specialist general surgeons from high-resource countries (personal communication with the ICRC's health unit). Their familiarity with basic orthopaedic, plastic and

Table 1 Gender and age distribution for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992–1995 and 2009–2012

Age and Gender	1992–1995 Total = 463 n (%)	Mean (SD)	2009–2012 Total = 535 n (%)	Mean (SD)	Univariate analysis P value
Gender					< 0.0001
Male	449 (97.0)		466 (87.1)		
Female	14 (3.0)		69 (12.9)		
Age		26.2 (10.8)		27.2 (13.9)	0.22
< 16 years	52 (11.2)		92 (17.2)		0.007
16–49	387 (83.6)		388 (72.5)		< 0.0001
> 49	24 (5.2)		55 (10.3)		0.003

Table 3 Injury patterns for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992–1995 and 2009–2012

Injury pattern	1992–1995 Total = 463 <i>n</i> (% of total)	2009–2012 Total = 535 <i>n</i> (% of total)	Univariate analysis <i>P</i> value
Type of injury			
Simple wound ^a ± fracture	188 (40.6)	267 (49.9)	0.003
Medium wound ^b + com. fracture ^d	151 (32.6)	168 (31.4)	0.68
Large wound ^c - fracture	15 (3.2)	16 (3.0)	0.82
Large wound ^c + com. fracture ^d	84 (18.2)	36 (6.7)	< 0.0001
Any fracture + vascular injury	15 (3.2)	37 (6.9)	0.009
Vascular injury - fracture	10 (2.2)	11 (2.1)	0.91
Tissue damage			
Small	144 (31.1)	225 (42.0)	0.0004
Medium	210 (45.4)	238 (44.5)	0.78
Extensive	109 (23.5)	72 (13.5)	< 0.0001
Location of injury			
Upper limb	97 (20.9)	132 (24.7)	0.16
Lower limb	298 (64.4)	331 (61.9)	0.42
Upper and lower limbs	68 (14.7)	72 (13.4)	0.58
Second wound			0.08
No	359 (77.5)	439 (82.1)	
Yes	104 (22.5)	96 (17.9)	

^a Simple wound = Entry and exit site less than 10 cm in total and no wound cavity

^b Medium wound = Entry and exit site less than 10 cm in total and wound cavity

^c Large wound = Entry and exit site more than 10 cm in total and wound cavity

^d Com. Fracture = Comminute fracture

vascular surgery may have varied. This is especially true for the later time period as current trends in surgical training in high-resource countries have favoured subspecialization with specific rather than general skill sets [28]. In a recent survey among ICRC health care staff on self-perceived training needs, 31% requested additional training in fracture surgery, 20% in reconstructive surgery and 19% in vascular surgery [29]. This together with our findings calls for adapting pre-deployment preparation and international team composition to better address context-varying injuries.

The decreased use of external fixation in 2009–2012 could also be due to a more restrictive use of this technique based on changes in ICRC's treatment practices between the time periods (personal communication with former ICRC coordinator of surgical programmes Åsa Molde). Fewer blood transfusions in 2009–2012 may be an effect of less severe limb injuries (Table 3).

The frequency of amputations in this study remained the same over time, whereas it has gradually decreased in civilian high-resource settings [14, 15]. This discrepancy in treatment routines between high- and low-resource settings

is not unusual and also occurs in other types of trauma [16]. Availability of equipment, medicines, as well as familiarity with reconstructive techniques including flaps will affect how the surgeons in the field choose to deal with complex limb injuries.

In 2009–2012, a larger proportion of the admitted patients were women and children, and fewer were treated for extensive soft tissue injuries and fracture comminution than in 1992–1995 (Tables 1 and 3). These differences in demographics and injury patterns might be explained by an increasingly indiscriminate nature of warfare over time resulting in more patients admitted with injuries from explosives, mortars, aerial bombings and fewer from APM (Table 2). Fewer limb injuries from APM could potentially be attributed to the implementation of the Ottawa treaty between the time periods. The treaty was endorsed by several fighting parties in the region [30]. It bans the use, stockpiling, production and transfer of APM and promotes their destruction [31]. For both time periods, a very low mortality for admitted patients in spite of severe limb injuries was observed (Table 4).

Table 4 Relationship between time period and surgical procedures, hospital stay and mortality for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992–1995 and 2009–2012

Time period	Univariate analysis Total = 998			Logistic regression analysis Total = 998			
	1992–1995 Total = 463 <i>n</i> (%)	2009–2012 Total = 535 <i>n</i> (%)	<i>P</i> value	All confounders ^a 1 = 1992–1995 Odds ratio (95% CI) for 2009–2012	<i>P</i> value	Final model 1 = 1992–1995 Odds ratio (95% CI) for 2009–2012	<i>P</i> value
In-hospital amputation			0.20				
No	417 (90.1)	494 (92.3)		1		1	
Yes	46 (9.9)	41 (7.7)		1.20 (0.69–2.09)	0.51	1.12 (0.66–1.91)	0.67
External fixation			0.008				
No	422 (91.1)	510 (95.3)		1		1	
Yes	41 (8.9)	25 (4.7)		0.63 (0.36–1.08)	0.09	0.56 (0.33–0.96)	0.04
Split skin graft			< 0.0001				
No	330 (71.3)	478 (89.3)		1		1	
Yes	133 (28.7)	57 (10.7)		0.31 (0.21–0.46)	< 0.0001	0.31 (0.21–0.45)	< 0.0001
Debridement/change of dressing			0.02				
0–2	310 (67.0)	393 (73.5)		1		1	
≥ 3	153 (33.0)	142 (26.5)		0.89 (0.65–1.22)	0.48	0.89 (0.66–1.20)	0.46
Blood transfusion			< 0.0001				
No	371 (80.1)	485 (90.7)		1		1	
Yes	92 (19.9)	50 (9.3)		0.41 (0.27–0.64)	< 0.0001	0.43 (0.28–0.66)	0.0001
Length of hospital stay			0.007				
< 21 days	228 (49.2)	309 (57.8)		1		1	
≥ 21 days	235 (50.8)	226 (42.2)		0.82 (0.62–1.10)	0.18	0.87 (0.66–1.14)	0.31
In-hospital mortality			0.18				
No	458 (98.9)	533 (99.6)		1		1	
Yes	5 (1.1)	2 (0.4)		0.47 (0.07–2.96)	0.42	0.32 (0.06–1.67)	0.18

^a Gender, age, time since injury, mean arterial pressure, degree of tissue damage, presence of second penetrating wound and vital vessel injury were also included as confounders in the logistic regression analysis, but not reported in this table. For full logistic regression analysis see Table 5–11 in Supplement

Limitations

This study has limitations. Considering the difficulties in reaching the hospitals for various reasons and the late presentations, it can be safely assumed that many severely injured patients died before reaching the respective hospitals. This should not affect the surgical treatment given to admitted patients with limb injuries during both time periods. In this study, we included patients from two different ICRC's hospitals, which also could lead to patient selection and treatment bias. Previously published data from both hospitals, however, demonstrate that patients' injury patterns were similar and both hospitals adhered to the ICRC's treatment protocols [24].

This study only includes patients treated at ICRC's hospitals in Pakistan. Therefore, the external validity of our findings may be limited. The patients were nonetheless treated according to ICRC's treatment protocols that are

generally accepted and similar to protocols followed by other humanitarian organizations and surgical care providers in conflicts [32–34]. Finally, the study is retrospective and consequently has inherent weaknesses that come with this study design, such as general selection bias and missing data. To mitigate selection bias, we used random sampling for the 1992–1995 time period and included all patients that meet the inclusion criteria for 2009–2012. Patients that were excluded due to missing confounding data did not differ significantly from analysed patients in terms of in-hospital amputations or other surgical procedures.

Conclusions

In this resource-scarce conflict setting, almost all admitted patients with weapon-related injuries survived. While the use of external fixation and split skin graft was less

common in 2009–2012 than in 1992–1995, the risk of amputation remained unchanged over time. These results contrast with the improved limb salvage results seen during the same time period in high-resource settings. It likely reflects the significant challenges of providing limb-preserving techniques in a resource-scarce conflict setting. Adjusting pre-deployment preparation and team composition could be one strategy to mitigate some of these challenges. Further investigation is needed to assess whether such strategies have the desired effect of improving surgical care in resource-scarce conflict settings.

Acknowledgements Open access funding provided by Linköping University. We thank former ICRC coordinator of surgical programmes Åsa Molde and former ICRC surgeon Günter Wimhöfer for providing background information on ICRC's activities in Afghanistan and Pakistan and Lars Valter, Forum Östergötland, Linköping University, Sweden, for statistical advice. Funding was provided by Sigurd and Elsa Goljes Memorial Foundation and ALF grants, Region Östergötland, Sweden.

Author contribution This study was conceived by MM and PA. The study design was made by MM, KH, JvS and PA. Data were collected by KH, MM and ZO and analysed by KH, MM and PA. Interpretation of the data was done by KH, MM, ZO, AW, JvS and PA. The first draft of the manuscript was made by MM and was critically revised by all authors.

Funding Sigurd and Elsa Goljes Memorial Foundation and ALF grants, Region Östergötland, Sweden (Grant Numbers LIO-799621, LA2016-0457).

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to declare. ZO has a long-term contract, AW has had a long-term contract, and PA intermittent short-term contract with the ICRC.

Ethical approval Exploration of data was approved by the Regional Ethical Review Board in Linköping, Sweden (Dnr. 2014/417–31, 2015/66–33, 2016/298–32).

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

- Cernak I, Savic J, Ignjatovic D et al (1999) Blast injury from explosive munitions. *J Trauma* 47:96–103
- Hinsley DE, Rosell PA, Rowlands TK et al (2005) Penetrating missile injuries during asymmetric warfare in the 2003 Gulf conflict. *BJS* 92:637–642
- Aboutanos MB, Baker SP (1997) Wartime civilian injuries: epidemiology and intervention strategies. *J Trauma* 43:719–726
- Trouwborst A, Weber BK, Dufour D (1987) Medical statistics of battlefield casualties. *Injury* 18:96–99
- Nerlander MP, Haweizy RM, Wahab MA et al (2019) Epidemiology of trauma patients from the Mosul Offensive, 2016–2017: results from a dedicated trauma center in Erbil, Iraqi Kurdistan. *World J Surg* 43:368–373. <https://doi.org/10.1007/s00268-018-4817-1>
- Islinger RB, Kuklo TR, McHale KA (2000) A review of orthopedic injuries in three recent U.S. military conflicts. *Mil Med* 165:463–465
- Stansbury LG, Lalliss SJ, Branstetter JG et al (2008) Amputations in U.S. military personnel in the current conflicts in Afghanistan and Iraq. *J Orthop Trauma* 22:43–46
- Andersson P, Muhrbeck M, Veen H et al (2018) Hospital workload for weapon-wounded females treated by the International Committee of the Red Cross: more work needed than for males. *World J Surg* 42:93–98. <https://doi.org/10.1007/s00268-017-4160-y>
- Vos T, Allen C, Arora M et al (2016) Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388:1545–1602
- McDonald C (2017) Global incidence of major, non-fatal amputation due to traumatic causes. Dept. of Global Health, University of Washington. Washington. <https://digital.lib.washington.edu/researchworks/handle/1773/40421?show=full> Accessed 12 Jan 2019
- Imam B, Miller WC, Finlayson HC et al (2017) Incidence of lower limb amputation in Canada. *Can J Public Health* 108:374–380
- Lange RH (1989) Limb reconstruction versus amputation decision making in massive lower extremity trauma. *Clin Orthop Relat Res* 243:92–99
- Saddawi-Konefka D, Kim HM, Chung KC (2008) A systematic review of outcomes and complications of reconstruction and amputation for type IIIB and IIIC fractures of the tibia. *Plast Reconstr Surg* 122:1796–1805
- Dillingham TR, Pezzin LE, MacKenzie EJ (2002) Limb amputation and limb deficiency: epidemiology and recent trends in the United States. *South Med J* 95:875–883
- Goodney PP, Tarulli M, Faerber AE et al (2015) Fifteen-year trends in lower limb amputation, revascularization, and preventive measures among medicare patients. *JAMA Surg* 150:84–86
- Hofman K, Primack A, Keusch G et al (2005) Addressing the growing burden of trauma and injury in low- and middle-income countries. *Am J Public Health* 95:13–17
- Palmieri D (2012) An Institution standing the test of time? *IRRC* 94:1273–1298
- Arreguin-Toft I (2001) How the weak win wars: a theory of asymmetric conflict. *Int Secur* 26:93–128
- Cuttat P, News bulletin, ICRC Hospital Peshawar (2009) International Committee of the Red Cross, Geneva. <https://www.icrc.org/en/doc/assets/files/otherbulletin-icrc-march-09.pdf>. Accessed 23 Nov 2018
- Madrazo A, News bulletin, Pakistan: ICRC activities to continue on reduced scale (2012) International Committee of the Red Cross, Geneva. Available at: <https://www.icrc.org/en/doc/resources/documents/news-release/2012/pakistan-news-2012-08-28.htm>. Accessed 23 November, 2018
- Maresca L, Casey-Maslen S (2000) The banning of anti-personnel landmines: the legal contribution of the International Committee of the Red Cross. Cambridge University Press, Cambridge, p 173
- Coupland RM (1992) The Red Cross classification of war wounds: the E.X.C.F.V.M. scoring system. *World J Surg* 16:910–917. <https://doi.org/10.1007/BF02066991>

23. Coupland RM, Brochure, The Red Cross wound classification (1993) International Committee of the Red Cross, Geneva, pp 1–15
24. Giannou C, Baldan M, Red Cross wound score and classification (2009) In: War surgery: working with limited resources in armed conflict and other situations of violence, vol I, International Committee of the Red Cross, Geneva, pp 81–91, 109
25. Generate pseudo-random numbers, version 9.6 (2016) Computer programming language, Phytion Software Foundation. Available at: <https://docs.python.org/2/library/>. Accessed 23 Nov 2018
26. Davidovic LB, Cinara IS, Ille T et al (2005) Civil and war peripheral arterial trauma: review of risk factors associated with limb loss. *Vascular* 13:141–147
27. Groth AT, Kirk KL, Shawen SB, et al (2011) Surgery Foot in War Trauma. In: Lerner A, Soundry M (eds) Armed conflict injuries to the extremities: a treatment manual. Springer, Heidelberg, p 301
28. Stitzenberg KB, Sheldon GF (2005) Progressive specialization within general surgery: adding to the complexity of workforce planning. *J Am Coll Surg* 201:925–932
29. Haverkamp FJC, Veen H, Hoencamp R et al (2018) Prepared for mission? A survey of medical personnel training needs within the International Committee of the Red Cross. *World J Surg* 42:3493–3500. <https://doi.org/10.1007/s00268-018-4651-5>
30. The International Campaign to Ban Landmines (2018) ICBL-CMC, Geneva. <https://www.icbl.org/en-gb/the-treaty/treaty-status.aspx>. Accessed 24 November, 2018
31. United Nation's Convention on the Prohibition of the use, stockpiling, production and transfer of anti-personnel mines and on their destruction (1997) APLC/MSP, United Nations New York. Available at: <https://www.unog.ch>. Accessed 3 December, 2018
32. Herard P, Boillot F (2012) Amputation in emergency situations: indications, techniques and Medecins Sans Frontieres France's experience in Haiti. *Int Orthop* 36:1979–1981
33. Mitkovic M, Bumbasirevic M, Grubor P et al (2013) Nature and results of treatment of war wounds caused by cluster bombs. *Acta Chir Iugosl* 60:41–47
34. Rew DA, Clasper J, Kerr G (2004) Surgical workload from an integrated UK field hospital during the 2003 Gulf conflict. *JRAMC* 150:99–106

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplement

Supplement Table 1 Comparison of risk for in-hospital amputation for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992-1995 and 2009-2012

In-hospital amputation	Univariate analysis			Logistic regression analysis			
	No n (%)	Yes n (%)	P value	All confounders Odds ratio (95% CI)	P value	Final model Odds ratio (95% CI)	P value
Gender			0.36				
Male	833 (91.0)	82 (9.0)					
Female	78 (94.0)	5 (6.0)		1 0.45 (0.15-1.38)	0.16		
Age			0.03				
<16 years	127 (88.2)	17 (11.8)					
16-49	717 (92.5)	58 (7.5)		1 0.69 (0.35-1.36)	0.28		
>49	67 (84.8)	12 (15.2)		0.88 (0.32-2.40)	0.80		
Time since injury			0.59				
<24 hours	352 (90.3)	38 (9.7)		1			
24-48	323 (91.5)	30 (8.5)		1.00 (0.55-1.82)	0.99		
49-168	236 (92.5)	19 (7.5)		0.97 (0.49-1.92)	0.92		
Mean arterial pressure			<0.0001				
≥65 mmHg	903 (91.8)	81 (8.2)		1		1 6.43 (1.69-24.38)	0.006
<65	8 (57.1)	6 (42.9)		5.84 (1.47-23.16)			
Degree of tissue damage			<0.0001				
Small	363 (98.4)	6 (1.6)		1		1	
Medium	428 (95.5)	20 (4.5)		2.51 (0.98-6.45)	0.06	2.53 (0.99-6.51)	0.05
Extensive	120 (66.3)	61 (33.7)		25.76 (10.49-63.25)	<0.0001	25.75 (10.55-62.87)	<0.0001
2nd wound present			<0.0001				
No	743 (93.1)	55 (6.9)		1		1	
Yes	168 (84.0)	32 (16.0)		2.55 (1.45-4.48)	0.001	2.56 (1.47-4.46)	0.0009
Vital vessel injury			<0.0001				
No	873 (93.3)	63 (6.7)		1		1	
Yes	38 (61.3)	24 (38.7)		7.61 (3.73-15.53)	<0.0001	7.54 (3.74-15.20)	<0.0001
Time period			0.20				
1992-1995	417 (90.1)	46 (9.9)		1		1	
2009-2012	494 (92.3)	41 (7.7)		1.20 (0.69-2.09)	0.51	1.12 (0.66-1.91)	0.67

Supplement Table 2 Comparison of risk for use of external fixation for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992-1995 and 2009-2012

