Recent Clinical Techniques, Results, and Research in Wounds

Melvin A. Shiffman
Mervin Low *Editors*

Vascular Surgery, Neurosurgery, Lower Extremity Ulcers, Antimicrobials, Wound Assessment, Care, Measurement and Repair

Springer

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Foreword[1]

It is a great honour for me to be invited to provide a foreword for the series of six books edited by Dr. Shiffman and Dr. Low, which cover a broad expanse of subjects relevant to and important in the care of patients with wounds.

Wounds have existed since the beginning of time and, until recent years, have received scant attention unless major conflicts developed which necessitated innovation in the treatment of patients with wounds. However, in recent years there has been an increasing interest in this subject as evidenced by the explosion of journals, meetings, societies and associations and initiatives that have been developed in this field.

The need for an academic underpinning of the subject of wound healing is without question. Research papers published in recent years have undoubtedly enhanced the scientific basis for wound healing. This, coupled with demographic changes in many countries around the world, has led to increasing numbers of patients developing wounds or wound healing problems. It is recognised that in the vast majority of geographies globally the number of patients with wounds is increasing in everything other than major burns where better health and safety initiatives have been an effective preventive strategy.

This series of books not only attempts to deal with subjects that are normally seen in wound healing text but also provides a huge amount of space to the management of wounds seen in surgical practice, both general and specialist surgery. The sections on infection are an attempt to deal with a very common but poorly managed clinical problem and one that requires urgent attention in view of the global challenge of antimicrobial stewardship. The tradition chronic wounds are also included and provide a medical as well as a nursing and paramedical focus on these subjects.

It is particularly pleasing to see books and chapters focused on specialised surgical practice as these are areas that are rarely covered in other educational products in this area. The opportunity for new therapies, measuring the range of effective and appropriate outcomes and the use of new technologies are all included.

For those of us who work in the area of wound healing, these books will unquestionably be an important reference source. For those readers who want to get an insight into this common, expensive and complex problem they will without doubt find the content of these books an important source of informed opinion and refer to the rapidly expanding evidence base that is developing in this subject area.

I would urge you to immerse yourself in these books. Read, reflect and consider how information that you have had access to can and will change your clinical practice.

Keith Harding
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UK

[1]P. S.

We, Melvin A. Shiffman and Mervin Low, are greatly enthralled by Keith Harding's willingness to write the Foreword for the books on wounds. Keith Harding is the Director of TIME Institute (Translation, Innovation, Methodology and Engagement) and Head of the Wound Healing Research Unit in the School of Medicine at Cardiff University. He is Clinical Lead for Wound Healing in the Cardiff and Vale NHS Trust. In September 2013 Harding was appointed Dean of Clinical Innovation at Cardiff University. From 2002 to 2005 he was Head of the Department of Surgery at Cardiff University. He is Editor-in-Chief of the *International Wound Journal*. Harding is a Past President of the European Tissue Repair Society. He was the first President of the European Pressure Ulcer Advisory Panel and first Recorder of the European Wound Management Association. He was Chair of the International Working Group on Wound Healing in Diabetic Foot Disease in 2003. He was Chair of the Expert Working Group that produced a range of International Consensus Documents from 2004 to 2011. Professor Harding was appointed a Commander of the Order of the British Empire in the 2013 New Year Honours for services to medicine and healthcare.

Preface

We are delighted to have the book on wounds extended into six volumes. There is so very much medical literature in journals and books that to cover the whole gamut of wounds would be virtually impossible. We tried to include as many of the experienced practitioners in wound care as possible, but many of them are too busy to spend the time committing to submitting a chapter.

The selection of topics in each of the volumes was decided by the number of authors responded to each of the subjects. As usual in editing a book, many authors who agreed to submit manuscripts finally were not available to complete the chapters. We contacted or tried to contact over 1500 authors and most of them did not respond or the responses were not as good as expected.

The volumes include:

1. Biofilm, Pilonidal Cysts and Sinuses
2. Burns, Infections and Wound Management
3. Pressure Injury, Diabetes and Negative Pressure Wound Therapy
4. Plastic and Thoracic Surgery, Orthopedics and Ophthalmology
5. Vascular Surgery, Neurosurgery, Lower Extremity Ulcers, Antimicrobials, Wound Assessment, Care, Measurement and Repair
6. Chronic Wounds, Wound Dressings and Wound Healing

There are many expert international contributors who have worked in various aspects of wound research as well as clinical practice. We have tried to have chapters that involved humans and in vivo results and avoided as much as possible animals and in vitro results. Chapter conclusions are those of the authors and may not be the same as those of the editors. At times the chapter may appear cumbersome, but the authors try to show some proof of their results. Language difficulties are common when translated into English so that grammar, spelling and sometimes words have to be corrected.

Hopefully, the reader will get information that adds to their care and treatment of patients. Researchers may gain knowledge of other researchers' progress and improve on the results or can continue their work in other directions. Controversy is many times a good thing since looking in other directions to prove or disprove a result can improve knowledge. We have a long way to go to be able to treat all wounds properly and successfully in as short a time as possible.

Tustin, CA, USA — Melvin A. Shiffman
Newport Beach, CA, USA — Mervin Low

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Part I

Vascular Surgery

The Gatti Score and the Risk of Deep Sternal Wound Infection After Bilateral Internal Thoracic Artery Grafting

Giuseppe Gatti, Andrea Perrotti,
Giuseppe Santarpino, and Fausto Biancari

1 Introduction

Sternal wound infections remain a major source of physical, emotional, and economic stress in cardiac surgery, though extensive use of negative pressure wound therapy and advances in reconstructive surgery of the sternum have improved results dramatically [1, 2]. The most serious form of this complication, namely, deep sternal wound infections (DSWI), occurs in 1% up to 4% of patients after coronary artery bypass grafts (CABG) surgery performed via a median sternotomy and is associated with increased early mortality and poor late outcomes [1–3].