External fixation	Univariate analysis			Logistic regression analysis		
	No n (%)	Yes n (%)	P value	All confounders Odds ratio (95% CI)	P value	Final model Odds ratio (95% CI)
Total=998						
Gender			0.04			
Male	850 (92.9)	65 (7.1)		1		
Female	82 (98.8)	1 (1.2)		0.21 (0.03-1.58)	0.13	
Age			0.006			
<16 years	140 (97.2)	4 (2.8)		1		
16-49	724 (93.4)	51 (6.6)		2.25 (0.78-6.47)	0.13	2.55 (0.90-7.25)
>49	68 (86.1)	11 (13.9)		5.74 (1.71-19.30)	0.005	6.28 (1.89-20.85)
Time since injury			0.29			
<24 hours	370 (94.9)	20 (5.1)		1		
24-48	325 (92.1)	28 (7.9)		1.39 (0.75-2.59)	0.29	
49-168	237 (92.9)	18 (7.1)		1.54 (0.76-2.97)	0.24	
Mean arterial pressure			0.94			
≥65 mmHg	919 (93.4)	65 (6.6)		1		
<65	13 (92.9)	1 (7.1)		0.93 (0.11-8.00)	0.95	
Degree of tissue damage			<0.0001			
Small	362 (98.1)	7 (1.9)		1		
Medium	412 (92.0)	36 (8.0)		4.27 (1.87-9.79)	0.0006	4.43 (1.94-10.12)
Extensive	158 (87.3)	23 (12.7)		6.56 (2.68-16.03)	<0.0001	6.82 (2.84-16.38)
2nd wound present			0.66			
No	751 (94.1)	47 (5.9)		1		
Yes	181 (90.5)	19 (9.5)		1.38 (0.77-2.48)	0.28	
Vital vessel injury			0.32			
No	876 (93.6)	60 (6.4)		1		
Yes	56 (90.3)	6 (9.7)		1.22 (0.48-3.10)	0.68	
Time period			0.008			
1992-1995	422 (91.1)	41 (8.9)		1		
2009-2012	510 (95.3)	25 (4.7)		0.63 (0.36-1.08)	0.09	0.56 (0.32-0.96)
						0.04

Supplement Table 3 Comparison of risk for use of split skin graft for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992-1995 and 2009-2012

Split skin graft		Univariate analysis			Logistic regression analysis		
		No n (%)	Yes n (%)	P value	All confounders Odds ratio (95% CI)	Total=998 P value	Final model Odds ratio (95% CI) P value
Gender	Male	744 (81.3)	171 (18.7)	0.35	1	0.03	1
	Female	64 (77.1)	19 (22.9)		2.04 (1.09-3.81)		2.18 (1.18-4.04) 0.01
Age	<16 years	108 (75.0)	36 (25.0)	0.13	1	0.15	0.17
	16-49	634 (81.8)	141 (18.2)		0.70 (0.43-1.13)		
	>49	66 (83.5)	13 (16.5)		0.58 (0.26-1.27)		
Time since injury	<24 hours	315 (80.8)	75 (19.2)	0.54	1	0.97	0.91
	24-48	281 (79.6)	72 (20.4)		0.99 (0.66-1.49)		
	49-168	212 (83.1)	43 (16.9)		0.97 (0.61-1.55)		
Mean arterial pressure	≥65 mmHg	800 (81.3)	184 (18.7)	0.02	1	0.29	
	<65	8 (57.1)	6 (42.9)		2.05 (0.54-7.80)		
Degree of tissue damage	Small	345 (93.5)	24 (6.5)	<0.0001	1	<0.0001	1
	Medium	369 (82.4)	79 (17.6)		2.89 (1.77-4.71)		2.89 (1.77-4.70) <0.0001
	Extensive	94 (51.9)	87 (48.1)		11.55 (6.82-19.55)		11.44 (6.81-19.21) <0.0001
2nd wound present	No	663 (83.1)	135 (16.9)	0.0007	1	0.05	1
	Yes	145 (72.5)	55 (27.5)		1.51 (1.00-2.26)		1.53 (1.02-2.30) 0.04
Vital vessel injury	No	759 (81.1)	177 (18.9)	0.69	1	0.45	
	Yes	49 (79.0)	13 (21.0)		0.76 (0.37-1.55)		
Time period	1992-1995	330 (71.3)	133 (28.7)	<0.0001	1	<0.0001	1
	2009-2012	478 (89.3)	57 (10.7)		0.31 (0.21-0.46)		0.31 (0.21-0.45) <0.0001

Supplement Table 4 Comparison of risk for use of debridement or change of dressing in operating theatre for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992-1995 and 2009-2012

Debridement/Change of dressing	Univariate analysis			Logistic regression analysis		
	0-2 n (%)	≥3 n (%)	P value	All confounders Odds ratio (95% CI)	P value	Final model Odds ratio (95% CI)
Gender			0.91			
Male	645 (70.5)	270 (29.5)		1		
Female	58 (69.9)	25 (30.1)		1.13 (0.65-1.96)	0.67	
Age			0.29			
<16 years	97 (67.4)	47 (32.6)		1		
16-49	555 (71.6)	220 (28.4)		0.91 (0.60-1.40)	0.68	
>49	51 (64.6)	28 (35.4)		1.08 (0.57-2.06)	0.82	
Time since injury			0.31			
<24 hours	285 (73.1)	105 (26.9)		1		
24-48	240 (68.0)	113 (32.0)		1.36 (0.95-1.93)	0.09	
49-168	178 (69.8)	77 (30.2)		1.54 (1.05-2.26)	0.03	
Mean arterial pressure			0.004			
≥65 mmHg	698 (70.9)	286 (29.1)		1		
<65	5 (35.7)	9 (64.3)		3.48 (1.01-12.00)	0.05	
Degree of tissue damage			<0.0001			
Small	313 (84.8)	56 (15.2)		1		1
Medium	306 (68.3)	142 (31.7)		2.50 (1.75-3.58)	<0.0001	2.48 (1.73-3.54)
Extensive	84 (46.4)	97 (53.6)		5.61 (3.84-8.64)	<0.0001	5.66 (3.69-8.69)
2nd wound present			<0.0001			
No	609 (76.3)	189 (23.7)		1		1
Yes	94 (47.0)	106 (53.0)		3.61 (2.56-5.11)	<0.0001	3.54 (2.52-4.98)
Vital vessel injury			0.0008			
No	671 (71.7)	265 (28.3)		1		1
Yes	32 (51.6)	30 (48.4)		2.05 (1.16-3.64)	0.01	1.98 (1.13-3.49)
Time period			0.02			
1992-1995	310 (67.0)	153 (33.0)		1		1
2009-2012	393 (73.5)	142 (26.5)		0.89 (0.65-1.22)	0.48	0.89 (0.66-1.20)

Supplement Table 5 Comparison of risk for use of blood transfusion for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992-1995 and 2009-2012

Blood transfusion	Univariate analysis		Logistic regression analysis			
	No n (%)	Yes n (%)	P value	All confounders Odds ratio (95% CI)	Final model Odds ratio (95% CI)	P value
Gender			0.95			
Male	785 (85.8)	130 (14.2)				
Female	71 (85.5)	12 (14.5)		1.36 (0.64-2.90)		0.43
Age			0.03			
<16 years	113 (78.5)	31 (21.3)				
16-49	674 (87.0)	101 (13.0)		0.54 (0.32-0.93)	0.54 (0.32-0.92)	0.02
>49	69 (87.3)	10 (12.7)		0.33 (0.13-0.84)	0.32 (0.13-0.82)	0.02
Time since injury			0.93			
<24 hours	336 (86.2)	54 (13.8)				
24-48	303 (85.8)	50 (14.2)		1.17 (0.72-1.89)		0.53
49-168	217 (85.1)	38 (14.9)		1.65 (0.97-2.79)		0.06
Mean arterial pressure			<0.0001			
≥65 mmHg	851 (86.5)	133 (13.5)				
<65	5 (35.7)	9 (64.3)		11.27 (2.94-43.18)	10.04 (2.67-37.74)	0.0006
Degree of tissue damage			<0.0001			
Small	351 (95.1)	18 (4.9)				
Medium	390 (87.1)	58 (12.9)		2.67 (1.50-4.78)	2.67 (1.49-4.76)	0.0009
Extensive	115 (63.5)	66 (36.5)		8.26 (4.49-15.21)	8.16 (4.44-14.97)	<0.0001
2nd wound present			<0.0001			
No	707 (88.6)	91 (11.4)				
Yes	149 (74.5)	51 (25.5)		2.74 (1.76-4.29)	2.62 (1.69-4.07)	<0.0001
Vital vessel injury			<0.0001			
No	826 (88.2)	110 (11.8)				
Yes	30 (48.4)	32 (51.6)		9.59 (511-18.00)	9.17 (4.92-17.08)	<0.0001
Time period			<0.0001			
1992-1995	371 (80.1)	92 (19.9)				
2009-2012	485 (90.7)	50 (9.3)		0.41 (0.27-0.64)	0.43 (0.28-0.66)	0.0001

Supplement Table 6 Comparison of risk of longer hospital stay for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992-1995 and 2009-2012

Length of hospital stay		Univariate analysis			Logistic regression analysis		
		Total=998		P value	Total=998		P value
		<21 days n (%)	≥21 days n (%)		All confounders Odds ratio (95% CI)	Final model Odds ratio (95% CI)	
Gender	Male	496 (54.2)	419 (45.8)	0.40	1 1.32 (0.79-2.20)	1	0.30
	Female	41 (49.4)	42 (50.6)				
Age	<16 years	69 (47.9)	75 (52.1)	0.03	1 0.76 (0.51-1.14)	1	0.18
	16-49	434 (56.0)	341 (44.0)				
	≥49	34 (43.0)	45 (57.0)				
Time since injury	<24 hours	215 (55.1)	175 (44.9)	0.49	1 1.21 (0.87-1.67)	1	0.26
	24-48	181 (51.3)	172 (48.7)				
	49-168	141 (55.3)	114 (44.7)				
Mean arterial pressure	≥65 mmHg	531 (54.0)	453 (46.0)	0.41	1 0.97 (0.29-3.28)	1	0.96
	<65	6 (42.9)	8 (57.1)				
Degree of tissue damage	Small	288 (78.0)	81 (22.0)	<0.0001	1 4.16 (3.04-5.69)	1 4.11 (3.01-5.61)	<0.0001
	Medium	204 (45.5)	244 (54.5)				
	Extensive	45 (24.9)	136 (75.1)				
2nd wound present	No	452 (56.6)	346 (43.4)	0.0003	1 1.57 (1.11-2.23)	1 1.58 (1.12-2.23)	0.009
	Yes	85 (42.5)	115 (57.5)				
Vital vessel injury	No	518 (55.3)	418 (44.7)	0.0002	1 2.18 (1.18-4.04)	1 2.12 (1.16-3.89)	0.02
	Yes	19 (30.6)	43 (69.4)				
Time period	1992-1995	228 (49.2)	235 (50.8)	0.007	1 0.82 (0.62-1.10)	1 0.87 (0.66-1.14)	0.31
	2009-2012	309 (57.8)	226 (42.2)				

Supplement Table 7 Comparison of risk of in-hospital mortality for patients treated for weapon-related limb injuries at ICRC's hospitals in Quetta and Peshawar, Pakistan, 1992-1995 and 2009-2012

In-hospital mortality	Univariate analysis			Logistic regression analysis		
	No n (%)	Yes n (%)	P value	All confounders Odds ratio (95% CI)	Total=998 P value	Final model Odds ratio (95% CI) P value
Gender			0.42			
Male	908 (99.2)	7 (0.8)		1		
Female	83 (100)	0 (0)		-	1.0	
Age			0.49			
<16 years	144 (100)	0 (0)		1		
16-49	769 (99.2)	6 (0.8)		-	1.0	
>49	78 (98.7)	1 (1.3)		-	1.0	
Time since injury			0.36			
<24 hours	389 (99.7)	1 (0.3)		1		
24-48	350 (99.2)	3 (0.8)		2.39 (0.24-23.84)	0.46	
49-168	252 (98.8)	2 (1.2)		4.69 (0.47-47.21)	0.19	
Mean arterial pressure			0.75			
≥65 mmHg	977 (99.3)	7 (0.7)		1		
<65	14 (100)	0 (0)		-	1.0	
Degree of tissue damage			0.01			
Small	369 (100)	0 (0)		1		
Medium	445 (99.3)	3 (0.7)		-	0.99	
Extensive	177 (97.8)	4 (2.2)		-	0.99	
2nd wound present			0.01			
No	795 (99.6)	3 (0.4)		1		
Yes	196 (98.0)	4 (2.0)		4.24 (0.84-21.40)	0.08	1 5.48 (1.19-25.18) 0.03
Vital vessel injury			0.01			
No	931 (99.5)	5 (0.5)		1		
Yes	60 (96.8)	2 (3.2)		4.82 (0.74-31.42)	0.1	1 7.52 (1.38-41.15) 0.02
Time period			0.18			
1992-1995	458 (98.9)	5 (1.1)		1		
2009-2012	533 (99.6)	2 (0.4)		0.47 (0.07-2.96)	0.42	1 0.32 (0.06-1.67) 0.18

PAPER **III**

RESEARCH ARTICLE

Open Access

Predicting surgical resource consumption and in-hospital mortality in resource-scarce conflict settings: a retrospective study

Måns Muhrbeck^{1,2*} , Zaher Osman³, Johan von Schreeb⁴, Andreas Wladis^{1,2} and Peter Andersson^{1,5}

Abstract

Background: In armed conflicts, civilian health care struggles to cope. Being able to predict what resources are needed is therefore vital. The International Committee of the Red Cross (ICRC) implemented in the 1990s the Red Cross Wound Score (RCWS) for assessment of penetrating injuries. It is unknown to what extent RCWS or the established trauma scores Kampala trauma Score (KTS) and revised trauma score (RTS) can be used to predict surgical resource consumption and in-hospital mortality in resource-scarce conflict settings.

Methods: A retrospective study of routinely collected data on weapon-injured adults admitted to ICRC's hospitals in Peshawar, 2009–2012 and Goma, 2012–2014. High resource consumption was defined as ≥ 3 surgical procedures or ≥ 3 blood-transfusions or amputation. The relationship between RCWS, KTS, RTS and resource consumption, in-hospital mortality was evaluated with logistic regression and adjusted area under receiver operating characteristic curves (AUC). The impact of missing data was assessed with imputation. Model fit was compared with Akaike Information Criterion (AIC).

Results: A total of 1564 patients were included, of these 834 patients had complete data. For high surgical resource consumption AUC was significantly higher for RCWS (0.76, 95% CI 0.74–0.78) than for KTS (0.53, 95% CI 0.50–0.56) and RTS (0.51, 95% CI 0.48–0.54) for all patients. Additionally, RCWS had lower AIC, indicating a better model fit. For in-hospital mortality AUC was significantly higher for RCWS (0.83, 95% CI 0.79–0.88) than for KTS (0.71, 95% CI 0.65–0.76) and RTS (0.70, 95% CI 0.63–0.76) for all patients, but not for patients with complete data.

Conclusion: RCWS appears to predict surgical resource consumption better than KTS and RTS. RCWS may be a promising tool for planning and monitoring surgical care in resource-scarce conflict settings.

Keywords: Armed conflicts, Health resources, Penetrating wounds

Background

In armed conflicts, civilian casualties often must rely on frail and fragmented health care systems that can only offer limited or no surgical resources to treat injured with potentially life-threatening injuries [1]. For

healthcare providers delivering surgical care in conflicts, it is therefore imperative to know how to use available surgical resources as efficiently as possible. The extent of surgical resource consumption or workload in resource-scarce conflict settings is often discussed in terms of the number of surgical procedures, amputations, use of blood products, and mortality rate [2–7]. However, to our knowledge, no method has been validated to predict these outcomes in conflict settings. Trauma scores developed and used in non-conflict trauma settings to

* Correspondence: mans.muhrbeck@regionostergotland.se

¹Department of Surgery in Norrköping, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

²Center for Disaster Medicine and Traumatology, University Hospital, Linköping, Sweden

Full list of author information is available at the end of the article

© The Author(s). 2021 **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

predict mortality could potentially be used for this purpose. The vital sign-based Revised Trauma Score (RTS) has been shown to predict need for surgery, haemorrhage, and mortality [8–11]. In resource-scarce trauma settings the Kampala Score (KTS), a simplified combination of age, RTS and the anatomical-based Injury Severity Score (ISS), has been validated to predict the need for admission and mortality [12]. However, the predictive abilities for RTS and KTS could be diminished in conflicts due to disintegrated infrastructure, lack of transportation means, non-existing prehospital care and long distance to hospital that cause a survival bias where injured with life-threatening, but treatable, injuries die before reaching the hospital. Furthermore, injury patterns in armed conflicts are different from those seen in civilian traumas. Penetrating injuries are more prevalent, whereas blunt injuries are more frequent in civilian traumas [13, 14].

The International Committee of the Red Cross (ICRC) is a humanitarian organization providing medical assistance to victims of war and other situations of violence, including independently running or supporting hospitals. In the early 1990s Mr. Robin Coupland, at the time Chief Surgeon for ICRC, developed a system for wound classification (Red Cross Wound Score, RCWS) in order to help surgeons systematically describe penetrating wounds in conflicts [15]. To classify a wound according to RCWS, the extent of tissue damage (RCWS grade) and type of tissue involved (RCWS type) must be assessed (Table 1). The RCWS grade corresponds to amount of energy transferred to the tissue at time of injury [4, 15]. Studies have demonstrated that RCWS grade is associated with need of surgery, number of surgical procedures, in-hospital amputation, and mortality [4, 16–19]. However, RCWS predictive ability regarding the extent of surgical resource consumption and mortality has not previously been examined.

The aim of the present study was therefore to determine if RCWS, KTS and RTS have predictive abilities to assess the extent of surgical resource consumption as well as in-hospital mortality in resource-scarce conflict settings. Surgical resource consumption was characterized by the number of surgical procedures, blood transfusions and limb amputation. A secondary aim was to assess if the individual components of RCWS are independent predictors of surgical resource consumption and in-hospital mortality. If any of these scores could be found to be reliable, they would provide an urgently needed instrument for planning and monitoring surgical interventions in resource-scarce conflict settings.

Patients and settings

Routinely collected patient data from ICRC’s hospitals in Peshawar, Pakistan, and Goma, the Democratic Republic

Table 1 Parameters included in the Red Cross Wound Score (RCWS) [4]

Wound feature	Definition
E (entry)	Entry wound in cm
X (exit)	Exit wound in cm (X = 0 if no exit)
C (cavity)	Can the cavity of the wound take two fingers before surgical excision? C0 = No C1 = Yes
F (fracture)	Fracture F0 = No fracture F1 = Simple fracture, hole or insignificant comminution F2 = Clinically significant comminution
V (vital structure)	Injury threatening life or threatening life or limb V0 = No vital structure injured VN = (neurological) penetration of the dura of the brain or spinal cord VT = (thorax or trachea) penetration of the pleura or of the larynx/trachea in the neck VA = (abdomen) penetration of the peritoneum VH = (haemorrhage) injury of a major peripheral blood vessel, down to the brachial artery in the arm or the popliteal artery in the leg or carotid artery in the neck

of the Congo (DRC), were retrospectively analysed for this study. Patients treated at ICRC’s hospital in Peshawar were predominantly injured in the conflict on both sides of the Pakistani–Afghanistan border. The hospital was closed in 2014 and had 116 beds, a critical care unit, three operating theatres, X-ray services and a laboratory [20]. Patients treated at the hospital in Goma were mainly injured in the ongoing conflict between several rivaling factions in the Kivu province in the DRC. Compared to the conflict along the Afghanistan–Pakistani border, less injuries from indiscriminate weapons such as grenades, bombs and anti-personnel mines have been reported [2, 20, 21]. The hospital is still operational and has 65 beds, one operating theatre, X-ray services and a laboratory. Both hospitals had access to physiotherapy for post-operative mobilization and rehabilitation with prosthesis fitting [20].

At both hospitals combatants as well as local citizens with weapon-related injuries were treated. Those seeking care were not asked whether they were civilians or combatants. Care at both hospitals was provided free of charge during the study period.

Method

The material consists of two matching databases of anonymized patient data from the ICRC’s hospitals in Peshawar and Goma. All patients treated between 18

February 2009–9 May 2012 (Peshawar) and 17 November 2012–17 September 2014 (Goma) were continuously included in the two databases (Excel® spreadsheet) at the time of patient discharge. Entries in the Peshawar database were done by two physicians whose primary job was to maintain the database. Entries in the Goma database were done by health care staff after receiving basic training. In total 3028 patients were recorded in the Peshawar database and 689 in Goma database. The two databases were merged and transferred to SPSS Statistical software version 25 (IBM Corporation, Armonk, NY, USA) for this study. The final database was validated with respect to the original databases by comparing data from 50 patients from each database without any differences identified. Patients that fulfilled the following inclusion criteria were extracted from the merged database: adult patients with weapon-related penetrating injuries, assessed RCWS and known discharge status. Adults were defined as age 15 or older in accordance with previous studies from similar settings [20, 22]. RCWS was determined by the surgeon at the time of the first surgical procedure according to ICRC guidelines [4]. Surgeons had received training in the use of RCWS prior to deployment. The wound with the highest RCWS grade and type was recorded as the first injury. To categorize a penetrating injury according to RCWS, the wound's grade 1–3 (wound's entry- and exit-diameter and if a two-fingers- wide cavity exists) and type (soft tissue, bones, threatening life or threatening life or limb) needs to be determined (Table 1).

Depending on the grade and type, a wound is assigned to one of twelve different categories (Additional Table 1) [4, 15, 23]. The presence of a second severe injury was dichotomously registered as existing or not. Severe injury was defined as a cavitating soft tissue injury with entry or exit 10 cm or more, or open fracture or injury to vital structure. This definition excludes RCWS soft tissue injury grade 1 and 2 and translates to an Abbreviated Injury Score of 2 or above for any given anatomical site, corresponding to the definition used in KTS [24]. In the case of multiple admissions only the first one was included. Patients with unspecified sex, time of injury and vital signs were considered to be incomplete cases and excluded in the primary analysis (Fig. 1).

ICRC characterizes surgical workload in conflict settings by severity of injuries, number of operations per patient, number of blood transfusions required and length of hospitalization [4]. To better reflect the actual surgical resource consumption, in the present study, this definition was modified to: number of surgical procedures under anaesthesia, blood transfusions and limb amputation. Length of hospitalization was excluded as this can be influenced by issues that are not related to the treatment of the patients' injuries. This modification

was done after advice from senior ICRC surgeons, and is consistent with how surgical resource consumption is characterized in previous studies [2–5, 7, 25].

High surgical resource consumption was dichotomously defined as:

- 1 Three or more surgical procedures under anaesthesia OR
- 2 Three or more blood transfusions OR
- 3 In-hospital amputation proximal to the metacarpophalangeal joints of the hand or the midtarsal joints of the foot.

Three or more surgical procedures were chosen because a study with 16,172 patients treated at ICRC's hospitals demonstrated that 66% of the patients were treated with two surgical procedures or fewer [4]. Three or more blood transfusions were chosen as a previous ICRC-study with 4470 weapon-injured patients demonstrated that 15.5% were transfused and an average of 2.9 units was given [25]. Furthermore, the ICRC recommends an availability of 100 units/100 patients if anti-personnel mines are used in the conflict [4]. In-hospital limb amputation was included as these patients require extensive resources postoperatively in terms of physiotherapy and prosthesis fitting. In-hospital mortality was defined as deceased during hospital stay.

Statistical methods

We estimated that 592 patients would be needed to detect a difference of 0.10 in the trauma scores' ability to detect high surgical resource consumption. A reference area under the receiver operating characteristic curve (AUC) of 0.75, confidence interval (CI) 95% and power 90% was used for this sample size calculation. Nonetheless, all eligible patients were included in the analysis.

Descriptive comparisons between the hospitals in Peshawar and Goma were analysed using Fisher's exact test for categorical variables and Mann-Whitney *U*-test for continuous variables. Values are given as mean with standard deviation (SD) where appropriate. To compare the predictive effect of the individual components of RCWS, KTS and RTS on surgical resource consumption and in-hospital mortality, binary logistic regression was performed. Sex, age (15–49 and > 49 yrs.), time since injury (0–6, 7–24 and > 24 h) systolic blood pressure (0–75, 76–89, and > 89 mmHg), respiratory rate (≤ 9 , 10–29, ≥ 30 /min), Glasgow Coma Scale (≤ 5 , 6–8, 9–12 and 13–15), RCWS grade, RCWS type and the existence of more than one severe injury were predictors included in the regression analysis as these are the components of RCWS, KTS and RTS or have in previous studies been identified as influencing the need for surgical intervention, blood transfusion or amputation [2, 26–29]. Vital

signs were taken upon admission, and the intervals used match the intervals used in RTS [8]. The procedure to calculate RTS and KTS has been described elsewhere [8, 12]. Forward Wald was used to calculate a final regression model. When comparing differences in resource consumption for the Peshawar and Goma hospital, hospital site was added to the final regression model. The impact of predictors is presented as odds ratio (OR) with 95% confidence intervals. *P*-values (two-tailed) less than 0.05 were considered significant for both the univariate and logistic regression analysis.

The sensitivity and specificity associated with the ability of RCWS, KTS and RTS to predict high surgical resource consumption and in-hospital mortality was

assessed by analysing of the AUCs. To enable analysis of patients with more than one severe injury, RCWS was adjusted for the existence of additional severe injuries. In making comparisons of the prognostic abilities of RCWS, KTS and RTS regarding high surgical resource consumption and in-hospital mortality the AUCs along with 95% CI were used. Overlapping confidence intervals were interpreted as indicating non-significant differences in the prognostic abilities of RCWS, KTS and RTS.

Comparison of goodness of model fit for RCWS, KTS and RTS in predicting high surgical resource consumption and in-hospital mortality was analysed by Akaike Information Criterion (AIC). A lower AIC score for a trauma score indicates a better fitting model in

comparison with the other evaluated trauma scores. Little's MCAR test and imputation with fully conditional specification was done to enable the analysis of incomplete cases and thereby assessing the impact of exclusion of patients with unknown sex, time of injury and vital signs. Mann-Whitney *U*-test with Fisher's exact test was used to evaluate why a patient ended up in the high resource group.

Results

Descriptive

A total of 657 of 1324 patients treated in Peshawar and 177 of 240 patients treated in Goma had complete data (Complete cases, Fig. 1). There were more men and older patients injured in Peshawar compared to Goma, and more patients had injuries from indiscriminate weapons such as bombs, shells and fragments or anti-personnel mines, and fewer from gunshots ($p \leq 0.001$ for all) (Table 2).

There was no difference in the RCWS type for the first injury recorded between the hospitals. However, patients in Goma were more likely to be admitted within 6 hours of their injury or have injuries to the upper limbs, while patients in Peshawar more frequently had injuries to the head, face or more than one severe injury ($p < 0.05$ for all) (Table 3).

Chest-tube insertion and fracture surgery were more frequent in Peshawar than in Goma ($p = 0.039$ and $p < 0.001$, respectively). There was no difference in frequencies of amputations, surgical procedures, blood transfusions and in-hospital mortality between the hospitals (Table 4).

Surgical resource consumption

The relationship between surgical resource consumption, sex, age, vital signs and RCWS are shown in Table 5. A larger portion of the patients with high surgical resource consumption had lower systolic blood pressure, higher RCWS grade, higher RCWS type and more frequently had more than one severe injury compared to patients with low surgical resource consumption ($p <$

0.01 for all). When adjusting for all predictors in the final logistic regression model RCWS grade, RCWS type and existence of more than one severe injury remained significant. Sex, age, time since injury, systolic blood pressure, respiratory rate and Glasgow Coma Scale did not affect a patient's surgical resource consumption.

For complete cases RCWS achieved significantly higher AUC and lower AIC, i.e., better predictive ability and model fit, than KTS and RTS for high surgical resource consumption (Table 6, Fig. 2). In the logistic regression model for all cases, using imputed data for incomplete cases, the same predictors plus age more than 49 years (OR 1.84, 95% CI 1.26–2.70, $p < 0.001$) were found to be significant (Additional Table 2). There was no statistically significant difference in the AUC for all cases compared to complete cases (Table 6 and Fig. 2).

Subgroup analysis of high resource consumption and hospital site

When hospital site was added to the final logistic regression model of complete cases, patients treated in Goma were more likely to have a high surgical resource

Table 3 Injury patterns for patients treated at ICRC's hospitals in Peshawar and Goma

Injury patterns	Peshawar Total = 657 n (% of total)	Goma Total = 177 n (% of total)	P value
Location of injury ^a			
Head or face	58 (8.8)	6 (3.4)	0.016
Neck	15 (2.3)	1 (0.6)	0.216
Thorax	69 (10.5)	13 (7.3)	0.255
Back	39 (5.9)	5 (2.8)	0.128
Abdomen	30 (4.6)	13 (7.3)	0.178
Pelvis ^b	26 (4.0)	9 (5.1)	0.527
Upper limbs	136 (20.7)	50 (28.2)	0.042
Lower limbs	276 (42.0)	76 (43.0)	0.864
Unknown	8 (1.2)	4 (2.3)	0.294
RCWS type ^a			
Soft tissue	225 (34.2)	62 (35.0)	0.859
Fracture	269 (40.9)	75 (42.4)	0.732
Penetration of peritoneum	38 (5.8)	17 (9.6)	0.086
Penetration of pleura ^c	47 (7.2)	9 (5.1)	0.399
Penetration of dura ^d	34 (5.2)	5 (2.8)	0.231
Major periph. blood vessel ^e	44 (6.7)	9 (5.1)	0.492
Additional severe injuries ^f			
No	562 (85.6)	165 (93.2)	0.005
Yes	95 (14.4)	12 (6.8)	

^aFirst injury recorded; ^bPelvis including buttocks, perineum, and genitals;

^cIncluding the larynx/trachea of the neck; ^dThe dura of the brain or spinal

cord; ^eDown to the brachial or popliteal or carotid arteries; ^fCorresponding to Abbreviated Injury Score ≥ 2

Table 2 Mechanism of injury for patients treated at ICRC's hospitals in Peshawar and Goma

Mechanism	Peshawar Total = 657 n (% of total)	Goma Total = 177 n (% of total)	P value
Gunshot	357 (54.3)	156 (88.1)	< 0.001
Bomb, shell, fragment	266 (40.5)	17 (9.6)	< 0.001
Anti-personnel mine	31 (4.7)	0	0.001
Arme blanche	1 (0.2)	3 (1.7)	0.031
Other	2 (0.3)	0	
Unknown	0	1 (0.6)	

Table 4 Surgical care and in-hospital mortality for patients treated at ICRC's hospitals in Peshawar and Goma

Surgical care and in-hospital morality	Peshawar Total = 657		Goma Total = 177		P value
	n (% of total)	Mean (SD)	n (% of total)	Mean (SD)	
Surgical procedure under anesthesia ^a					
Craniotomy	21 (3.2)		2 (1.1)		0.195
Thoracotomy	8 (1.2)		0		0.214
Chest-tube	67 (10.2)		9 (5.1)		0.039
Laparotomy	82 (12.5)		14 (7.9)		0.111
Peri. vasc. Repair	31 (4.7)		4 (2.3)		0.148
Arm-amputation	11 (1.7)		3 (1.7)		1
Leg-amputation	34 (5.2)		5 (2.8)		0.122
Any amputation	38 (5.8)	0.06 (0.23)	8 (4.5)	0.05 (0.21)	0.513 ^d
Any frac. surgery ^b	157 (23.9)	0.24 (0.43)	18 (10.2)	0.10 (0.30)	< 0.001 ^d
Any soft tissue surgery ^c	608 (92.5)	0.93 (0.26)	170 (96.0)	0.96 (0.20)	0.098 ^d
Any type of procedure	637 (97.0)	0.97 (0.17)	168 (94.9)	0.95 (0.22)	0.188 ^d
Blood transfusion		0.19 (0.56)		0.34 (1.19)	0.521 ^d
0 units	543 (82.6)		153 (86.4)		0.356
1–2	75 (11.4)		15 (8.5)		0.278
≥ 3	34 (5.2)		9 (5.1)		1
Unknown	5 (0.8)		0		0.590
In-hospital mortality		0.03 (0.16)		0.02 (0.13)	0.431 ^d
No	639 (97.3)		174 (98.3)		
Yes	18 (2.7)		3 (1.7)		

^aSome patients have been subjected to several procedures which results in more procedures than patients and sum of percentages more than 100; ^bExternal fixation, Kirchner wire, traction, or manipulation; ^cDebridement, split skin graft, delayed primary closure, burn care, or change of dressing; ^dP-value for comparison of mean

consumption than patients treated in Peshawar (OR 2.01, 95% CI 1.36–2.98, $p < 0.001$). After adjusting surgical procedures shown in Table 3 for the total number of injured sites, patients in Goma were more frequently exposed to soft tissue surgery (Goma: mean 3.58 times/patient, SD 3.39, and Peshawar: 2.18, SD 1.75, $p < 0.001$) and also to any type of surgical procedures under anaesthesia than in Peshawar (Goma: mean 3.71 times/patient, SD 3.71, and Peshawar: 1.96, SD 1.55, $p < 0.001$). When comparing Goma and Peshawar regarding patients with high surgical resource consumption the distribution between hospitals was similar regarding the number of surgical procedures, blood transfusions and amputation rates. Three or more surgical procedures most strongly explained why most patients ended up in the high resource consumption group for both hospital sites (Goma: 86 of 102 patients, 84.3%, and Peshawar: 197 of 260 patients, 75.8%, $p = 0.09$).

In-hospital mortality

For in-hospital mortality, there was no difference between the hospitals (Peshawar: 18 patients, 2.7% and

Goma: 3 patients, 1.7%, $p = 0.781$). At both hospitals, a larger portion of the patients who died in hospital arrived sooner after injury, had systolic blood pressure < 90 mmHg, GCS < 13 and higher RCWS type than those discharged alive ($p < 0.05$ for all) (Additional Table 3). In the logistical regression model for complete cases, no predictors were significant for in-hospital mortality. RCWS AUC and AIC for in-hospital mortality was not significantly better than KTS and RTS (Table 7, Fig. 3).

In the logistic regression model for all cases, using imputed data for incomplete cases, systolic blood pressure 76–89 mmHg and RCWS types threatening life and threatening life or limb were found to be significant predictors for in-hospital mortality (systolic blood pressure 76–89 mmHg: OR 4.63, 95% CI 1.59–13.44, threatening life: OR 10.72, 95% CI 4.42–26.01 and threatening life or limb: 8.43, 95% CI 3.36–21.18, $p < 0.01$ for all) (Additional Table 3). In the analysis of AUC for all cases RCWS demonstrated a significantly higher AUC and lower AIC, i.e. better predictive ability and model fit, than KTS and RTS for in-hospital mortality (Table 7 and Fig. 3).