Throughout the years, many studies have been performed to identify the predictors of sternal wound infections after CABG surgery [3–10]. Baseline patient characteristics, surgical techniques, postoperative complications, and various protocols of perioperative management of patients have been examined. On the basis of the results of almost all of these investigations, the simultaneous use of both internal thoracic arteries (ITAs) as coronary grafts for myocardial revascularization, i.e., bilateral ITA (BITA) grafting, was an independent predictor of sternal wound infections, although skeletonizing the grafts has been proven useful in reducing the incidence mainly in diabetic patients [10–20].

Throughout the years, many statistical models have been devised to predict the risk of developing sternal wound infections after median sternotomy [2, 21–26]. However, these models arose from cohorts of patients undergoing different surgical procedures, or preselected series of CABG patients where most of the patients have received single ITA (and saphenous vein or radial artery) grafts for myocardial revascularization. Besides, some models were tested for every surgical site infection after CABG surgery including also leg wound complications. Unfortunately, the predictive power of these models is limited mainly due to the complex pathogenesis of sternal wound infections, which involves specific comorbidities, periprocedural factors, and postoperative complications. Also according to these analyses, the use of BITA grafting was confirmed to be a strong predictor of sternal complications, and concerns about the high risk of DSWI have limited its more extensive use in CABG surgery.

Consequently, in order to minimize sternal complications, BITA grafts should be used only in selected patients without the well-known risk fac-

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tors for sternal wound infection, such as female gender, obesity, diabetes mellitus, chronic lung disease, renal impairment, and peripheral vascular disease [2, 13, 14, 21–26]. However, this strict selection would deprive too many patients from the long-term survival benefits derived from BITA use [15–17, 19, 20]. Moreover, patients suffering from diabetes or renal failure are the patients who would most benefit from the good long-term patency rates of the BITA grafts even in the presence of these two serious comorbidities [12, 15–19].

In this context, it seemed ever more urgent the need for a predictive scoring system focused specifically on sternal wound infections following BITA grafting.

In 2015, Gatti et al. [27] reviewed retrospectively the outcomes of nearly 3000 consecutive BITA patients who had been operated on at the Cardiovascular Department of the University Hospital of Trieste, Trieste, Italy. A new, weighted scoring system based on the results of this analysis was specifically created to predict DSWI risk after BITA grafting.

2 Methods

Between 1999 and 2013, a total of 4160 consecutive patients with multivessel coronary artery disease underwent isolated CABG surgery at the Cardiovascular Department of the University Hospital of Trieste, Trieste, Italy. A BITA grafting was performed in 2936 (70.6%) cases (Table 1) [27, 28].

Table 1 The Italian original series: preoperative patients' characteristics and risk profiles [27][a]

Characteristic	Patients $n = 2936$
Age, years	66.3 ± 9.0
<70	1752 (59.7)
70–80	1054 (35.9)
>80	130 (4.4)
Female gender	460 (15.7)
Hypertension	2109 (71.8)
Former smoker	654 (22.3)
Current smoker	112 (3.8)
Hyperlipidemia	2620 (89.2)
BMI, kg/m^2	27.2 ± 3.6
<20	37 (1.3)
>30	586 (20.0)
Diabetes	865 (29.5)
On oral hypoglycemic agents	652 (22.2)
On insulin	213 (7.3)
Poor glycemic control[b]	137 (4.7)
Serum hemoglobin, g/L	13.3 ± 1.6
<12	708 (24.1)
Poor mobility[c]	14 (0.5)
Chronic lung disease[c]	134 (4.6)
eGFR, mL/min[d]	78.5 ± 27.7
50–85[c]	1515 (51.6)
<50[c]	365 (12.4)
Chronic dialysis	35 (1.2)
Extracardiac arteriopathy[c]	192 (6.5)
Atrial fibrillation	12 (0.4)
Congestive heart failure	135 (4.6)
Unstable angina	860 (29.3)
Silent myocardial ischemia	47 (1.6)
Recent myocardial infarction[c]	366 (12.5)
Coronary artery disease	
Left main	1062 (36.2)
One-vessel	14 (0.5)
Two-vessel	375 (12.8)
Three-vessel	2547 (86.8)
LVEF, %	55.2 ± 10.3
30–50[c]	710 (24.2)
<30[c]	85 (2.9)
Previous PCI	94 (3.2)
Previous cardiac operation[c]	31 (1.1)
Previous CABG surgery	12 (0.4)
Cardiogenic shock	5 (0.2)
Aborted sudden death	7 (0.2)
Use of IABP	96 (3.3)
Urgent surgical priority[c]	1750 (59.6)
Emergency[c]	53 (1.8)
Expected operative risk (by EuroSCORE II [28]), %	2 (1.1–3.9)

BMI body mass index, *BSG* basal serum glucose, *CABG* coronary artery bypass grafts, *EuroSCORE* European System for Cardiac Operative Risk Evaluation, *GFR* glomerular filtration rate, *IABP* intra-aortic balloon pumping, *LVEF* left ventricular ejection fraction, *PCI* percutaneous coronary intervention, *SD* standard deviation

[a]Values are number of patients, mean ± SD, or median, with the percentage or the range between the first and the third quartile in brackets

[b]BSG >200 mg/dL at three consecutive measurements

[c]Definitions and cutoff values are those employed for EuroSCORE II [28]

[d]The creatinine clearance rate, calculated according to the Cockcroft–Gault formula, was used for approximating the GFR

Surgery was carried out via a median sternotomy either with cardiopulmonary bypass, with or without cross-clamping the aorta or off-pump technique. When a period of myocardial ischemia was used, myocardial protection was usually achieved with multidose cold blood cardioplegia delivered in both antegrade and retrograde mode. A single-dose crystalloid solution (Custodiol–histidine–tryptophan–ketoglutarate® solution; Essential Pharma, Newtown, Pennsylvania, PA) was sometimes preferred, especially when longer ischemic times were expected.