Table 5 Relationship between surgical resource consumption, sex, age, vital signs and Red Cross Wound Score (RCWS)

Surgical resource consumption	Univariate analysis Complete cases Total = 834			Logistic regression analysis Complete cases Total = 834			
	Low Total = 472 n (%)	High ^a Total = 362 n (%)	P value	All confounders 1 = High ^a Odds ratio (95% CI)	P value	Final model (Wald) 1 = High ^a Odds ratio (95% CI)	P value
Sex			1.000				
Male	418 (88.6)	320 (88.4)		1			
Female	54 (11.4)	42 (11.6)		1.14 (0.68–1.90)	0.626		
Age			0.710				
15–49 years	433 (91.7)	329 (90.9)		1			
> 49	39 (8.3)	33 (9.1)		1.23 (0.70–2.17)	0.466		
Time since injury			0.152				
0–6 h	15 (3.2)	21 (5.8)		1			
7–24 h	203 (43.0)	159 (43.9)		0.68 (0.30–1.54)	0.352		
> 24 h	254 (53.8)	182 (50.3)		0.66 (0.29–1.52)	0.327		
Systolic blood pressure			0.004				
> 89 mmHg	464 (98.3)	341 (94.2)		1			
76–89	5 (1.1)	10 (2.8)		1.16 (0.33–4.11)	0.931		
0–75	3 (0.6)	11 (3.0)		1.65 (0.44–6.20)	0.821		
Respiratory rate			0.446				
10–29/min	458 (97.0)	347 (95.9)		1			
≤ 9	–	–		–	–		
≥ 30	14 (3.0)	15 (4.1)		0.88 (0.37–2.09)	0.772		
Glasgow Coma Scale			0.077				
13–15	465 (98.5)	347 (95.8)		1			
9–12	4 (0.9)	10 (2.8)		1.09 (0.31–3.87)	0.896		
6–8	2 (0.4)	4 (1.1)		1.60 (0.22–11.65)	0.644		
≤ 5	1 (0.2)	1 (0.3)		0.84 (0.05–15.76)	0.906		
RCWS grade			< 0.001				
1 (simple)	266 (56.4)	83 (22.9)		1		1	
2 (medium)	185 (39.2)	178 (49.2)		2.60 (1.81–3.74)	< 0.001	2.63 (1.84–3.76)	< 0.001
3 (large)	21 (4.4)	101 (27.9)		11.60 (6.61–20.36)	< 0.001	11.94 (6.85–20.80)	< 0.001
RCWS type			< 0.001				
Soft tissue	220 (46.6)	67 (18.5)		1		1	
Fracture	164 (34.8)	180 (49.7)		2.39 (1.62–3.54)	< 0.001	2.32 (1.58–3.41)	< 0.001
Threatening life	59 (12.5)	45 (12.4)		2.16 (1.27–3.67)	0.005	2.20 (1.31–3.72)	0.003
Threatening life/limb	29 (6.1)	70 (19.4)		4.67 (2.61–8.34)	< 0.001	4.80 (2.77–8.33)	< 0.001
Additional severe injuries ^b			< 0.001				
No	439 (93.0)	288 (79.6)		1		1	
Yes	33 (7.0)	74 (20.4)		2.90 (1.83–4.89)	< 0.001	3.00 (1.85–4.88)	< 0.001

^aDefined as ≥ 3 surgical procedures under anesthesia or ≥ 3 blood transfusions or limb amputation; ^bCorresponding to Abbreviated Injury Score ≥ 2

Discussion

To our knowledge, this is the first study to reveal that RCWS could have predictive abilities for high surgical resource consumption in adult patients with weapon-related injuries. This finding is consistent with previous

studies that have demonstrated a relationship between RCWS and the need for surgery, number of procedures, in-hospital amputation and mortality [16–19]. Furthermore, in a recent study from our group findings suggest that RCWS grade is correlated with number of surgeries,

Table 6 Analysis of AUC and AIC for individual scores for high surgical resource consumption

High surgical resource consumption	Complete cases Total = 834		All cases ^a Total = 1555 ^b	
	AUC (95% CI)	AIC	AUC (95% CI)	AIC
RCWS ^c	0.77 (0.74–0.81)	904	0.76 (0.74–0.78)	1777
- Grade ^c	0.74 (0.70–0.77)	948	0.73 (0.71–0.76)	1833
- Type ^c	0.70 (0.67–0.74)	1010	0.67 (0.65–0.70)	1979
KTS	0.59 (0.55–0.63)	1114	0.53 (0.50–0.56)	2072
RTS	0.54 (0.50–0.58)	1145	0.51 (0.48–0.54)	2096

^aUsing imputed data for incomplete cases; ^b9 patients excluded due to unknown surgical resource consumption; ^cAdjusted for existence of additional severe injuries, corresponding to Abbreviated Injury Score ≥ 2

blood transfusions and hospital stay in paediatric patients with weapon-related extremity wounds [30].

When comparing RCWS with KTS and RTS, commonly used trauma scores in non-conflict settings, RCWS had a better ability to predict high surgical resource consumption in conflict settings [11, 24, 31]. KTS and RTS were chosen for comparison as they have been demonstrated to be able to predict resource-related outcomes in trauma settings [10–12, 32, 33]. Furthermore, RCWS, KTS and RTS are similar in the aspect that they can be assessed without the need of advanced diagnostic equipment or calculations. A source of error is that we had a large number of missing vital signs in our dataset

Table 7 Analysis of AUC and AIC for individual scores for in-hospital mortality

In-hospital mortality	Complete cases Total = 834		All cases ^a Total = 1559 ^b	
	AUC (95% CI)	AIC	AUC (95% CI)	AIC
RCWS ^c	0.77 (0.67–0.86)	180	0.83 (0.79–0.88)	481
- Grade ^c	0.56 (0.42–0.70)	176	0.59 (0.52–0.66)	582
- Type ^c	0.73 (0.62–0.84)	193	0.78 (0.73–0.84)	506
KTS	0.74 (0.62–0.87)	183	0.71 (0.65–0.76)	537
RTS	0.67 (0.54–0.81)	182	0.70 (0.63–0.76)	534

^aUsing imputed data for incomplete cases; ^b5 patients excluded due to unknown discharge status; ^cAdjusted for existence of additional severe injuries, corresponding to Abbreviated Injury Score ≥ 2

potentially affecting the predictive abilities for KTS and RTS. To address this issue, we performed the logistic regression model with imputed data for missing vital signs without any substantial changes in vital signs impact on surgical resource consumption.

Patients treated in Peshawar were more frequently injured by indiscriminate weapons, arrived later to the hospital and more often had more than one severe injury than in Goma. Therefore, it was unexpected to find that the patients in Goma more often required high surgical resources than in Peshawar. The reason for the observed difference in surgical resource consumption is unclear as both hospitals followed the same treatment protocol and were similarly equipped [20]. A possible explanation

could be that patients treated in Goma were in a poorer general condition with impaired healing ability due to being more frequently affected by malnutrition or endemic diseases such as tuberculosis and malaria compared to Peshawar [34]. This difference in consumption of surgical resources between Goma and Peshawar illustrates how RCWS potentially could be used to systematically evaluate treatment facilities use of surgical resources in relation to injuries treated.

Consistent with other studies from armed conflicts, a low in-hospital mortality was observed at both hospitals [4, 20, 35]. In the analysis of all cases systolic blood pressure 76–89 mmHg, RCWS types threatening life and threatening life or limb were found to be significant predictors for in-hospital mortality rendering significantly better predictive ability for RCWS than KTS and RTS. The grade component of RCWS has previously been found to be associated with mortality in patients with conflict-related abdominal wounds with penetration of the peritoneum [19]. Otherwise, little has previously been known regarding RCWS predictive ability for mortality. In non-conflict settings better predictive abilities for KTS and RTS regarding mortality following weapon-related injuries have been reported [36, 37]. The worse predictive ability observed in our study could be explained by the survival bias where patients with the most altered vital signs die before reaching the hospital. The low in-hospital mortality and that over 90% of the patients in our material arrived more than 6 hours after

the injury occurred supports this assumption. The conclusion that RCWS has better predictive ability for in-hospital mortality than KTS and RTS in conflicts must therefore be made with caution.

The difficulty to foresee what resources will be needed in conflicts coupled with fragile and unreliable supply chains can potentially lead to disastrous effects in terms of morbidity and mortality [4, 38]. Our findings support the use of RCWS as an instrument for evaluation of surgical resource needs and in-hospital mortality in relation to injuries treated. RCWS could consequently be used to ensure that the surgical resources provided meet the actual treatment needs. The systematic use of RCWS could also enable quality audits and research in conflict settings. The inclusion of RCWS in the minimum data set recommended by the Consensus Framework for the Humanitarian Surgical Response to 21st Century Warfare should therefore be considered [39]. However, prospective studies from different armed conflicts are needed to assess the external validity of our findings and determine the predictive ability for each RCWS category. Furthermore, the interobserver reliability for RCWS needs to be evaluated.

Limitations

The use of routinely collected patient data limits the possibility of controlling for potentially confounding factors, such as variations in workload, adherence to treatment protocols, access to drugs, blood products,

disposable material, and missing data. To mitigate the issue with missing data, we also analysed the predictive abilities for RCWS, KTS and RTS using an imputed dataset, with only minor alterations in the results. Nevertheless, alternative explanations for our findings could exist. A prospective cohort study design would have allowed for better control of confounding factors. However, prospective data collection in conflict settings is often difficult due to restraints in infrastructure and frequent rotation of health care staff. Additionally, participation in a study can have negative implications for participating health care staff and patients.

Conclusion

Our findings indicate that RCWS was able to predict high surgical resource consumption better than and in-hospital mortality at least equal to KTS and RTS. In addition to facilitating systematic evaluation of penetrating injuries RCWS could consequently be a useful instrument in planning and monitoring of surgical care facilities in resource-scarce conflict settings. However, future studies are needed to determine RCWS predictive ability and interobserver variability in different conflicts and for different care providers.

Abbreviations

AIC: Akaike Information Criterion; AUC: Area under the receiver operating characteristic curve; CI: Confidence interval; ICRC: International Committee of the Red Cross; ISS: Injury Severity Score; KTS: Kampala Trauma Score; OR: Odds ratio; RCWS: Red Cross Wound Score; RTS: Revised Trauma Score; SD: Standard deviation

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12873-021-00488-2>.

Additional file 1: Table 1. Explanation on how to classify penetrating injuries according to Red Cross Wound Score (RCWS).

Additional file 2: Table 2. Relationship between surgical resource consumption, sex, age, vital signs and Red Cross Wound Score (RCWS). Table of univariate and logistic regression analysis examining the relationship between surgical resource consumption, sex, age, vital signs, RCWS grade and type for patients treated at ICRC's hospitals in Peshawar and Goma.

Additional file 3: Table 3. Relationship between in-hospital mortality, sex, age, vital signs and Red Cross Wound Score (RCWS). Table of univariate and logistic regression analysis examining the relationship between in-hospital mortality, sex, age, vital signs RCWS grade and type for patients treated at ICRC's hospitals in Peshawar and Goma.

Acknowledgements

Preliminary results were presented orally at the Swedish Surgical Society annual meeting, August 22–26, 2016, Malmö, Sweden, and at the 6th World Society of Emergency Surgery annual meeting, June 26–28, 2019, Nijmegen, the Netherlands, and an abstract was orally presented at the 20th WADEM Congress on Disaster and Emergency Medicine, April 25–28, 2017, Toronto, Canada. Open access funding was provided by Linköping University. We thank former ICRC chief surgeons Robin M. Coupland and Harald Veen for providing indispensable input on how to define surgical resource consumption and Lars Valter, Forum Östergötland, Linköping University, Sweden, for statistical advice.

Authors' contributions

This study was conceived and designed by MM, JvS and PA. Data were collected by ZO in Peshawar and ICRC-staff in Goma and analysed by MM and PA. Interpretation of data was done by MM, ZO, JvS, AW and PA (all authors). First draft of the manuscript was made by MM and was critically revised by all authors. Final version of the manuscript was read and approved by all authors.

Funding

This study was supported by grants from Region Östergötland (ALF LIO-79962 and RALF 06000908), Sweden. Region Östergötland did not have any influence in the design of the study or in the collection, analysis, and interpretation of data or in writing of the manuscript. Open Access funding provided by Linköping University.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The data utilized in the present study emanates from areas with ongoing armed conflict where academic institutions are non- or malfunctioning, making local ethics approvals not possible to obtain. Furthermore, as this study is a secondary analysis of previously collected non-identifiable quality control data from ICRC-operated hospitals local ethics approvals and consents were not deemed necessary. However, to ensure that ethical standards were met, ethical approval for exploration and compilation of data for scientific presentation was obtained from the Regional Ethical Review Board in Linköping, Swedish Ethical Review Authority, approval numbers 2014/417–31, 2015/66–33 and 2016/298–32. The ICRC approved the study and gave all necessary administrative permissions. The study was performed in accordance with the ethical standards stipulated by Linköping University and the Declaration of Helsinki.

Consent for publication

Publication of this study was approved by the ICRC.

Competing interests

The authors have no conflicts of interest to declare. ZO has a long-term contract at the ICRC, AW was Chief Surgeon at the ICRC 2017–2019 and PA has had several intermittent contracts with the ICRC.

Author details

¹Department of Surgery in Norrköping, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden. ² Center for Disaster Medicine and Traumatology, University Hospital, Linköping, Sweden. ³International Committee of the Red Cross, Geneva, Switzerland. ⁴Department of Global Public Health, Karolinska Institutet, Stockholm, Sweden. ⁵International Medical Programme, Center for Disaster Medicine and Traumatology, University Hospital, Linköping, Sweden.

Received: 13 December 2020 Accepted: 30 July 2021

Published online: 11 August 2021

References

1. Leaning J, Guha-Sapir D. Natural disasters, armed conflict, and public health. *N Engl J Med*. 2013;369(19):1836–42. <https://doi.org/10.1056/NEJMr1109877>.
2. Andersson P, Muhrbeck M, Veen H, Osman Z, von Schreeb J. Hospital workload for weapon-wounded females treated by the International Committee of the red Cross: more work needed than for males. *World J Surg*. 2018;42(1):93–8. <https://doi.org/10.1007/s00268-017-4160-y>.
3. Gertsch P. Assessment of hospital workload in war surgery. *Br J Surg*. 1987; 74(9):831–3. <https://doi.org/10.1002/bjs.1800740928>.
4. Giannou C, Baldan M. War surgery: working with limited resources in armed conflict and other situations of violence, vol. 1. Geneva: International Committee of the Red Cross; 2019.
5. Coupland RM, Korver A. Injuries from antipersonnel mines. The experience of the International Committee of the red Cross. *BMJ*. 1991;303(6816):1509–12. <https://doi.org/10.1136/bmj.303.6816.1509>.

6. Ramalingam T. Extremity injuries remain a high surgical workload in a conflict zone: experiences of a British field hospital in Iraq. 2003. *JRAMC*. 2004;150(3):187–90 <https://doi.org/10.1136/jramc-150-03-06>.
7. Korver AJ. Injuries of the lower limbs caused by antipersonnel mines: the experience of the International Committee of the red Cross. *Injury*. 1996; 27(7):477–9 [https://doi.org/10.1016/0020-1383\(96\)00066-6](https://doi.org/10.1016/0020-1383(96)00066-6).
8. Champion HR, Sacco WJ, Copes WS, Gann DS, Gennarelli TA, Flanagan ME. A revision of the trauma score. *J Trauma*. 1989;29(5):623–9 <https://doi.org/10.1097/00005373-198905000-00017>.
9. Roy N, Gerdin M, Schneider E, Kizhakke Veetil DK, Khajanchi M, Kumar V, et al. Validation of international trauma scoring systems in urban trauma centres in India. *Injury*. 2016;47(11):2459–64 <https://doi.org/10.1016/j.injury.2016.09.027>.
10. Emerman CL, Shade B, Kubincanek J. A comparison of EMT judgment and prehospital trauma triage instruments. *J Trauma*. 1991;31(10):1369–75 <https://doi.org/10.1097/00005373-199110000-00009>.
11. Raux M, Sartorius D, Le Manach Y, David JS, Riou B, Vivien B. What do prehospital trauma scores predict besides mortality? *J Trauma*. 2011;71(3): 754–9 <https://doi.org/10.1097/ta.0b013e3181fd0dae>.
12. Kobusingye OC, Lett RR. Hospital-based trauma registries in Uganda. *J Trauma*. 2000;48(3):498–502 <https://doi.org/10.1097/00005373-200003000-00022>.
13. Wild H, Stewart BT, LeBoa C, Stave CD, Wren SM. Epidemiology of injuries sustained by civilians and local combatants in contemporary armed conflict: an appeal for a shared trauma registry among humanitarian actors. *World J Surg*. 2020;44(6):1863–73 <https://doi.org/10.1007/s00268-020-05428-y>.
14. Søreide K. Epidemiology of major trauma. *Br J Surg*. 2009;96(7):697–8 <https://doi.org/10.1002/bjs.6643>.
15. Coupland RM. The red cross classification of war wounds: the E.X.C.F.V. M. scoring system. *World J Surg*. 1992;16(5):910–7 <https://doi.org/10.1007/bf02066991>.
16. Coupland RM. Hand grenade injuries among civilians. *JAMA*. 1993;270(5): 624–6 <https://doi.org/10.1001/jama.1993.03510050090034>.
17. Muhrbeck M, Holmgren K, Osman Z, von Schreeb J, Wladis A, Andersson P. Trends in demographics and surgical treatment of weapon-related limb injuries over two decades in a resource-scarce setting. *World J Surg*. 2019; 43(11):2681–8 <https://doi.org/10.1007/s00268-019-05110-y>.
18. Hinsley DE, Rosell PA, Rowlands TK, Clasper JC. Penetrating missile injuries during asymmetric warfare in the 2003 gulf conflict. *Br J Surg*. 2005;92(5): 637–42 <https://doi.org/10.1002/bjs.4911>.
19. Coupland RM. Abdominal wounds in war. *Br J Surg*. 1996;83(11):1505–11 <https://doi.org/10.1002/bjs.1800831106>.
20. Haverkamp FJC, van Gennip L, Muhrbeck M, Veen H, Wladis A, Tan E. Global surgery for paediatric casualties in armed conflict. *World J Emerg Surg*. 2019;14(1):55. <https://doi.org/10.1186/s13017-019-0275-9>.
21. Chu K, Havet P, Ford N, Trelles M. Surgical care for the direct and indirect victims of violence in the eastern Democratic Republic of Congo. *Confl Health*. 2010;4(1):6. <https://doi.org/10.1186/2f1572-1505-4-6>.
22. Wagner Z, Heft-Neal S, Wise PH, Black RE, Burke M, Boerma T, et al. Women and children living in areas of armed conflict in Africa: a geospatial analysis of mortality and orphanhood. *Lancet Glob Health*. 2019;7(12):e1622–e31 [https://doi.org/10.1016/s2214-109x\(19\)30407-3](https://doi.org/10.1016/s2214-109x(19)30407-3).
23. Coupland RM. The red cross wound classification. Geneva: International Committee of the Red Cross; 1993.
24. MacLeod JBA, Kobusingye O, Frost C, Lett R, Kirya F, Shulman CA. Comparison of the Kampala trauma score (KTS) with the revised trauma score (RTS), injury severity score (ISS) and the TRISS method in a Ugandan trauma registry. *Eur J Trauma*. 2003;29(6):392–8 <https://doi.org/10.1007/s00068-003-1277-5>.
25. Eshaya-Chauvin B, Coupland RM. Transfusion requirements for the management of war injured: the experience of the International Committee of the red Cross. *Br J Anaesth*. 1992;68(2):221–3 <https://doi.org/10.1093/bja/68.2.221>.
26. Forrester JD, Forrester JA, Basimounie JP, Tahir MZ, Trelles M, Kushner AL, et al. Sex disparities among persons receiving operative care during armed conflicts. *Surgery*. 2017;162(2):366–76 <https://doi.org/10.1016/j.surg.2017.03.001>.
27. Azad AD, Kong VY, Clarke DL, Laing GL, Bruce JL, Chao TE. Use of vital signs in predicting surgical intervention in a south African population: a cross-sectional study. *Int J Surg*. 2020;77(9):300–4 <https://doi.org/10.1016/j.ijsu.2020.05.013>.
28. Kotwal RS, Howard JT, Orman JA, Tarpey BW, Bailey JA, Champion HR, et al. The effect of a golden hour policy on the morbidity and mortality of combat casualties. *JAMA Surg*. 2016;151(1):15–24 <https://doi.org/10.1001/jamasurg.2015.3104>.
29. Davidovic LB, Cinar A, Ille T, Kostic DM, Dragas MV, Markovic DM. Civil and war peripheral arterial trauma: review of risk factors associated with limb loss. *Vascular*. 2005;13(3):141–7 <https://doi.org/10.1258/rsmvasc.13.3.141>.
30. van Gennip L, Haverkamp FJC, Muhrbeck M, Wladis A, Tan E. Using the red cross wound classification to predict treatment needs in children with conflict-related limb injuries: a retrospective database study. *World J Emerg Surg*. 2020;15(1):52 <https://doi.org/10.1186/s13017-020-00333-0>.
31. Weeks SR, Juillard CJ, Monono ME, Etoundi GA, Ngamby MK, Hyder AA, et al. Is the Kampala trauma score an effective predictor of mortality in low-resource settings? A comparison of multiple trauma severity scores. *World J Surg*. 2014;38(8):1905–11 <https://doi.org/10.1007/s00268-014-2496-0>.
32. Haac B, Varela C, Geyer A, Cairns B, Charles A. The utility of the Kampala trauma score as a triage tool in a sub-Saharan African trauma cohort. *World J Surg*. 2015;39(2):356–62 <https://doi.org/10.1007/s00268-014-2830-6>.
33. Gardner A, Forson PK, Odoro G, Stewart B, Dike N, Glover P, et al. Diagnostic accuracy of the Kampala trauma score using estimated abbreviated injury scale scores and physician opinion. *Injury*. 2017;48(1):177–83 <https://doi.org/10.1016/j.injury.2016.11.022>.
34. Institute for Health Metrics and Evaluation (IHME). GBD Compare Data Visualization. Seattle, WA: IHME, University of Washington. Available from <http://vizhub.healthdata.org/gbd-compare> (Accessed 15 Apr 2020).
35. Trelles M, Stewart BT, Hemat H, Naseem M, Zaher S, Zakir M, et al. Averted health burden over 4 years at Medecins sans Frontieres (MSF) trauma Centre in Kunduz, Afghanistan, prior to its closure in 2015. *Surgery*. 2016; 160(5):1414–21 <https://doi.org/10.1016/j.surg.2016.05.024>.
36. Aspelund AL, Patel MQ, Kurland L, McCaul M, van Hoving DJ. Evaluating trauma scoring systems for patients presenting with gunshot injuries to a district-level urban public hospital in Cape Town, South Africa. *Afr J Emerg Med*. 2019;9(4):193–6 <https://doi.org/10.1016/j.afjem.2019.07.004>.
37. Alikan AA, Selcuk E, Bayraktar FA. Predicting outcomes of penetrating cardiovascular injuries at a rural center by different scoring systems. *Braz J Cardiovasc Surg*. 2020;35(2):198–205 <https://doi.org/10.21470/1678-9741-2019-0403>.
38. Debarre A. Hard to reach: providing healthcare in armed conflict. New York: International Peace Institute; 2018. Available from https://www.ipinst.org/wp-content/uploads/2018/12/1812_Hard-to-Reach.pdf (Accessed 10 Dec 2020).
39. Wren SM, Wild HB, Gurney J, Amirtharajah M, Brown ZW, Bulger EM, et al. A consensus framework for the humanitarian surgical response to armed conflict in 21st century warfare. *JAMA Surg*. 2019;155(2):114–21 <https://doi.org/10.1001/jamasurg.2019.4547>.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions

Additional table 1 Explanation of Red Cross Wound Score (RCWS) [4]

RCWS is an anatomical scoring system that was designed by the International Committee of the Red Cross (ICRC) to quickly and relatively easily give guidance on a penetrating wound's significance. RCWS is based on size, tissue involved and location. Thus, RCWS is not dependent on the type of weapons or projectile that caused the wound. The following variables are included in the assessment of a wound according to RCWS:

- E Entry wound in centimetres
- X Exit wound in centimetres
- C Presence of cavity >2 fingers wide (0=No and 1=Yes)
- F Fracture (0=No fracture, 1=Simple fracture, hole or insignificant comminution and 2=Clinically significant comminution)
- V Vital structure (0=No penetration of vital structure, N=Penetration of the dura of the brain or spinal cord, A=Peritoneum, T=Pleura and H=Major peripheral vessels extending to the brachial or popliteal or carotid arteries)

Type	Grade 1	Grade 2	Grade 3
ST Soft tissue wounds	Small simple wound E+X<10cm C0 F0 V0 1ST	Medium sized soft tissue wound E+X<10cm C1 F0 V0 2ST	Large soft tissue wound E+X≥10cm C1 F0 V0 3ST
F Wounds with fractures	Small wound with simple fracture E+X<10cm C0 F1 V0 1F	Medium sized wound with important fracture E+X<10cm C0 F2 V0 2F	Large wound with significant fracture E+X≥10cm C1 F2 V0 3F
V Vital wounds	Small wound threatening life E+X<10cm C0 F0 V=N OR T OR A OR H 1V	Medium sized wound threatening life E+X<10cm C1 F0 V= N OR T OR A OR H 2V	Large wound threatening life E+X≥10cm C1 F0 V= N OR T OR A OR H 3V
VF Vital wounds with fractures	Small wound threatening limb or life E+X<10cm C0 F1 V= N OR T OR A OR H 1VF	Medium wound threatening limb or life E+X<10cm C1 F2 V= N OR T OR A OR H 2VF	Large wound threatening limb or life E+X≥10cm C1 F2 V= N OR T OR A OR H 3VF

4.Giannou C, Baldan M. War surgery: working with limited resources in armed conflict and other situations of violence. Vol 1. Geneva: International Committee of the Red Cross; 2019.

Additional table 2 Relationship between surgical resource consumption, sex, age, vital signs and Red Cross Wound Score (RCWS)

Surgical resource consumption	Univariate analysis			Logistic regression analysis			
	Complete cases Total=834		P value	Complete cases Total=834		All cases ^a Total=1555 ^b	
	Low Total=472 n (%)	High ^c Total=362 n (%)		All confounders 1=High ^c Odds ratio (95% CI)	P value	All confounders 1=High ^c Odds ratio (95% CI)	P value
Sex			1.000				
Male	418	320 (88.4)		1		1	
Female	54 (11.4)	42 (11.6)		1.14 (0.68-1.90)	0.626	1.14 (0.79-1.63)	0.491
Age			0.710				
15-49 years	433	329 (90.9)		1		1	
>49	39 (8.3)	33 (9.1)		1.23 (0.70-2.17)	0.466	1.84 (1.26-2.70)	0.002
Time since injury			0.152				
0-6 hours	15 (3.2)	21 (5.8)		1		1	
7-24 hours	203	159 (43.9)		0.68 (0.30-1.54)	0.352	1.03 (0.52-2.06)	0.933
>24 hours	254	182 (50.3)		0.66 (0.29-1.52)	0.327	0.97 (0.49-1.95)	0.938
Systolic blood pressure			0.004				
>89 mmHg	464	341 (94.2)		1		1	
76-89	5 (1.1)	10 (2.8)		1.16 (0.33-4.11)	0.931	1.52 (0.61-3.75)	0.362
0-75	3 (0.6)	11 (3.0)		1.65 (0.44-6.20)	0.821	1.23 (0.51-2.97)	0.640
Respiratory rate			0.446				
10-29/min	458	347 (95.9)		1		1	
≤9	-	-		-	-	-	-
≥30	14 (3.0)	15 (4.1)		0.88 (0.37-2.09)	0.772	0.72 (0.42-1.26)	0.222
Glasgow Coma Scale			0.077				
13-15	465	347 (95.8)		1		1	
9-12	4 (0.9)	10 (2.8)		1.09 (0.31-3.87)	0.896	1.01 (0.19-5.37)	0.811
6-8	2 (0.4)	4 (1.1)		1.60 (0.22-11.65)	0.644	0.85 (0.20-3.59)	0.825
≤5	1 (0.2)	1 (0.3)		0.84 (0.05-15.76)	0.906	0.90 (0.37-2.18)	0.985
RCWS grade			<0.001				
1 (simple)	266	83 (22.9)		1		1	
2 (medium)	185	178 (49.2)		2.60 (1.81-3.74)	<0.001	2.51 (1.93-3.26)	<0.001
3 (large)	21 (4.4)	101 (27.9)		11.60 (6.61-20.36)	<0.001	10.76 (7.41-15.63)	<0.001
RCWS type			<0.001				
Soft tissue	220	67 (18.5)		1		1	
Fracture	164	180 (49.7)		2.39 (1.62-3.54)	<0.001	2.12 (1.60-2.82)	<0.001
Threatening life	59 (12.5)	45 (12.4)		2.16 (1.27-3.67)	0.005	1.92 (1.31-2.83)	0.001
Threatening life/limb	29 (6.1)	70 (19.4)		4.67 (2.61-8.34)	<0.001	3.05 (2.04-4.54)	<0.001
Additional severe injuries ^d			<0.001				
No	439	288 (79.6)		1		1	
Yes	33 (7.0)	74 (20.4)		2.90 (1.83-4.89)	<0.001	2.43 (1.72-3.44)	<0.001

^aUsing imputed data for incomplete cases; ^b9 patients with unknown surgical resource consumption excluded; ^cDefined as ≥3 surgical procedures under anesthesia or ≥3 blood transfusions or limb amputation; ^dCorresponding to Abbreviated Injury Score ≥2

Additional table 3 Relationship between in-hospital mortality, sex, age, vital signs and Red Cross Wound Score (RCWS)

In-hospital mortality	Univariate analysis			Logistic regression analysis		
	Complete cases Total=834		P value	Complete cases Total=834		All cases ^a Total=1559 ^b
	No Total=813 n (%)	Yes Total=21 n (%)		All confounders 1=In-hospital mortality Odds ratio (95%CI)	P value	All confounders 1=In-hospital mortality Odds ratio (95% CI)
Sex			1.000			
Male	719 (88.4)	19 (90.0)		1		1
Female	94 (11.6)	2 (10.0)		0.82 (0.17-4.04)	0.815	1.38 (0.65-2.92)
Age			0.701			
15-49 years	743 (91.4)	19 (90.5)		1		1
>49	70 (8.6)	2 (9.5)		1.48 (0.31-7.00)	0.620	1.31 (0.60-2.85)
Time since injury			0.010			
0-6 hours	33 (4.1)	3 (14.3)		1		1
7-24 hours	354 (43.5)	8 (38.1)		0.32 (0.50-1.99)	0.222	0.37 (0.12-1.16)
>24 hours	426 (52.4)	10 (47.6)		0.41 (0.07-2.61)	0.347	0.65 (0.21-2.00)
Systolic blood pressure			0.033			
>89 mmHg	787 (96.8)	18 (85.7)		1		1
76-89	13 (1.6)	2 (9.5)		2.46 (0.26-23.29)	0.433	4.63 (1.59-13.44)
0-75	13 (1.6)	1 (4.8)		1.04 (0.08-13.41)	0.978	2.34 (0.64-8.65)
Respiratory rate			0.163			
10-29/min	786 (96.7)	19 (90.5)		1		1
≤9	-	-		-	-	1.90 (0.95-3.80)
≥30	27 (3.3)	2 (9.5)		3.02 (0.55-16.58)	0.203	3.00 (0.82-11.06)
Glasgow Coma Scale			<0.001			
13-15	796 (97.9)	16 (76.2)		1		1
9-12	12 (1.5)	2 (9.5)		6.55 (1.00-43.13)	0.084	2.42 (0.27-21.47)
6-8	5 (0.6)	1 (4.8)		5.25 (0.44-62.71)	0.190	4.41 (0.78-24.75)
≤5	0	2 (9.5)		-	-	2.08 (0.62-6.98)
RCWS grade			0.656			
1 (simple)	338 (41.6)	11 (52.4)		1		1
2 (medium)	355 (43.7)	8 (38.1)		0.56 (0.17-1.78)	0.323	1.37 (0.73-2.58)
3 (large)	120 (14.8)	2 (9.5)		0.37 (0.07-2.03)	0.250	1.39 (0.65-2.97)
RCWS type			0.007			
Soft tissue	283 (34.8)	4 (19.0)		1		1
Fracture	339 (41.7)	5 (23.8)		1.48 (0.32-6.78)	0.612	1.12 (0.42-3.00)
Threatening life	98 (12.1)	6 (28.6)		3.88 (0.88-17.02)	0.073	10.72 (4.42-26.01)
Threatening life/limb	93 (11.4)	6 (28.6)		2.89 (0.49-17.12)	0.241	8.43 (3.36-21.18)
Additional severe injuries ^c			0.174			
No	711 (87.5)	16 (76.2)		1		1
Yes	102 (12.5)	5 (23.8)		2.09 (0.67-6.52)	0.204	1.41 (0.75-2.65)

^aUsing imputed data for incomplete cases; ^b5 patients with unknown discharge status excluded; ^cCorresponding to Abbreviated Injury Score ≥2

PAPER IV

Contents lists available at ScienceDirect

Injury

journal homepage: www.elsevier.com/locate/injury

Efficacy of topical honey compared to systemic gentamicin for treatment of infected war wounds in a porcine model: A non-inferiority experimental pilot study

Måns Muhrbeck^{a,b,1,*}, Andreas Wladis^{b,c}, Maria Lampi^b, Peter Andersson^{b,c}, Johan P.E. Junker^{b,d}

^a Department of Surgery in Norrköping, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

^b Department of Biomedical and Clinical Sciences, Center for Disaster Medicine and Traumatology, Linköping University, Linköping, Sweden

^c Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden

^d Department of Biomedical and Clinical Sciences, Laboratory of Experimental Plastic Surgery, Linköping University, Linköping, Sweden

ARTICLE INFO

Article history:

Accepted 18 October 2021

Available online xxx

Keywords:

Developing countries

Armed conflicts

Penetrating wounds

Wound infection

Honey

Antibiotics

Experimental animal model

ABSTRACT

Background: In armed conflicts, infected wounds constitute a large portion of the surgical workload. Treatment consists of debridements, change of dressings, and antibiotics. Many surgeons advocate for the use of honey as an adjunct with the rationale that honey has bactericidal and hyperosmotic properties. However, according to a Cochrane review from 2015 there is insufficient data to draw any conclusions regarding the efficacy of honey in treatment of wounds. We, therefore, decided to evaluate if honey is non-inferior to gentamicin in the treatment of infected wounds in a highly translatable porcine wound model.

Material and methods: 50 standardized wounds on two pigs were infected with *S. aureus* and separately treated with either topically applied Manuka honey or intramuscular gentamicin for eight days. Treatment efficacy was evaluated with quantitative cultures, wound area measurements, histological, immunohistochemical assays, and inflammatory response.

Results: Topically applied Manuka honey did not reduce bacterial count or wound area for the duration of treatment. Intramuscular gentamicin initially reduced bacterial count (geometric mean $5.59 \cdot 0.37 - 4.27 \cdot 0.80 \log_{10}$ (GSD) CFU/g), but this was not sustained for the duration of the treatment. However, wound area was significantly reduced with intramuscular gentamicin at the end of treatment (mean $112.8 \pm 30.0 - 67.7 \pm 13.2$ (SD) mm²). ANOVA-analysis demonstrated no variation in bacterial count for the two treatments but significant variation in wound area ($p < 0.0001$). The inflammatory response was more persistent in the pig with wounds treated with topically applied Manuka honey than in the pig treated with intramuscular gentamicin.

Conclusion: At the end of treatment *S. aureus* count was the same with topically applied Manuka honey and intramuscular gentamicin. The wound area was unchanged with topically applied Manuka honey and decreased with intramuscular gentamicin. Topically applied Manuka honey could consequently be non-inferior to intramuscular gentamicin in reducing *S. aureus* colonization on the wound's surface, but not

Abbreviations: ANOVA, analysis of variance; ATCC, American type culture collection; CFU/g, colony forming units per gram of tissue; °C, degree Celsius; CCD, charged-coupled device; CD68, protein highly expressed in macrophages and other cells in the monocyte lineage; EDTA, ethylenediaminetetraacetic acid; EMEA, European agency for evaluation of medical products; FDA, US federal drug administration; H&E, haematoxylin and eosin staining; GSD, geometric standard deviation; IM, intramuscular; LMIC, low- and middle-income countries; MDR, multidrug-resistance; MIC, minimum inhibitory concentration; MGO, methylglyoxal, MRSA, methicillin-resistant *S. aureus*; PBS, phosphate-buffered saline; RPM, revolutions per minute; SAA, serum amyloid A; SD, standard deviation; UMF, Unique Manuka factor, a measurement of methylglyoxal concentration; WHO, World Health Organization; W/v, weight by volume.

* Corresponding author at: Department of Surgery in Norrköping, and Department of Biomedical and Clinical Sciences, Linköping University, Linköping, Sweden.