The details as the selection criteria of patients, rate of BITA use, ITA harvesting technique, use of prophylactic antibiotics, preoperative skin preparation, the choice either of off-pump or on-pump technique, sternal closure, wound care, and perioperative management of hyperglycemia are summarized in Table 2 [27–38].

All perioperative data were prospectively and meticulously recorded for every patient in a computerized data registry.

Table 2 The Italian (original and prospective), the French, and the German series: perioperative management of patients, surgical techniques, and sternal wound care

Measure	The Italian original (n = 2936) [27] and prospective series (n = 304) [29]	French series [30] (n = 255)	German series [31] (n = 53)
Study period	The original series, 1999–2013, 15 years; the prospective series, January 2014–July 2015, 19 months	2015, 12 months	January 2014–November 2016, 35 months
Institution	Cardiovascular Department, University Hospital of Trieste, Trieste, Italy	Department of Thoracic and Cardiovascular Surgery, University Hospital Jean Minjoz, Besançon, France	Department of Cardiac Surgery, Cardiovascular Center, Paracelsus Medical University, Klinikum Nürnberg, Nuremberg, Germany
Rate of BITA use	The original series, 2936/4160 patients, 70.6%; the prospective series, 304/335 patients, 90.7%	255/255 patients, 100%	53/1313 patients, 4%
Preoperative BITA evaluation	To evaluate the suitability of both ITAs to be used as coronary grafts, all patients had undergone bilateral selective angiography of the subclavian artery during preoperative coronary angiography	None	None
Selection criteria of the patients	All patients with multivessel coronary disease who required left-sided myocardial revascularization were candidates for BITA grafting, the sole exceptions being the cases in which one or both ITAs were unsuitable as coronary grafts, or when there was an unexpected operative finding of severe cardiac dysfunction, or when rapid worsening of hemodynamics due to ischemia required immediate institution of CPB. Actually, there have been even some cases where a second ITA graft was harvested during CPB	All patients with multivessel coronary disease had BITA grafting, the sole exceptions being the cases in which one or both ITAs were unsuitable as coronary grafts. Actually, there have been even some cases where a second ITA graft was harvested during CPB	The use either of one or both ITAs depended on the surgeon's choice

(continued)

Table 2 (continued)

Measure	The Italian original (n = 2936) [27] and prospective series (n = 304) [29]	French series [30] (n = 255)	German series [31] (n = 53)
Use of prophylactic antibiotics	A first-generation cephalosporin (cefazolin) was chosen. Vancomycin was used if there was a severe allergy to β-lactam antibiotics or in the event of mediastinal re-exploration; in the last case, the addition of an aminoglycoside was considered	A second-generation cephalosporin (cefuroxime) was chosen. Vancomycin was used if there was a severe allergy to β-lactam antibiotics. In the event of mediastinal re-exploration, the same protocol was used	A first-generation cephalosporin (cefazolin) was chosen. Vancomycin was used if there was a severe allergy to β-lactam antibiotics. In the event of mediastinal re-exploration, the same protocol was used
Skin preparation	A careful skin preparation was performed with alcoholic iodine solution. Chlorhexidine–alcohol was used only for patients with iodine allergy. A microbial sealant that immobilizes bacteria was adopted	A careful skin preparation was performed with povidone-iodine. Chlorhexidine–alcohol was used only for patients with iodine allergy. A microbial sealant that immobilizes bacteria was adopted	A careful skin preparation was performed with alcoholic solution
ITA harvesting technique	Both ITAs were harvested as skeletonized conduits with low-intensity cautery and bipolar coagulation forceps, extending distally just to include either the superior epigastric or the musculophrenic artery [32]	Both ITAs were harvested as skeletonized conduits with low-intensity cautery; the collaterals were divided between vascular clips. Distal bifurcation was spared, bilaterally	Both ITAs were harvested as pedicled conduits with low-intensity cautery; the collaterals were divided between vascular clips. Distal bifurcation was spared, bilaterally
Use of off-pump and beating heart on-pump techniques	Off-pump and beating heart on-pump techniques were adopted only in the presence of a diffusely atherosclerotic ascending aorta (by intraoperative EAS) [33]	The use either of off-pump or on-pump technique depended on the surgeon's choice. Some surgeons did exclusively off-pump, and others surgeons did exclusively on-pump, except for diffusely atherosclerotic ascending aorta, where off-pump (or beating heart on-pump) technique was used	Only the on-pump technique was used
Sternal closure	Standard single-loop sternal wiring technique was used as a sternal closure method until 2009. Since 2010, the Erdinc double-loop sternal wiring technique [34] was adopted systematically. Bone wax was forbidden. Neither platelet gel nor topical antibiotics were used. Skin staples were used	All patients were closed by the Sutherland sternal wiring technique [35]. Bone wax was used depending on the surgeon's preference. Neither platelet gel nor topical antibiotics were used. A subcuticular suture was used	All patients were closed by standard sternal wiring technique. Bone wax was used according to the surgeon's choice. Neither platelet gel nor topical antibiotics were used. A subcuticular suture was used
Wound care	Traditional gauze dressings were applied immediately after the surgery to closed surgical incisions. Patients were monitored daily for symptoms of wound infection. Wounds were inspected immediately after removal of the dressings (postoperative day 2), early before hospital discharge, and at postoperative day 15 in a specifically dedicated surgical outpatient	Idem	The Prevena™ Therapy system [36] was applied immediately after surgery to closed surgical incisions. A negative pressure of −75 mmHg was applied. The system was continuously active for a period of 5 days. Patients were monitored daily for symptoms of wound infection. Wounds were inspected immediately after removal of the system, early before hospital discharge, and at postoperative day 30 in a specifically dedicated surgical outpatient

Table 2 (continued)

Measure	The Italian original ($n = 2936$) [27] and prospective series ($n = 304$) [29]	French series [30] ($n = 255$)	German series [31] ($n = 53$)
Management of hyperglycemia	All diabetic patients were treated during operation and then in ICU with a continuous intravenous insulin infusion in order to maintain serum glucose <180–200 mg/dL [37]	Idem	Idem
Other surgical details	Both ITAs were used as in situ grafts when possible (based on the double-source concept). The right ITA was preferentially directed to the left anterior descending coronary artery and the left ITA to the posterolateral cardiac wall. Sometimes, the right ITA was taken down and used as a free graft from either the in situ left ITA (Y-graft) or (rarely) the proximal (aortic) end of a saphenous vein graft. The anteaortic crossover right ITA bypass graft was protected by means of a pedicled flap taken from the thymic remnants [38]. Additional coronary bypasses, usually for the right coronary artery, were performed with saphenous vein grafts	In every patient, the right ITA was taken down and used as the second branch of a Y-graft with the in situ left ITA. The BITA grafting alone technique was used	Both ITAs were used as in situ grafts when possible (based on the double-source concept). The left ITA was preferentially directed to the left anterior descending coronary artery and the right ITA to the posterolateral cardiac wall. Sometimes, the right ITA was taken down and used as a free graft from the in situ left ITA (Y-graft). Additional coronary bypasses, usually for the right coronary artery, were performed with saphenous vein grafts

BITA bilateral internal thoracic artery, *CPB* cardiopulmonary bypass, *EAS* epiaortic ultrasonography scan, *ICU* intensive care unit, *ITA* internal thoracic artery

3 Definitions

Unless otherwise stated, definitions of preoperative clinical variables were those employed for the European System for Cardiac Operative Risk Evaluation II (EuroSCORE II) [28].

The Centers for Disease Control and Prevention classification of the surgical site infections was adopted to define sternal wound infections [39]. In brief, superficial incisional infection involves only skin or subcutaneous tissues, deep incisional infection involves deep soft tissues (fascial and muscle layers) with or without the sternal bone, and organ/space infection involves the mediastinum (i.e., mediastinitis). For the purposes of this treatise, deep incisional infection and mediastinitis were considered to be DSWI.

Poor preoperative glycemic control was defined as basal serum glucose >200 mg/dL at three consecutive measurements before surgery. Atherosclerosis of the ascending aorta was demonstrated using the epiaortic ultrasonography scan, which was performed intraoperatively in every patient. A porcelain aorta was defined as a diffusely calcified and unclampable ascending aorta [27–33]. The risk profile for each patient was calculated according to EuroSCORE II [28].

Postoperatively, low cardiac output was defined as three consecutive cardiac index measurements <2.0 L/min/m^2 despite adequate preload, afterload and inotropic support, or intra-aortic balloon pumping. Acute kidney injury was defined as postoperative serum creatinine >2.0 mg/L in the patients without preoperative renal impairment and postoperative increase in serum creatinine of at least 1.0 mg/L above baseline in the patients with preoperative renal impairment [27].

4 Statistical Methods

Data from patients with DSWI were compared with data from patients without sternal complications. Preoperative clinical characteristics of the patients, operative data, and perioperative complications were compared using the chi-square or Fisher's exact test for dichotomous variables and the Student's t-test or the Mann–Whitney U-test for continuous variables. All variables from the univariable analysis with a p-value <0.1 were entered into a backward stepwise multivariable logistic regression analysis. Risk indices were constructed from the independent risk factors identified from the final multivariable logistic regression model. Variables were eligible for inclusion at p-value <0.1. Each of the risk indices had the variable weighted according to its regression coefficient. The function "nomogram" in the "rms" package for R was used to convert the multivariable model into a scoring system [40]. Two multivariable analysis models and two corresponding models of a new predictive scoring system for DSWI were created. The preoperative model included only preoperative characteristics of the patients. The combined model included both preoperative and intraoperative and postoperative variables. The predictive power of the models was assessed using Goodman–Kruskal's nonparametric correlation coefficient G. According to Haley [41], the predictive power was defined as low ($G < 0.3$), moderate (G, 0.3–0.5) and high ($G > 0.5$). The discrimination power of the models was assessed with the receiver-operating characteristic (ROC) curve and the calculation of the area under the ROC curve (AUC). According to arbitrary guidelines [42], the accuracy of prediction was defined as low (AUC, 0.5–0.7), moderate (AUC, 0.7–0.9), and high (AUC, 0.9–1). The new predictive scoring system was compared (using DeLong's method [43]) with some existing scoring systems for surgical site infection following cardiac surgery [21–26]. An internal validation procedure based on the 0.632 bootstrap method was performed for both models. Finally, three studies on new validation samples of patients were carried out; the Hosmer–Lemeshow test and the ROC curve analysis were adopted to assess the goodness-of-fit and the discriminatory power, respectively, of the score. Correspondence between actual and expected DSWI risk was evaluated as well. Statistical analyses were performed using SPSS for Windows, version 13.0 (SPSS, Inc., Chicago, IL, USA) [27].