¹ Present address: Department of Surgery, Vrinnevi Hospital, Norrköping 603 79, Sweden.

E-mail address: mans.muhrbeck@regionostergotland.se (M. Muhrbeck).

<https://doi.org/10.1016/j.injury.2021.10.019>

0020-1383/© 2021 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

Please cite this article as: M. Muhrbeck, A. Wladis, M. Lampi et al., Efficacy of topical honey compared to systemic gentamicin for treatment of infected war wounds in a porcine model: A non-inferiority experimental pilot study, *Injury*, <https://doi.org/10.1016/j.injury.2021.10.019>

in reducing wound size. The use of Manuka honey dressings to prevent further progression of a wound infection may therefore be of value in armed conflicts, where definite care is not immediately available.

© 2021 The Author(s). Published by Elsevier Ltd.

This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>)

Introduction

In armed conflicts infected wounds are common and contribute to a significant portion of the surgical workload [1,2]. Current treatment protocols consist of repeated debridements, change of dressings, and administration of antibiotics to mitigate infection [3,4]. Final wound closure is subsequently achieved with delayed primary closure, flaps, split-thickness skin grafts, or a combination thereof. Successful treatment of wounds in armed conflicts depends on supply chains that can provide enough dressing material and antibiotics, even in times of a surge of injured. In low- and middle-income countries (LMIC), these supply chains are fragile, and the onset of an armed conflict many times causes a complete disruption of essential supplies to health care facilities [5–7]. Health care staff then have to rely on materials and antibiotics they have at hand. Locally available antibiotics can be of questionable origin, effectiveness and could even be harmful to patients [8].

Furthermore, uncontrolled and non-systematic use of antibiotics significantly accelerates the globally increasing problem with antimicrobial resistance [9,10]. There is, therefore, much to gain in developing robust and effective treatment strategies that do not rely on antibiotics or expensive dressing materials. Many health-care professionals, particularly in resource-scarce settings, have for many years advocated the use of honey dressings in the treatment of infected wounds [11,12]. The rationale for the use of honey is that it empirically has been found to have bactericidal and hyperosmotic properties. There are some scientific reports to support this perception [13–15]. However, a Cochrane review from 2015 stated that: “it is difficult to draw overall conclusions regarding the effects of honey as a topical treatment for wounds due to the heterogeneous nature of the patient populations and comparators studied and the mostly low quality of the evidence” [16]. We, therefore, decided to evaluate if topical honey is non-inferior to intramuscular (IM) gentamicin in the treatment of infected wounds in a well-established model for human wound healing, the porcine wound model [17–19]. Response to treatments was assessed during eight days with quantitative tissue cultures, wound surface area measurements, histological and immunohistochemical assays, as well as analysis of local and systemic inflammatory responses.

Material and methods

Treatments and bacteria

Staphylococcus aureus (S. aureus, ATCC 29,213, Manassas, VA, US) was chosen as it is one of the most commonly detected bacteria in infected wounds in armed conflicts and is prone to develop antimicrobial resistance [1,20–22]. Several recent studies from armed conflicts have reported a high proportion of wounds being infected with methicillin-resistant S. aureus (MRSA) [1,23]. Furthermore, S. aureus is able to develop a biofilm that limits the efficacy of antimicrobial treatment [24].

Manuka honey UMF 15+ (Comvita, Maidenhead, UK) from New Zealand was chosen as it has in previous studies been demonstrated to disrupt biofilms and has a bactericidal effect on S. aureus [15,25,26]. Additionally, dressings with Manuka honey have been approved by the US Federal Drug Administration (FDA) for treatment of wounds [27].

Gentamicin 40 mg/ml (Sanofi, Paris, France) was chosen as it has bactericidal properties against S. aureus, is generally available in LMIC and is recommended treatment for severe cutaneous infections by organizations that provide health care in armed conflicts [3,4,28].

Animals

Two female Swedish native breed pigs (*Sus scrofa domestica*, Persbo gård, Ransta, Sweden), both weighing 52 kg, were used for the study. Animals were socially housed and acclimatized for more than 72 h prior to any procedures. The pigs were kept in boxes measuring 2 × 2.5 m² with a light/dark cycle of 12 h/12 h and an ambient temperature of 18–20 °C. The pig's wellbeing and skin temperature were monitored at least three times daily. All animal experiments were performed under ethical approval from the regional animal ethics board (ID1418) and in strict accordance with guidelines postulated by the Medical Faculty at Linköping University.

Porcine wound model

The porcine model was chosen as the pig's skin has been demonstrated to have similar cellular composition, morphology, and immunological properties as human skin [29,30]. Prior to any procedure, animals were sedated with IM administration of 3 µg/kg of zolazepam and tiletamine (Zoletil; Virbac A/S, Kolding, Denmark) and 10 µg/kg dexmedetomidine (Dexdomitor; Orion Pharma AB, Danderyd, Sweden). At the time of wound creation, inoculation, and termination (on days 0 and 10) general anaesthesia and analgesia were maintained with intravenous infusion of 3–7.5 mg/kg pentobarbital sodium (Pentobarbitalnatrium vet.; Apotek Produktion & Laboratorier AB, Kungens kurva, Sweden) in combination with 0.5–0.75 µg/kg Fentanyl (Fentanyl; Orion Pharma AB, Danderyd, Sweden) as needed. Animals were intubated with endotracheal tubes connected to automatic ventilators. Vital parameters were monitored by pulse oximetry, capnography, and oral thermometer. Signs of postoperative pain were treated with IM administration of 50–75 µg fentanyl and 40 mg meloxicam (Loxicom; N-Vet AB, Uppsala, Sweden). The dorsum of the pigs was waxed and shaved. Skin was disinfected with 10% povidone-iodine scrub (Jodopax vet.; Pharmaxim AB, Helsingborg, Sweden). The spine was marked with a pen to allow accurate placement of wounds. For the pig predetermined for IM gentamicin treatment, 20 wounds full thickness circular wounds down to the panniculus carnosus, each measuring 78.5 mm², was created using a 10 mm punch biopsy tool (Acu-Punch; Acuderm Inc, Fort Lauderdale, FL, US). For the pig predetermined for topical honey treatment, 30 wounds were created in identical fashion (Fig. 1A). The 10 additional wounds created on the pig predetermined for honey treatment was due to the anticipated exclusion of wounds due to leakage of the applied honey. For both pigs, wounds were separated with 4 cm of healthy tissue to prevent cross-contamination between adjacent wounds. All wounds were inoculated with 10⁸ colony-forming units (CFU) of S. aureus (ATCC 29,213, Manassas, VA, US) diluted in 1 ml saline by tunnelling through approx. 1 cm of adjacent healthy tissue with an 18-gauge needle (Fig. 1B). This method of inoculation was chosen to avoid leakage and cross-contamination.

Fig. 1. Illustrations of animal procedures (A) Overview of wounds before inoculation (day 0). (B) Inoculation procedure. (C) Application of honey. (D) Wounds covered with occlusive and elastic dressing prior to terminating anaesthesia.

Wounds were covered with transparent and occlusive film (Tegaderm; Mölnlycke, Sweden) and an elastic dressing (Elastic bandage, Hansbo sport, Västra Frölunda, Sweden) (Fig. 1A, D). On days 0, 2, 4, 7, and 10 after inoculation, venous blood was drawn from ear or groin veins and collected in 2 ml EDTA tubes, and 3 ml polymer gel and lithium heparin tubes (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ, US) and oral temperature was measured. All wounds were macroscopically inspected and photographed with a ruler for later area calculations. On days 2, 4, 7, and 10, biopsies were taken with a 4 mm punch biopsy tool (Biopsy punch; Paramount Surgimed Ltd, New Delhi, India) and scalpel with a margin of approx. 3 mm and involving 50% of the wound circumference. Both biopsies included healthy underlying tissue. Punch biopsies were placed in dry, sterile cryogenic vials and frozen. Scalpel biopsies were immediately fixated in 4% neutral-buffered paraformaldehyde (HistoLab, Askim, Sweden). Biopsied wounds were subsequently excluded. 6–8 wounds were biopsied at each time point from the pig receiving topical honey treatment, and 5 wounds were biopsied at each time point from the pig receiving IM gentamicin. On day 10, the pigs were euthanized by intravenous injection of 400 mg/kg pentobarbital sodium (Pentobarbitalnatrium vet.; Apotek Produktion & Laboratorier AB, Kungens kurva, Sweden).

Treatments

For the pig with wounds predetermined for honey, treatment was initiated on day 2 after inoculation by completely filling wound cavities with approx. 2 ml undiluted honey per wound (Fig. 1C). On days 4 and 7 after inoculation, all wound cavities were superficially cleaned with cotton gauze to remove the honey and any wound secretion. Wound cavities were then immediately refilled with undiluted honey (Fig. 1C). This interval and method were chosen to mimic dressing change procedures in clinical settings. For the pig with wounds predetermined for IM gentamicin, treatment was also initiated on day 2 after inoculation with 5 mg/kg at 12 h intervals for the first day and subsequently once daily until day 10 when the experiment was terminated. Wound cavities were cleaned at the same time points and in an identical fashion as the honey-treated wounds. The administration route and length of gentamicin treatment were set to resemble treatment

protocols used in LMIC and armed conflicts [3,4]. Since no universally accepted guidelines for gentamicin treatment in pigs exists, dosage and interval were set after the recommendation from a gentamicin manufacturer (Biovet JSC, Peshtera, Bulgaria), European Agency for Evaluation of Medical Products (EMA), and veterinarian's advice [31]. Gentamicin was administered to the right lateral side of neck or to the hind to avoid interaction with other IM medications, such as the anaesthetic, which was administered to the left lateral side of neck.

Quantitative *S. aureus* cultures in wound tissue

The frozen 4 mm punch biopsies were thawed, weighed, homogenized, serially diluted, and plated overnight on Mannitol salt agar plates and counted for *S. aureus* colonies yielding log₁₀ colony-forming units per gram of tissue (CFU/g).

Control group for quantitative *S. aureus* cultures in wound tissue

As a control, we used data from saline-treated wounds obtained from a previous study done by our group (unpublished data). The method and materials used in this study were identical to the present study, except that the wound cavities were filled with sterile saline 0.9% solution. No other treatment was given. 8–10 wounds on days 2, 4, 7, and 10 after inoculation were biopsied and cultured in the same manner as in the present study.

Measurement of wound surface area

Wound diameter was measured using ImageJ software 1.53f (National Institutes of Health, Bethesda, Maryland, US). The diameter was defined as border to border of macroscopically healthy skin. The largest diameter for all wounds was used for the calculation of area.

Tissue preparation

The fixed scalpel biopsies were dehydrated by immersion in a series of ethanol-xylene, embedded in paraffin, cut into 8–10 µm thick sections using a microtome (RM2255; Leica Biosystems, Wetzlar, Germany) and mounted on microscopy slides for subsequent staining.

Histological examination and reepithelialization

Slides from all wounds and time points were deparaffinized in a xylene-ethanol series, rehydrated and stained with Haematoxylin and eosin (H&E) (Sigma-Aldrich Sweden AB, Stockholm, Sweden), and mounted. Two evaluators independently assessed all slides for the appearance of neopeidermis in the central part of the wound. Representative images were chosen.

Immunohistochemical analysis

Staining for *S. aureus* was done with the Vectastin Elite ABC-HRP Kit (Vector Laboratories Inc, Burlingame, CA, US). Following deparaffinization and rehydration, slides were incubated with normal goat serum (15 µl/ml; Vector Laboratories Inc, Burlingame, CA, US) in PBS, and thereafter with rabbit polyclonal antibodies for enterotoxins from *S. aureus* as validated immunogen (dilution 1:800, 4–5 mg/ml; Thermo Fisher Scientific Inc., Waltham, MA, US) for 30 min at room temperature [32]. Following several rinses in PBS, slides were incubated with biotinylated goat anti-rabbit IgG antibodies (15 µl/ml; Vector Laboratories Inc, Burlingame, CA, US) for 30 min at room temperature. After subsequently washing in PBS, slides were incubated with an avidine-horseradish peroxidase complex and developed using Vector VIP (Vector Laboratories Inc, Burlingame, CA, US) as peroxidase substrate. The stained slides were examined using a light microscope (BX41; Olympus Corp., Tokyo, Japan) and images captured with a CCD camera (MTR3CDD, TouoTek Photonics Corp., Zhejiang, China).

Staining for macrophages was done in a similar fashion with normal horse serum (15 µl/ml; Vector Laboratories Inc, Burlingame, CA, US), mouse CD68 antibodies with a subcellular fraction of human macrophages as validated immunogen (dilution 1:200, 0.2 mg/ml; Thermo Fisher Scientific Inc., Waltham, MA, US), biotinylated horse anti-mouse IgG antibodies (15 µl/ml, Vector Laboratories Inc., Burlingame, CA, US), and an avidine-horseradish peroxidase complex, using Vector VIP (Vector Laboratories Inc, Burlingame, CA, US) as peroxidase substrate [33].

Analysis of macrophages in wound tissue

The CD68 stained slides from all wounds were examined using a light microscope (BX41; Olympus Corp.), and images were captured with a CCD camera (MTR3CDD, TouoTek Photonics Corp.). Two representative images from each treatment arm and time point were chosen by the authors. Chosen images were blinded, and the relative concentration of macrophages, 1–3 (low–high), was visually assessed by five independent experienced evaluators through a web-based questionnaire.

Analysis of systemic parameters

The EDTA tubes were immediately sent to Evidensia Valla Djursjukhus (Linköping, Sweden) for analysis of haemoglobin, total count of white blood cells, and neutrophils. The IDEEX ProCyt DX (IDEEX Laboratories Inc., Westbrook, Maine, US) was used for the analysis. The polymer gel and lithium heparin tubes were centrifuged for 10 min at 2000 rpm. The serum was then extracted and frozen at -20°C for later analysis of serum amyloid A (SAA). SAA is an acute-phase protein, similar to C reactive protein, and will rapidly increase in the event of trauma, infection, or other systemic stressors in pigs [34]. SAA concentration was assessed using the PHASE RANGE Multispecies SAA ELISA Kit (Tridelata Development Ltd., Maynooth, Ireland). The manufacturer's protocol was followed. From each time point and treatment arm, 4 samples at a dilution of 1:500 were analysed. The absorbance of each sample and standards were read at 450 nm and also at 630 nm to

adjust for background using a microplate spectrometer (Epoch2; BioTek, Winooski, Vermont, US). A constant was calculated by plotting the absorbance of the adjusted standards at 450 nm against their known concentrations on a standard graph. Concentrations of SAA for the samples were then calculated by multiplying the adjusted absorbance value with the constant. 1 out of 80 samples was excluded due to absorbance considerably deviating from other samples from the same time point (day 0) and treatment arm (topical honey). The ELISA was repeated with two samples with a dilution of 1:500 and one sample with a dilution of 1:1000 from each time point and treatment arm with confirming results.

Statistical analysis

Power calculation was done with G*Power 3.1 (Heinrich Heine Universität, Düsseldorf, Germany) [35]. The primary endpoint of reduction of bacteria in tissue after eight days of treatment was used for the power calculation. Reduction to $\leq 3 \log_{10}$ CFU/g of tissue after eight days of treatment was considered successful. The rationale being that the potential of wound healing is significantly impaired, i.e., infected, with bacterial bioburden of $> 5 \log_{10}$ CFU/g, and the bacterial bioburden of non-infected skin is considered to be $2\text{--}3 \log_{10}$ CFU/g [36,37]. Solitary treatment with antibiotics and topical honey have separately been demonstrated to reduce the bacterial bioburden in wounds from $> 5 \log_{10}$ CFU/g to $\leq 3 \log_{10}$ CFU/g within eight days [38–40]. We expected that a reduction to $\leq 3 \log_{10}$ CFU/g of tissue would be seen in 95% of the wounds for both the honey and antibiotic treatment arms after eight days of treatment. With a non-inferiority margin of 0.21, to allow for error in the application and leakage of honey, we calculated that 19 samples were needed in each of the two treatment arms to demonstrate a non-inferiority at 90% power. Therefore, five samples were needed at each of the four time points to achieve a total sample size of 20 for each treatment arm and to allow for some margin of error. In total 20 wounds were created on the pig predetermined for IM gentamicin treatment and 30 wounds on pig predetermined for topical honey treatment. As previously mentioned, the difference in number of the wounds for each treatment arm was due to the expected leakage of the applied honey. Data was collected from all wounds.

Statistical analysis was performed using GraphPad Prism 8.4 (GraphPad Software, San Diego, CA, US). Values are given as mean $\pm$ standard deviation (SD), except for the results on the quantitative cultures of *S. aureus* in wound tissue over time (Fig. 2) where instead geometrical mean $\pm$ geometric standard deviation (GSD) are given. This was done to include all observations and to diminish misleading visual effects caused by the log10 scale. Statistical comparisons between treatment groups were performed using a mixed-model ANOVA with Greenhouse-Geiser correction. P-values (two-tailed) less than 0.05 were considered significant.

Results

Quantitative cultures of *S. aureus* in wound tissue

Two days after inoculation, prior to initiation of treatment (day 2), all wounds exhibited *S. aureus* counts consistent with infection ($> 5 \log_{10}$ CFU/g tissue), with geometric means 5.85 ± 0.55 (GSD) $\log_{10}$ CFU/g ($n = 6$) and 5.59 ± 0.37 (GSD) $\log_{10}$ CFU/g ($n = 3$) for wounds subsequently treated with topical honey or systemic gentamicin, respectively (Figs. 2 and 3). Treatment with topical honey did not reduce *S. aureus* counts ($n = 6\text{--}8$ /time point) at any of the time points during the experiment. IM gentamicin administration resulted in a significant decline in *S. aureus* count after two days of treatment (day 4), geometric mean 5.59 ± 0.37 (GSD) to $4.27 \pm$

Fig. 2. Quantitative *S. aureus* cultures from wound biopsies *S. aureus* infected wounds treated with either topical honey or intramuscular (IM) gentamicin were analysed for amount of bacteria, in colony-forming units/gram of tissue (CFU/g). IM gentamicin significantly reduced bacterial count after two days of treatment (day 4), but not in the following time points. Topical honey did not reduce bacterial count at any time point. Cultures from wounds treated with saline, from a previous study, were included as a reference (unpublished data). Clinical infection was defined as bacterial count $>10^5$ per gram of tissue [34,35]. Symbols and error bars represent geometrical mean and geometric standard deviation (GSD).