5 Results

5.1 Risk Factors for DSWI and Multivariable Analysis Models

A total of 129 (4.4%) patients suffered from DSWI. These patients were compared with 2743 (93.4%) patients who experienced no sternal complications. Older age, female gender, obesity, diabetes, poor glycemic control, severe anemia, chronic lung disease, severe renal impairment, chronic dialysis, extracardiac arteriopathy, congestive heart failure, left ventricular dysfunction, previous CABG surgery, urgent surgical priority, high expected operative risk (by EuroSCORE II), use of chlorhexidine–alcohol, porcelain aorta, and postoperative prolonged invasive ventilation, atrial fibrillation, low cardiac output, acute kidney injury, blood transfusion, multiple blood transfusion, and mediastinal re-exploration were risk factors for DSWI according to the univariable analysis. Using these dependent risk factors for DSWI, two multivariable analysis models were created to examine either preoperative alone or combined (preoperative, intraoperative, and postoperative) risk factors. Female gender, body mass index >30 kg/m^2, diabetes, poor glycemic control, chronic lung disease, and urgent surgical priority were the predictors of DSWI common to both models (Table 3) [27].

5.2 The New Predictive Scoring System for DSWI After BITA Grafting

According to the corresponding multivariable analysis models (Table 3), two models, preoper-

Table 3 The Italian original series: risk factors for DSWI [39] (multivariable analysis) (n = 2872) [27][a]

	Preoperative evaluation		Combined evaluation	
Risk factor	OR (95% CI)	p-value	OR (95% CI)	p-value
Female gender	2.96 (2.01–4.37)	<0.0001	3.00 (2.02–4.46)	<0.0001
BMI >30 kg/m^2	1.43 (0.95–2.17)	0.087	1.49 (0.98–2.26)	0.061
Diabetes on oral hypoglycemic agents	1.71 (1.12–2.63)	0.014	1.69 (1.10–2.61)	0.017
Diabetes on insulin	2.63 (1.52–4.56)	0.0005	2.45 (1.41–4.26)	0.0014
Poor glycemic control[b]	1.88 (0.99–3.59)	0.055	2.14 (1.12–4.10)	0.021
Chronic lung disease[c]	2.98 (1.56–5.69)	0.0009	2.83 (1.47–5.47)	0.0019
Chronic dialysis	2.73 (0.97–7.69)	0.057	–	–
Congestive heart failure	1.89 (1.00–3.57)	0.05	–	–
Urgent surgical priority[c]	1.69 (1.13–2.53)	0.011	1.61 (1.07–2.41)	0.022
Use of chlorhexidine–alcohol	–	–	2.35 (0.95–5.80)	0.063
Porcelain aorta (by intraoperative EAS [33])	–	–	1.83 (0.99–3.36)	0.053
Postoperative				
Low cardiac output[d]	–	–	5.34 (1.95–14.61)	0.0011
Multiple blood transfusion (>2 RBCs)	–	–	1.79 (1.07–2.99)	0.026
Mediastinal re-exploration[e]	–	–	1.94 (0.98–3.85)	0.059

BMI body mass index, *BSG* basal serum glucose, *CI* confidence interval, *DSWI* deep sternal wound infection, *EAS* epiaortic ultrasonography scan, *EuroSCORE* European System for Cardiac Operative Risk Evaluation, *IABP* intra-aortic balloon pumping, *OR* odds ratio, *RBCs* packed red blood cells, *SD* standard deviation

[a]Both patients with superficial incisional sternal wound infection and patients with sternal separation without infection were excluded from this analysis

[b]BSG >200 mg/dL at three consecutive measurements

[c]Definitions were those employed for EuroSCORE II (Ref. [28])

[d]Defined as three consecutive cardiac index measurements <2.0 l/min/m2 despite adequate preload, afterload and inotropic support, or IABP

[e]Through resternotomy or subxifoid window

ative and combined, of a new scoring system (the Gatti score) were created to predict DSWI after BITA grafting (Fig. 1). The predictive and the discriminatory power of both models were moderate (Table 4). The preoperative model of the Gatti score was equivalent to the corresponding combined model and the preoperative model of the Society of Thoracic Surgeons risk score (Fig. 2) [24, 27]. It was superior to the sternal wound infection prediction scale [22], the Northern New England Cardiovascular Disease Study Group prediction rule for mediastinitis [21], the additive EuroSCORE [26], the Friedman score [25], and the Alfred Hospital risk index A [23]. The combined model of the Gatti score was superior to the combined model of the Society of Thoracic Surgeons risk score [24], the sternal wound infection prediction scale-revisited [22], and the Alfred Hospital risk index B [23]. All the Gatti score variables remained significant by bootstrap internal validation [27].

5.3 Validation Studies

Although there were significant differences with the Italian original series (Tables 2 and 5) [27], the Gatti score has proven to be effective even in other cohorts of patients, which were the validation samples (Tables 4 and 6) [29–31]. When tested, correspondence between actual and expected DSWI risk was good for low- and high-risk patients [29, 30].

6 Discussion

The Gatti score is a weighted scoring system that was specifically created to predict DSWI risk after BITA grafting. It derives from a consecutive series of nearly 3000 BITA patients who had been operated on at an Italian institution between 1999 and 2013. In these patients, BITA grafts had been used on a routine basis, the rate of BITA use being increased from about 60% in 1999 to over

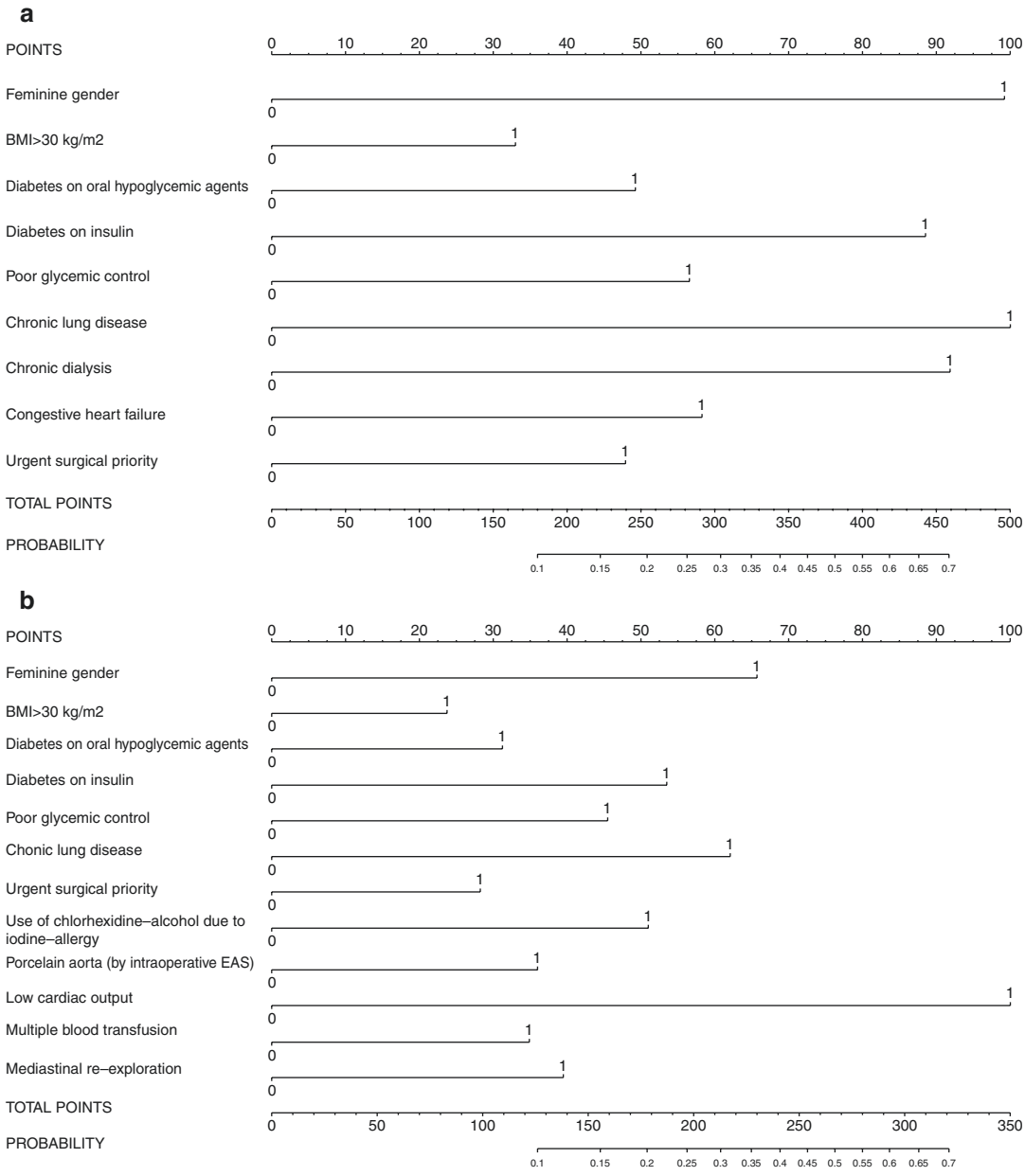

Fig. 1 The Gatti score. (**a**) Preoperative. (**b**) Combined model. Nomogram

80% in the last years. In 4.4% of the cases, any DSWI occurred [27]. It was an expected and frequent postoperative complication. It was more frequent than reported in the cohort of patients who had received single ITA grafts at the same institution during the same period of the study (1.8%). It was more frequent than reported by other authors [1–12, 14–16]. The higher rate of sternal complications of the present series was due to the liberal use of BITA grafts, without any preoperative selection of candidates for left-sided BITA grafting, the high prevalence among the study patients of obesity, diabetes and urgent surgical priority, and the relatively high rate of postoperative

Table 4 The Italian (original and prospective), the French, and the German series: performance of the Gatti score (preoperative and combined)[a]

Risk factor	Italian original series (n = 2872) [27]	Italian prospective series (n = 301) [29]	French series (n = 239) [30]	German series (n = 47) [31]
Preoperative model				
Goodman–Kruskal's coefficient G	0.76	–	–	–
The Hosmer–Lemeshow goodness-of-fit test				
Chi-square	–	–	–	4.8
Degrees of freedom	–	–	–	7
p-value	–	0.61	0.59	0.69
Discriminatory power				
AUC	0.72	0.82	0.78	0.84
95% CI	0.7–0.73	0.72–0.91	0.64–0.92	0.71–0.92
Combined model				
Goodman–Kruskal's coefficient G	0.84	–	–	–
The Hosmer–Lemeshow goodness-of-fit test				
Chi-square	–	–	–	–
Degrees of freedom	–	–	–	–
p-value	–	0.81	0.82	–
Discriminatory power				
AUC	0.73	0.8	0.84	–
95% CI	0.72–0.75	0.71–0.9	0.69–0.98	–

AUC area under the receiver-operating characteristic curve, *CI* confidence interval

[a]Both patients with superficial incisional sternal wound infection and patients with sternal separation without infection were excluded from this analysis

complications such as multiple blood transfusion and mediastinal re-exploration [27].

Two multivariable analysis models were created to examine preoperative factors either alone or in combination with intraoperative and postoperative factors. The female gender, obesity, diabetes, poor glycemic control, chronic lung disease, and urgent surgical priority were the predictors of DSWI common to both models. Both models showed moderate predictive power and accuracy of prediction. The preoperative model performed better than five of six scoring systems for sternal wound infection that were considered [21–27]; the combined model performed better than three considered scoring systems [22–24]. A positive internal validation procedure based on bootstrap was performed for both models [27]. Three validation studies have been performed successfully, though there were significant differences between the Italian original series and each one of the three validation samples [29–31].