Fig. 3. Staining for *S. aureus* in wound tissue. Immunohistochemistry with polyclonal antibodies for enterotoxins from *S. aureus* on biopsies from *S. aureus* infected wounds treated with either topical honey or intramuscular gentamicin. Infiltration of *S. aureus* in wound tissue was observed in wounds from both treatment groups by day 4, indicated by arrows in the figure. Scale bars represent 500 μ m.

Fig. 4. Surface area of wounds over time. *S. aureus* infected wounds were treated with either topical honey or intramuscular (IM) gentamicin. Wounds treated with IM gentamicin exhibit a significant decrease of wound area at terminal endpoint (day 10), compared to wounds treated with topical honey ($p < 0.0001$). The largest diameter was used for calculation of area. The diameter was defined as border to border of macroscopically healthy skin. Symbols and error bars represent mean and standard deviation (SD).

0.80 log₁₀ CFU/g ($n = 5$), but thereafter counts increased and remained above the level of infection until terminal endpoint. There was no difference in the bacterial count at the terminal endpoint (day 10) when comparing topical honey treatment, IM Gentamicin treatment with saline treatment. In the mixed-model ANOVA no significant difference in variation of *S. aureus* counts between treatment with topical honey and IM gentamicin was observed ($p = 0.56$).

Reduction of wound surface area

Two days after inoculation and prior to initiation of treatment (day 2) an increase of mean wound area was seen in all wounds, with means 78.5 mm^2 to $118.2 \text{ mm}^2 \pm 39.2$ (SD) ($n = 30$) and 78.5 mm^2 to $112.8 \text{ mm}^2 \pm 30.0$ (SD) ($n = 20$) for wounds later treated with topical honey or IM gentamicin, respectively (Figs. 4 and 5). Treatment with topical honey did not cause any changes in the size of the mean wound area, as it remained largely unchanged until the terminal endpoint (day 10), mean $112.2 \text{ mm}^2 \pm 10.4$ (SD) ($n = 10$). However, treatment with IM gentamicin resulted in a significant decrease in mean wound area observed at terminal endpoint (day 10), mean $112.8 \text{ mm}^2 \pm 30.0$ (SD) ($n = 20$) to $67.7 \text{ mm}^2 \pm 13.2$ ($n = 5$), compared to wounds treated with topical honey ($p < 0.0001$).

Reepithelialization

IM gentamicin, but not topical honey, treated wounds displayed ongoing reepithelialization at the terminal endpoint (day 10) (Fig. 6).

Local inflammatory response

A tendency towards higher concentrations of macrophages in wound tissue was seen in wounds treated with topical honey from day 4 until the terminal endpoint (day 10) (Fig. 7, Table 1). For

wounds treated with IM gentamicin, no substantial changes in the concentration of macrophages were observed until the terminal endpoint (day 10).

Systemic inflammatory response

From two days after inoculation and until the terminal endpoint (day 10) both pigs exhibited behaviour consistent with illness: loss of appetite, lethargy, and refraining from nesting. Consistent with an acute systemic inflammatory response, an increase in oral temperature, concentration of SAA, concentrations of total leukocytes, neutrophils, and a decrease in haemoglobin concentration was observed in both pigs two days after inoculation and prior to initiation of treatment (day 2) (Fig. 8A-E) [41]. SAA increased from mean $23.5 \text{ } \mu\text{g/ml} \pm 78.1$ (SD) to $947.1 \text{ } \mu\text{g/ml} \pm 58.2$ and $94.7 \text{ } \mu\text{g/ml} \pm 159.4$ (SD) to $967.4 \text{ } \mu\text{g/ml} \pm 131.6$ for pigs receiving topical honey or IM gentamicin, respectively (Fig. 8B). Following treatment with topical honey, SAA remained elevated after two days of treatment (day 4), mean $969.7 \text{ } \mu\text{g/ml} \pm 58.2$ (SD), and a slower decline of SAA was seen compared to treatment with IM gentamicin. For the pig that received IM gentamicin treatment, SAA concentration decreased after 2 days of treatment (day 4), $967.4 \text{ } \mu\text{g/ml} \pm 131.6$ (SD) to $647.9 \text{ } \mu\text{g/ml} \pm 194.4$ and returned to within normal range after five days of treatment (day 7), $342.0 \text{ } \mu\text{g/ml} \pm 194.3$ (SD). These differences in changes of SAA concentrations were confirmed in the mixed model ANOVA ($p = 0.04$).

Concentrations of total leukocytes and neutrophils normalized for both pigs after two days of treatment (day 4) (Fig. 8C, D). For the pig with wounds treated with topical honey, these concentrations subsequently increased until the terminal endpoint (day 10). For the pig with IM gentamicin treated wounds total leukocytes and neutrophils remained normal after 5 days of treatment (day 7), and then increased at the terminal endpoint (day 10). Concentrations of haemoglobin remained depressed for both pigs until the terminal endpoint (day 10) (Fig. 8E).

Fig. 5. Appearance of wounds over time. *S. aureus* infected wounds were treated with either topical honey or intramuscular gentamicin. Scale bars represent 5 mm.

Table 1

Visual assessment of macrophages in wound tissue. *S. aureus* infected wounds were treated with either topical honey or intramuscular (IM) gentamicin. Slides from wound biopsies were stained with CD68 monoclonal antibodies for a subcellular fraction of macrophages. Two slides per treatment group and time point were visually assessed by five independent experienced evaluators through a web-based questionnaire. Wounds treated with topical honey demonstrated a tendency towards higher concentration of macrophages in wound tissue from day 4 until terminal endpoint (day 10), compared to wounds treated with IM gentamicin.

Time points (days)	Concentration of macrophages Estimated, 1 (low) – 3 (high)			
	Treatment initiated			Terminal endpoint
	2 n = 10 mean (SD)	4 n = 10 mean (SD)	7 n = 10 mean (SD)	10 n = 10 mean (SD)
Treatment				
Topical honey	1.3 (0.5)	2.2 (0.6)	2.0 (0.9)	2.9 (0.3)
IM gentamicin	1.9 (0.9)	2.1 (0.6)	1.7 (0.7)	2.1 (1.0)

Discussion

Despite honey's established antimicrobial properties *in vitro* models, similar data from *in vivo* models and humans are scarce [16,42]. In a study by Medeiros et al., a significant reduction of the bacterial count could be demonstrated with *Melipona scutellaris* honey treatment compared to saline treatment of methicillin-resistant *S. aureus* (MRSA) infected wounds in rats [40]. In this study, inoculation was done through application of a gauze with 5×10^7 CFU of MRSA (ATCC 43300) in the wounds, which were then closed for 24 h. Wounds were then opened, cavities drained, and treatment was initiated. The bacterial count was assessed only by quantitative cultures. No immunohistochemical analyses were done to assess bacterial infiltration in the tissue. Given the method of inoculation and the short period before initiation of treatment in Medeiros study, there is reason to suspect that the infection of MRSA was superficial and therefore accessible to topical treatment.

In the present study, we choose to inoculate with a *S. aureus* strain without any acquired drug resistance (ATCC 29,213). The reason for not inoculating with a multidrug-resistant (MDR) bac-

terium, such as MRSA, was to avoid introducing a confounder that could have had a significant impact when establishing honey's efficacy in a complex wound model where previous data is lacking. The porcine model was chosen as it is a pragmatic approach to simulate the pathophysiology of infected traumatic wounds seen in humans without the interference of confounding factors such as co-morbidity, nutritional state, time since injury and mechanism of injury that otherwise could have influenced the results [17,18,43]. Furthermore, to better resemble infected wounds in armed conflict we choose a different inoculation method than the one used in Medeiros study. Inoculation was done by tunnelling through adjacent healthy tissue, wounds were not closed and more importantly treatment was initiated after 48 h. In our model, we did not observe any reduction in bacterial count in wounds treated with topical honey for the duration of the study (Fig. 2). This is most likely due to honey's inability to reach bacteria that had infiltrated in the tissue. Tissue infiltration of *S. aureus* was confirmed in the immunohistochemical analysis as we could identify growth of bacteria in the wound tissue and not only on the wound's surface (Fig. 3). For the wounds treated with IM gentamicin, a reduction

Wounds treated with topical honey

Wounds treated with intramuscular gentamicin

Fig. 6. Haematoxylin and eosin-staining of wounds *S. aureus* infected wounds were treated with either topical honey or intramuscular (IM) gentamicin. IM gentamicin, but not topical honey, treated wounds displayed ongoing reepithelialization at the terminal endpoint (day 10). Indicated by an arrow in the figure. Scale bars represent 500 µm.

in the bacterial count was observed after two days of treatment (day 4), which included the first day of treatment when gentamicin was administered twice compared to once daily for the remaining treatment. This reduction in the bacterial count was not sustained for the duration of the study, conceivably attributed to gentamicin's inability to reach bacteria in devitalized tissue, the dosage of antibiotics was too low or a combination thereof. Serum concentrations of gentamicin could have been measured to confirm that a therapeutic dosage was given. However, according to a report by EMEA the pharmacokinetics of gentamicin in pigs is poorly studied, and to our knowledge no established therapeutic concentra-

tions exists [31]. To mitigate this issue, we choose to give gentamicin in a dosage in the higher end of the recommendations from EMEA [31].

Consistent with the lack of reduction in bacterial count, we did not observe a reduction in wound surface area with topical honey treatment. Conversely, we did not observe an increase in wound surface area, which would be expected if honey did not have any effect on the bacterial activity. Honey could therefore have exerted an antimicrobial effect on the bacteria colonizing the surface of the wound, but not on the bacteria that had infiltrated the tissue. For wounds treated with IM gentamicin, we observed a reduction

Fig. 7. Staining for macrophages in wound tissue. Immunohistochemistry with CD68 monoclonal antibodies for a subcellular fraction of macrophages on biopsies from *S. aureus* infected wounds treated with either topical honey or intramuscular gentamicin. Scale bars represent 500 μm .

Fig. 8. Systemic immune response in pigs with *S. aureus* infected wounds. Wounds were treated with either topical honey or intramuscular gentamicin on separate pigs. Two days after inoculation (day 2), both pigs exhibit systemic measurements consistent with an acute systemic inflammatory response. This response was more persistent for the duration of treatment for the pig that received topical honey treatment. (A) Oral temperature was taken prior to tissue sampling at each time point. Normal temperature range modified from Soerensen et al. *Acta Vet Scand* 2015;57:5. (B) SAA was analysed from serum using the PHASE RANGE Multispecies SAA ELISA kit (Tridelta Development Ltd.). Normal range according to manufacturer. Symbols and error bars represent mean and standard deviation (SD). (C-E) Leucocytes, neutrophils, and haemoglobin were analysed from blood with the IDEEX ProCyte DX (IDDEX Laboratories Inc.). Normal ranges according to the manufacturer.

in wound surface area and signs of reepithelization (Figs. 5 and 6). This was probably because IM gentamicin was able to inhibit the growth of *S. aureus* in surrounding tissue and thereby enable wound repair. Our negative findings regarding the efficacy of honey on the healing of infected wounds are in line with the conclusion of the Cochrane review from 2015, as only one study was identified that could provide moderate evidence for honey's efficacy as treatment for infected wounds in humans [44]. In this randomized controlled trial, by Al-Waili et al., severely infected wounds following caesarean section or total abdominal hysterectomies were treated with either topical honey or antiseptic washes. Wounds treated with honey dressings exhibited a shorter time to eradication of bacterial infection, wound healing, and smaller scar formation [44]. However, both groups were treated with systemic antibiotics, and studies have indicated that the chosen antiseptic may have a negative effect on wound healing [45,46]. Therefore, it is unclear if Al-Waili et al. could demonstrate any additional benefit with topical honey compared to systemic antibiotics only.

The WHO has stated that: "the global community must encourage sustainable investments in new medicines, diagnostic tools, vaccines, and alternative interventions. The majority of pharmaceutical companies are no longer researching a new antibiotic which is a global concern for human and animal health. Research and development are needed to produce new treatments that can be deployed against multi-drug resistant infections" [47]. As a result of the ever-spreading antimicrobial resistance, an increasing number of non-healing wounds globally are infected with MDR bacteria, causing morbidity and adding burden to already strained health care systems, especially in armed conflicts [20,48,49]. A recent cohort study of Syrian patients with conflict-related injuries treated in Jordan found MDR bacteria in excess of 70% of infected wounds and was associated with a poorer outcome and high resource consumption [1]. This highlights the need to identify treatment strategies that do not rely on antibiotics. Honey's antimicrobial properties, low cost, almost indefinite shelf life, and readily availability worldwide makes it an ideal candidate. Moreover, honey has been demonstrated to be efficient against MDR bacteria *in vitro* studies [50,51].

The antimicrobial properties of honey are not fully understood and will vary depending on floral source. However, most honey types share the following antimicrobial properties: 1. Hydrogen peroxide activity causing a breakdown of bacterial walls. 2. Hyperosmolality that draws moisture from the wound and bacteria, causing a decrease of water activity (free water) to 0.56–0.59. 3. Acid pH ranging between 3.2 and 4.5, causing a hostile environment for bacteria. 4. Increase phagocytosis and bacterial destruction by stimulating macrophage activity and cytokines (IL-1 β , IL-6 and TNF- α) production. 5. Promoting healing by oxygenation, angiogenesis, and fibroblast proliferation [12,42].

The hydrogen peroxide activity is a crucial component of honey's antimicrobial effect. Hydrogen peroxide is produced by breakdown of glucose by the enzyme glucose oxidase. Non-active glucose oxidase is a natural component of honey. Its activation is pH-dependant (5.5–8.0) and will not occur unless the honey (pH 3.2–4.5) is exposed to a more basic environment, such as a wound. Hydrogen peroxide eliminates bacteria through oxidation that cause DNA damage and change the permeability of the bacterial wall [12,42,52]. Some bacteria, such as *S. aureus*, produce the enzyme catalase that breakdown hydrogen peroxide [53]. However, depending on the floral source of the honey, phytochemical components can add antimicrobial activity that is stable in the presence of catalase. This non-peroxide activity has been demonstrated in Manuka honey from New Zealand used in the present study [15]. Manuka honey was chosen due to its established broad antimicrobial spectrum, including commonly found wound pathogens in armed conflicts [13,20,50,52,54]. The Manuka

honey is a monofloral honey produced by the *Apis mellifera* honeybees that collect nectar from the flowers of the Manuka tree (*Leptospermum scoparium*). Manuka honey contains high concentrations of methylglyoxal (MGO), which has been demonstrated to be strongly antimicrobial, even in the presence of catalase [13–15,42,55]. The concentration of MGO will vary in different kinds of Manuka honey and is measured in Unique Manuka Factor (UMF, range 0–25+), which is equivalent to% (w/v) phenol in antimicrobial activity against *S. aureus* in an agar well diffusion assay [50,56]. However, how MGO exerts its antimicrobial effect is not fully understood. Studies using scanning electron microscopy have demonstrated loss of flagella and fimbriae in *E. coli* and *B. subtilis*, in the presence of MGO [57]. A shrinking and rounding of these rod-shaped bacteria were also observed [57].

Consequently, higher UMF has been demonstrated to correlate with lower minimum inhibitory concentration (MIC) for flagella and fimbriae-carrying bacteria, such as *E. coli* and *P. aeruginosa* [58,59]. The same correlation has not been demonstrated for *S. aureus*, which is lacking flagella and fimbriae and is coccidial in shape. It has therefore been suggested that Manuka honey exerts its antimicrobial effect on *S. aureus* through hydrogen peroxide or other phytochemical components, such as the protein bee defensin-1 [52]. The MGO concentration (UMF) may therefore have little relevance for the antimicrobial effect on *S. aureus*. In the present study, a MGO concentration of UMF 15+ was chosen as most FDA-approved Manuka honey dressings are in the range of UMF 13–18 [60]. However, it is worth noting that several *in vitro* studies have demonstrated that Manuka honey with lower UMF exhibit lower MIC for *S. aureus* [52].

Our findings of unchanged wound size and *S. aureus* count in wound tissue suggest that topical Manuka honey may be beneficial to prevent further progression of the wound infection and subsequent complications. This notion is especially interesting in settings where antibiotics and surgical care are not immediately available, such as in armed conflicts. Manuka honey dressings could be used at the site of injury, at trauma stabilization points, and before transports to inhibit the progression of a potential wound infection until definite care can be undertaken. Manuka honey may also constitute a valuable adjunct to antibiotics and surgical debridement. *In vitro* and *ex vivo* studies have demonstrated increased antimicrobial activity against *P. aeruginosa*, *S. aureus*, and MRSA when using antibiotics combined with Manuka honey [61–63]. Furthermore, a study by Jenkins and Cooper showed that subinhibitory concentrations of Manuka honey made MRSA susceptible to oxacillin again, i.e., reverse the resistance [64]. Essential to this synergistic effect of antibiotics and Manuka honey is Manuka honey's ability to interrupt biofilms produced by bacteria, thereby increasing the bacteria's susceptibility to antibiotics [25,26]. Honey's antimicrobial and antibiofilm activity could also be of benefit in the most fundamental part of the treatment of infected wounds in armed conflicts, the surgical debridement. Applying honey dressings at the end of the debridement may inhibit recolonization of pathogenic bacteria on the wound surface and subsequently hinder redevelopment of devitalized tissue. This could possibly result in a diminished need for repeated debridements and a shorter time to achieve wound closure. However, this needs to be extensively evaluated *in vivo* studies before honey can become relevant in the treatment of infected wounds in armed conflicts.