Of course, there are some limitations and chances for improvement. Since serum levels of glycated hemoglobin have not been available, preoperatively, for every patient, basal serum glucose >200 mg/dL at three consecutive measurements before surgery was adopted as the marker of poor preoperative glycemic control. The predictive system has to be implemented with the use of preoperative glycated hemoglobin according to internationally agreed guidance [1] and more recent evidence [4]. The analysis from which the score was derived did not evaluate the contribution to DSWI risk of potentially important factors such as causative pathogens, antibiotic prophylaxis, and preoperative patient preparation. The impact of operative methods such as off-pump technique on the risk of DSWI was not analyzed. However, off-pump and beating heart on-pump techniques were adopted only in the presence of a calcified ascending aorta. There is some arbitrariness in the adopted definitions. More studies have to be performed in order to strengthen the evidence of these first external validations.

Despite these limitations, the Gatti score could be a useful tool for the surgeon for decision-

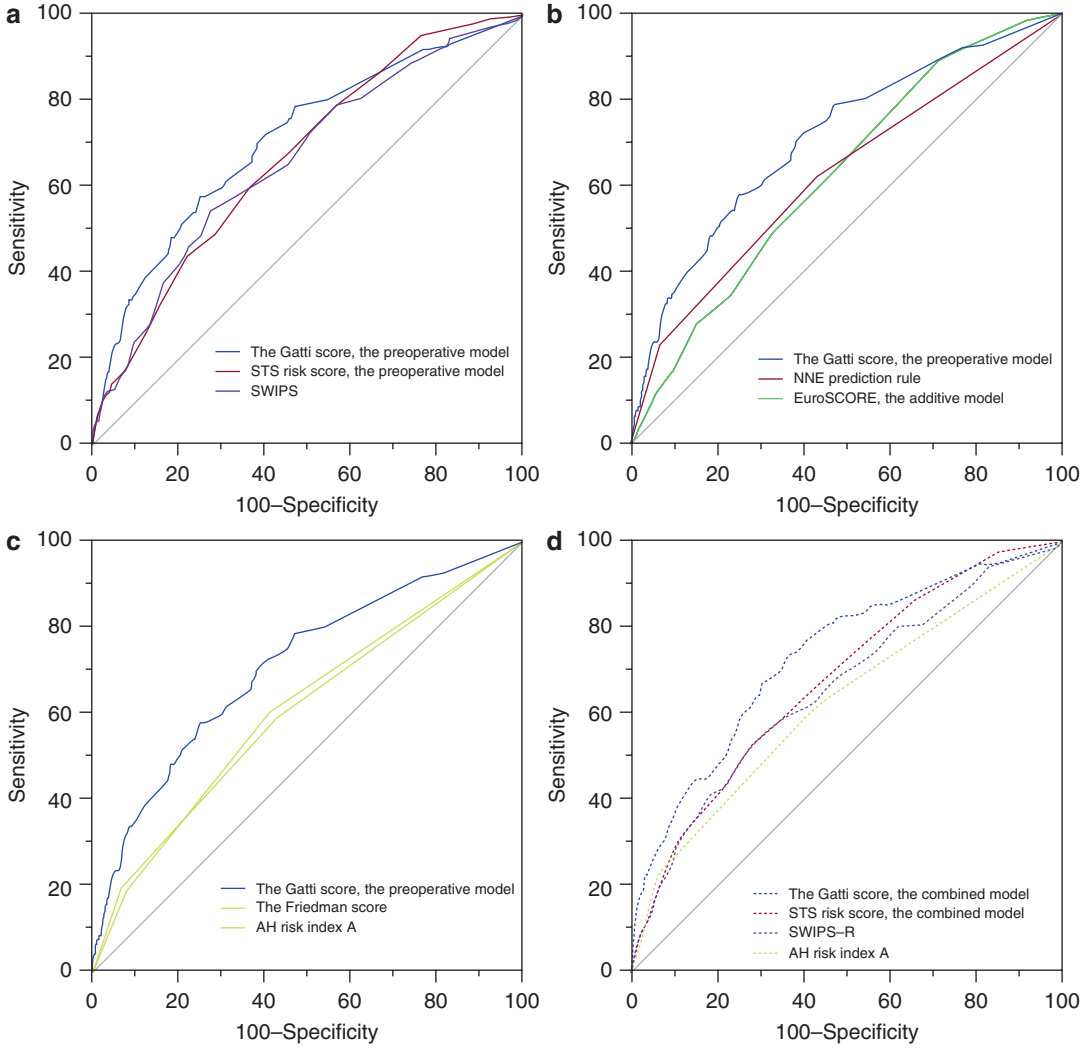

Fig. 2 The new predictive scoring system for DSWI after BITA grafting (the Gatti score, the preoperative model; AUC = 0.72, 95% CI: 0.7–0.73) versus (**a**) STS risk score, the preoperative model (AUC = 0.69, 95% CI: 0.67–0.71; $p = 0.14$) and SWIPS (AUC = 0.65, 95% CI: 0.64–0.67; $p = 0.012$); (**b**) NNE prediction rule for mediastinitis (AUC = 0.65, 95% CI: 0.63–0.67; $p = 0.0046$) and EuroSCORE, the additive model (AUC = 0.62, 95% CI: 0.6–0.64; $p = 0.0007$) and (**c**) the Friedman score (AUC = 0.62, 95% CI: 0.6–0.63; $p = 0.0002$) and AH risk index A (AUC = 0.59, 95% CI: 0.57–0.61; $p < 0.0001$). (**d**) The new predictive scoring system for DSWI after BITA grafting (the Gatti score, the combined model; AUC = 0.73, 95% CI: 0.72–0.75) versus STS risk score, the combined model (AUC = 0.66, 95% CI: 0.64–0.68; $p = 0.002$); SWIPS-R (AUC = 0.64, 95% CI: 0.63–0.66; $p = 0.0012$) and AH risk index B (AUC = 0.6, 95% CI: 0.58–0.61; $p < 0.0001$). *AH* Alfred Hospital, *AUC* area under the receiver-operating characteristic curve, *BITA* bilateral internal thoracic artery, *CI* confidence interval, *DSWI* deep sternal wound infection, *EuroSCORE* the European System for Cardiac Operation Evaluation, *NNE* the Northern New England Cardiovascular Disease Study Group, *STS* the Society of Thoracic Surgeons, *SWIPS(−R)* sternal wound infection prediction scale (−revisited)

making. Actually, there are many ways of using the score by the surgeon for her/his patient, depending on the relative weight in the choice of the following variables: the age of the patient, the depth of surgeon's persuasion about the long-term survival benefits from the use of BITA grafting, the rate of DSWI after CABG surgery at the surgeon's institution, and the percentage of successful treatment. For example, (1) the surgeon persuaded of the long-term survival benefits from BITA