Limitations

The lack of previous studies regarding honey's efficacy as a treatment for infected wounds in humans and *in vivo* models poses restraints on designing a study that could have direct implications for how infected wounds are treated. It can be expected that the antimicrobial effect of honey previously reported from primarily

in vitro models will be diminished in more complex *in vivo* models, such as the porcine model used, due to honey's inability to reach bacteria that have infiltrated into the tissue [13,55,65]. It would therefore have been rational to study honey's efficacy as an adjunct to surgical debridement or antibiotics. However, given the lack of existing data from more complex *in vivo* models, we found it essential to first quantitatively assess honey's efficacy without any confounding factors, such as surgical debridement or antibiotics. Based on the results from the power calculation and per the principles of the 3Rs (Replacement, Reduction, and Refinement), the lowest number of animals were used to obtain the needed data for a valid statistical comparison between topical honey and IM gentamicin [66]. Nevertheless, the use of only two animals poses limitations in the external validity of the study. The use of different numbers of infected wounds per treatment arm and pig could have affected our comparison of systemic inflammatory response for the two treatments. However, this should not have substantially impacted our primary outcome measure of reduction in bacterial counts per wound at terminal endpoint. The internal validity of the study is therefore robust, but the generalizability is limited.

Conclusion

In the porcine wound model used, intramuscular gentamicin was initially more effective than topically applied Manuka honey in reducing *S. aureus* in infected wounds. However, after eight days of treatment, *S. aureus* count was the same with both treatments. The wound area decreased with gentamicin and was unchanged with Manuka honey. Topically applied Manuka honey could therefore be non-inferior to intramuscular gentamicin in treating *S. aureus* colonization on the wound's surface, but not in reducing wound size. The use of Manuka honey dressings to prevent further progression of a wound infection prior to transports may therefore be of value in armed conflicts, where definite care is not immediately available.

Acknowledgements

We are immensely grateful to infectious disease consultant Anita Hållgren, MD PhD, for advice on choice of bacteria and antibiotics; Responsible veterinarian Anders Sandberg, DVM, for monitoring animal health; Danne Linghammar for technical assistance and animal care; Researcher Jonathan Rakar, PhD, for scientific and technical assistance; Annika Starkenberg, MLS, and Kristina Briheim, MLS, for technical assistance; Elvira Lindholm, PhD-student, and Alexander Larsson, MD, for assisting in the assessment of concentration of macrophages; Statistician Lars Valters, MA, for statistical advice; Anna Nordström, DVM, and Per Wallgren, DVM PhD at the National Veterinary Institute, for experimental design; Inger Lilliehöök, DVM PhD at the Swedish University of Agricultural Science for experimental design and Evidensia Valla Animal Hospital for blood sample analysis. Linköping University provided open access funding.

Funding

This work was supported by grants from the Kamprad Family Foundation (20170287) and the Swedish state under the agreement between the Swedish government and the county councils, the ALF-agreement (LIO-700121).

References

- [1] Alga A, Wong S, Shoaib M, Lundgren K, Giske CG, von Schreeb J, et al. Infection with high proportion of multidrug-resistant bacteria in conflict-related injuries is associated with poor outcomes and excess resource consumption: a cohort study of Syrian patients treated in Jordan. *BMC Infect Dis* 2018;18:233. doi:10.1186/s12879-018-3149-y.
- [2] Murray CK, Hinkle MK, Yun HC. History of infections associated with combat-related injuries. *J Trauma* 2008;64:221–31. doi:10.1097/TA.0b013e318163c40b.
- [3] Book Giannou C, Baldan M. War surgery: working with limited resources in armed conflict and other situations of violence. 1 International Committee of the Red Cross, Geneva; 2019.
- [4] Clinical guidelines: diagnostic and treatment manual. Paris Book. Médecins sans frontières; 2019.
- [5] Leaning J, Guha-Sapir D. Natural disasters, armed conflict, and public health. *N Engl J Med* 2013;369:1836–42. doi:10.1056/NEJMr1109877.
- [6] Matowe LW, Vwaako P, Adome RO, Kibwage I, Omary M, Bienvenu E. A strategy to improve skills in pharmaceutical supply management in East Africa: the regional technical resource collaboration for pharmaceutical management. *Hum Resour Health* 2008;6:30. doi:10.1186/1478-4451-6-30.
- [7] United Nations. Every-Woman-Every-Child/UN Commission on life-saving commodities for women and children. New York: United Nations; 2012. <https://www.unfpa.org/> [Accessed 3 December 2019].
- [8] Kelesidis T, Falagas ME. Substandard/counterfeit antimicrobial drugs. *Clin Microbiol Rev* 2015;28:443–64. doi:10.1128/CMR.00072-14.
- [9] Laxminarayan R, Duse A, Wartal C, Zaidi AK, Wertheim HF, Sumpradit N, et al. Antibiotic resistance—the need for global solutions. *Lancet Infect Dis* 2013;13:1057–98. doi:10.1016/S1473-3099(13)70318-9.
- [10] World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva: World Health Organization; 2014. <https://www.who.int/> [Accessed 10 January 2021].
- [11] Armon PJ. The use of honey in the treatment of infected wounds. *Trop Dr* 1980;10:91. doi:10.1177/004947558001000219.
- [12] Saikaly SK, Khachemoune A. Honey and wound healing: an Update. *Am J Clin Dermatol* 2017;18:237–51. doi:10.1007/s40257-016-0247-8.
- [13] Cooper RA, Molan PC, Harding KG. Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. *J R Soc Med* 1999;92:283–5. doi:10.1177/014107689909200604.
- [14] Irish J, Blair S, Carter DA. The antibacterial activity of honey derived from Australian flora. *PLoS One* 2011;6:e18229. doi:10.1371/journal.pone.0018229.
- [15] Johnston M, McBride M, Dahiya D, Owusu-Apenten R, Nigam PS. Antibacterial activity of Manuka honey and its components: an overview. *AIMS Microbiol* 2018;4:655–64. doi:10.3934/microbiol.2018.4.655.
- [16] Jull AB, Cullum N, Dumville J, Westby MJ, Deshpande S, Walker N, et al. Honey as a topical treatment for wounds. *Cochrane Database Syst Rev* 2015;CD005083. doi:10.1002/14651858.CD005083.pub4.
- [17] Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. *Wound Repair Regen* 2001;9:66–76. doi:10.1046/j.1524-475x.2001.00066.x.
- [18] Junker JPE, Lee CCY, Samaan S, Hackl F, Kiwanuka E, Minasian RA, et al. Topical delivery of ultrahigh concentrations of gentamicin is highly effective in reducing bacterial levels in infected porcine full-thickness wounds. *Plast Reconstr Surg* 2015;135:151–9. doi:10.1097/PRS.0000000000000801.
- [19] Daly LT, Tsai DM, Singh M, Nuutila K, Minasian RA, Cameron CYL, et al. Topical Minocycline effectively decontaminates and reduces inflammation in infected porcine wounds. *Plast Reconstr Surg* 2016;138:68e 856e-. doi:10.1097/PRS.00000000000002633.
- [20] Eardley WG, Brown KV, Bonner TJ, Green AD, Clasper JC. Infection in conflict wounded. *Philos Trans R Soc Lond B Biol Sci* 2011;366:204–18. doi:10.1098/rstb.2010.0225.
- [21] Petersen K, Riddle MS, Danko JR, Blazes DL, Hayden R, Tasker SA, et al. Trauma-related infections in battlefield casualties from Iraq. *Ann Surg* 2007;245:803–11. doi:10.1097/01.sla.0000251707.32332.c1.
- [22] Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest* 2003;111:1265–73. doi:10.1172/JCI18535.
- [23] Nawfal Dagher T, Al-Bayarsari C, Diene SM, Azar E, Rolain JM. Bacterial infection during wars, conflicts and post-natural disasters in Asia and the Middle East: a narrative review. *Expert Rev Anti Infect Ther* 2020;18:511–29. doi:10.1080/14787210.2020.1750952.
- [24] Refuvel F, Josse J, Vallé Q, Mongaret C, Gangloff SC. *Staphylococcus aureus* biofilms and their impact on the medical field. The rise of virulence and antibiotic resistance in *Staphylococcus aureus*. Enany S, editor. London: In-technOpen Ltd; 2017. doi:10.5772/66380.
- [25] Maddocks SE, Lopez MS, Rowlands RS, Cooper RA. Manuka honey inhibits the development of *Streptococcus pyogenes* biofilms and causes reduced expression of two fibronectin binding proteins. *Microbiology* 2012;158:781–90.
- [26] Campeau ME, Patel R. Antibiophilic activity of Manuka honey in combination with antibiotics. *Int J Bacteriol* 2014;2014:795281. doi:10.1155/2014/795281.
- [27] USFDA 510(k) premarket notification Food and drug administration. Rockville, MD: US: Dept of Health and Human Services; 2007.
- [28] Tam VH, Kabbara S, Vo G, Schilling AN, Coyle EA. Comparative pharmacodynamics of gentamicin against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2006;50:2626–31. doi:10.1128/AAC.01165-05.
- [29] Vardaxis NJ, Brans TA, Boon ME, Kreis RW, Marres LM. Confocal laser scanning microscopy of porcine skin: implications for human wound healing studies. *J Anat* 1997;190:601–11. doi:10.1046/j.1469-7580.1997.19040601.x.
- [30] Lavker RM, Dong G, Zheng PS, Murphy G. Hairless micropig skin. A novel model for studies of cutaneous biology. *Am J Pathol* 1991;138:687–97.
- [31] Committee of veterinary medicinal products/Gentamicin, summary report (EMA/MRL/803/01-Final). London: The European agency for the evaluation of medicinal products. (EMA) 2001 <https://www.ema.europa.eu>. [Accessed 25 September 2019].

- [32] Zhu X, Liu D, Singh AK, Drolia R, Bai X, Tenguria S, et al. Tunicamycin mediated inhibition of wall teichoic acid affects *Staphylococcus aureus* and *Listeria monocytogenes* cell morphology, biofilm formation and virulence. *Front Microbiol* 2018;9:1352. doi:10.3389/fmicb.2018.01352.
- [33] Finkin S, Yuan D, Stein I, Taniguchi K, Weber A, Unger K, et al. Ectopic lymphoid structures function as microniches for tumor progenitor cells in hepatocellular carcinoma. *Nat Immunol* 2015;16:1235–44. doi:10.1038/ni.3290.
- [34] Cray C, Zaias J, Altman NH. Acute phase response in animals: a review. *Comp Med* 2009;59:517–26.
- [35] Faul F, Erdfelder E, Lang AC, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007;39:175–91. doi:10.3758/bf03193146.
- [36] Robson MC, Lea CE, Dalton JB, Heggers JP. Quantitative bacteriology and delayed wound closure. *Surg Forum* 1968;19:501–2.
- [37] Bendy RH, Nuccio PA, Wolfe E, Collins B, Tamburro C, Glass W, et al. Relationship of quantitative wound bacterial counts to healing of decubiti: effect of topical gentamicin. *Antimicrob Agents Chemother* 1964;10:147–55.
- [38] Scher KS, Peoples JB. Combined use of topical and systemic antibiotics. *Am J Surg* 1991;161:422–5. doi:10.1016/0002-9610(91)91103-p.
- [39] Erbil B, Ersoy G, Ozkutuk A, Akarca FK, Korkmaz T, Demir OF, et al. The effects of oral antibiotics on infection prophylaxis in traumatic wounds. *Ulus Travma Acil Cerrahi Derg* 2014;20:231–5. doi:10.5505/tjes.2014.63993.
- [40] Medeiros Vde F, Azevedo IM, Rego AC, Egitto ES, Araujo-Filho I, Medeiros AC, et al. Antibacterial properties and healing effects of *Melipona scutellaris* honey in MRSA-infected wounds of rats. *Acta Cir Bras* 2016;31:327–32. doi:10.1590/S0102-865020160050000006.
- [41] Robertson CM, Coopersmith CM. The systemic inflammatory response syndrome. *Microbes Infect* 2006;8:1382–9. doi:10.1016/j.micinf.2005.12.016.
- [42] Hixon KR, Klein RC, Eberlin CT, Linder HR, Ona WJ, Gonzalez H, et al. A critical review and perspective of honey in tissue engineering and clinical wound healing. *Adv Wound Care* 2019;8:403–15. doi:10.1089/wound.2018.0848.
- [43] Tsai DM, Tracy LE, Lee CC, Hackl F, Kiwanuka E, Minasian RA, et al. Full-thickness porcine burns infected with *Staphylococcus aureus* or *Pseudomonas aeruginosa* can be effectively treated with topical antibiotics. *Wound Repair Regen* 2016;24:356–65. doi:10.1111/wrr.12409.
- [44] Al-Waili NS, Saloom KY. Effects of topical honey on post-operative wound infections due to gram positive and gram negative bacteria following caesarean sections and hysterectomies. *Eur J Med Res* 1999;4:126–30.
- [45] Leaper DJ, Simpson RA. The effect of antiseptics and topical antimicrobials on wound healing. *J Antimicrob Chemother* 1986;17:135–7. doi:10.1093/jac/17.2.135.
- [46] Atiyeh BS, Dibo SA, Hayek SN. Wound cleansing, topical antiseptics and wound healing. *Int Wound J* 2009;6:420–30. doi:10.1111/j.1742-481X.2009.00639.x.
- [47] World Health Organization Global action plan on antimicrobial resistance. Geneva: World Health Organization; 2017. <https://www.who.int>. [Accessed 26 February 2019].
- [48] Mulani MS, Kamble EE, Kumkar SN, Tawre MS, Pardesi KR. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. *Front Microbiol* 2019;10:539. doi:10.3389/fmicb.2019.00539.
- [49] Sahli ZT, Bizri AR, Abu-Sittah GS. Microbiology and risk factors associated with war-related wound infections in the Middle East. *Epidemiol Infect* 2016;144:2848–57. doi:10.1017/S0950268816000431.
- [50] Girma A, Seo W, She RC. Antibacterial activity of varying UMF-graded Manuka honeys. *PLoS ONE* 2019;14:e0224495. doi:10.1371/journal.pone.0224495.
- [51] Glasser JS, Guymon CH, Mende K, Wolf SE, Hostenpeth DR, Murray CK. Activity of topical antimicrobial agents against multidrug-resistant bacteria recovered from burn patients. *Burns* 2010;36:1172–84. doi:10.1016/j.burns.2010.05.013.
- [52] Nolan VC, Harrison J, Wright JEE, Cox JAG. Clinical significance of Manuka and medical-grade honey for antibiotic-resistant infections: a systematic review. *Antibiotics* 2020;9. doi:10.3390/antibiotics9110766.
- [53] Foster T, Baron S. *Staphylococcus*. Medical microbiology. 4th ed. Galveston: University of Texas; 1996. chapter 12.
- [54] Patton T, Barrett J, Brennan J, Moran N. Use of a spectrophotometric bioassay for determination of microbial sensitivity to Manuka honey. *J Microbiol Methods* 2006;64:84–95. doi:10.1016/j.mimet.2005.04.007.
- [55] Carter DA, Blair SE, Cockeett NN, Bouzo D, Brooks P, Schothauer R, et al. Therapeutic Manuka honey: no longer So alternative. *Front Microbiol* 2016;7:569. doi:10.3389/fmicb.2016.00569.
- [56] Allen KL, Molan PC, Reid GM. A survey of the antibacterial activity of some New Zealand honeys. *J Pharm Pharmacol* 1991;43:817–22. doi:10.1111/j.2042-7158.1991.tb03186.x.
- [57] Rabie E, Serem JC, Oberholzer HM, Gaspar AR, Bester MJ. How methylglyoxal kills bacteria: an ultrastructural study. *Ultrastruct Pathol* 2016;40:107–11. doi:10.3109/01913123.2016.1154914.
- [58] Tan HT, Rahman RA, Gan SH, Halim AS, Hassan SA, Sulaiman SA, et al. The antibacterial properties of Malaysian tualang honey against wound and enteric microorganisms in comparison to Manuka honey. *BMC Complement Altern Med* 2009;9:34. doi:10.1186/1472-6882-9-34.
- [59] Sherlock O, Dolan A, Athman R, Power A, Gethin G, Cowman S, et al. Comparison of the antimicrobial activity of Ulmo honey from Chile and Manuka honey against methicillin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complement Altern Med* 2010;10:47. doi:10.1186/1472-6882-10-47.
- [60] Molan P, Rhodes T. Honey: a biologic wound dressing. *Wounds* 2015;27:141–51.
- [61] Roberts AEL, Powell LC, Pritchard MF, Thomas DW, Jenkins RE. Antipseudomonad activity of Manuka honey and antibiotics in a specialized *ex vivo* model simulating cystic fibrosis lung infection. *Front Microbiol* 2019;10:869. doi:10.3389/fmicb.2019.00869.
- [62] Jenkins R, Cooper R. Improving antibiotic activity against wound pathogens with Manuka honey *in vitro*. *PLoS ONE* 2012;7:e45600. doi:10.1371/journal.pone.0045600.
- [63] Liu MY, Cockeett NN, Lu J, Turnbull L, Carter DA, Whitchurch CB, et al. Rifampicin-Manuka honey combinations are superior to other antibiotic-Manuka honey combinations in eradicating *Staphylococcus aureus* biofilms. *Front Microbiol* 2017;8:2653. doi:10.3389/fmicb.2017.02653.
- [64] Jenkins RE, Cooper R. Synergy between oxacillin and Manuka honey sensitizes methicillin-resistant *Staphylococcus aureus* to oxacillin. *J Antimicrob Chemother* 2012;67:1405–7. doi:10.1093/jac/dks071.
- [65] Brudzynski K, Sjaarda C. Honey glycoproteins containing antimicrobial peptides, Jelleins of the Major Royal Jelly Protein 1, are responsible for the cell wall lytic and bactericidal activities of honey. *PLoS ONE* 2015;10:e0120238. doi:10.1371/journal.pone.0120238.
- [66] Book Russell WMS, Burch RL. The principles of humane experimental technique Methuen, London; 1959.

The background of the entire page is a photograph of a bamboo fence. In the foreground, a medical table is partially visible, extending from the left towards the center. The table has a dark surface and a large wheel on the left side. To the right of the table, there is a white bag or container with some text on it, including '000500' and 'LINKÖP'.

FACULTY OF MEDICINE AND HEALTH SCIENCES

Linköping University Medical Dissertation No. 1791, 2021
Department of Biomedical and Clinical Sciences

Linköping University
SE-581 83 Linköping, Sweden

www.liu.se