Table 5 The Italian (original and prospective), the French, and the German series: the rates of the Gatti score risk factors for DSWI [39][a, b]

Risk factor	Italian original series (n = 2872) [27]	Italian prospective series (n = 301) [29]	French series (n = 239) [30]	German series (n = 47) [31]
Female gender	441 (15.4)	29 (9.6)	47 (19.7)	3 (6.4)
BMI >30 kg/m^2	572 (19.9)	63 (20.9)	55 (23)	20 (42.6)
Diabetes on oral hypoglycemic agents	638 (22.2)	82 (27.2)	65 (27.2)	4 (8.5)
Diabetes on insulin	204 (7.1)	23 (7.6)	22 (9.2)	3 (6.4)
Poor glycemic control				
BSG >200 mg/dL at three consecutive measurements	135 (4.7)	16 (5.3)	–	–
Glycated hemoglobin ≥6.5%	–	13 (4.3)	67 (28)	4 (8.5)
Chronic lung disease[c]	128 (4.5)	24 (8)	9 (3.8)	0
Chronic dialysis	34 (1.2)	4 (1.3)	3 (1.3)	0
Congestive heart failure	141 (4.9)	15 (5)	9 (3.8)	10 (21.3)
Urgent surgical priority[c]	1711 (59.6)	233 (77.4)	75 (31.4)	21 (44.7)
Use of chlorhexidine–alcohol	56 (1.9)	22 (7.3)	0	0
Diffusely atherosclerotic ascending aorta[d]	220 (7.7)	43 (14.3)	24 (10)[e]	3 (6.4)[e]
Porcelain aorta[d]	168 (5.8)	17 (5.6)	–	–
Postoperative				
Low cardiac output[f]	25 (0.9)	5 (1.7)	0	0
Multiple blood transfusion (>2 RBCs)	315 (11)	28 (9.3)	14 (5.9)	3 (6.4)
Mediastinal re-exploration[g]	136 (4.7)	28 (9.3)	3 (1.3)	3 (6.4)
The Gatti score				
The preoperative model (points)	79.3 ± 65.1	88.2 ± 64.8	81.4 ± 75.9	68.9 ± 58.4
The combined model (points)	59.4 ± 48.1	70 ± 51.1	58.8 ± 53.2	44 ± 32.2

BMI body mass index, *BSG* basal serum glucose, *DSWI* deep sternal wound infection, *EAS* epiaortic ultrasonography scan, *EuroSCORE* European System for Cardiac Operative Risk Evaluation, *IABP* intra-aortic balloon pumping, *RBCs* packed red blood cells, *SD* standard deviation

[a]Both patients with superficial incisional sternal wound infection and patients with sternal separation without infection were excluded from this analysis

[b]Values are number of patients with percentages in brackets or the mean ± SD

[c]Definitions were those employed for EuroSCORE II (Ref. [28])

[d]By intraoperative EAS (Ref. [23])

[e]By intraoperative palpation

[f]Defined as three consecutive cardiac index measurements <2.0 L/min/m^2 despite adequate preload, afterload and inotropic support, or IABP

[g]Through resternotomy or subxifoid window

Table 6 The Italian prospective and the French series: performance of the Gatti score (preoperative and combined)[a]

	Italian prospective series (n = 301) [29]		French series (n = 239) [30]	
DSWI risk [39]	Preoperative	Combined	Preoperative	Combined
Low risk				
Actual (%)	3.8	4.5	2.9	2.4
Expected (%)	<10	<10	<10	<10
Middle risk				
Actual (%)	20	14.3	22.2	27.6
Expected (%)	10–20	10–20	10–20	10–20
High risk				
Actual (%)	50	25	50	33.3
Expected (%)	>20	>20	>20	>20

DSWI deep sternal wound infection

[a]Both patients with superficial incisional sternal wound infection and patients with sternal separation without infection were excluded from this analysis

grafting, but concerned about the risk of DSWI due to the high rate of sternal complications after CABG surgery at her/his institution, would choose the preoperative model of the scoring system in order to exclude from the use of BITA graft patients aged 70 (or 75) or older with an expected risk of DSWI >10%; (2) the surgeon persuaded of the long-term benefits from BITA use, and working at an institution with a low rate of sternal complications, would use the preoperative model of the scoring system to exclude patients aged 70 (or 75) or older with an expected risk of DSWI >15% and every patient with an expected risk of DSWI >20% (regardless of age); (3) the surgeon firmly convinced of the BITA benefits would adopt BITA grafting for every patient regardless of age and use the combined model of the scoring system to identify the high-risk patients for DSWI in order to follow them closely on early after surgery and to perform a more aggressive treatment of superficial wound infections [27].

Conclusions

To limit the risk of sternal wound infection after BITA grafting, three requirements are needed for the surgeon: (1) to know about the risk factors for sternal wound infection of the patient that he/she will operate on, (2) to perform a reasonable preoperative selection of the patients according to these risk factors, and (3) to adopt effective measures of prevention and treatment. It was Dr. Gatti's intention to create a predictive scoring system in order to reduce the rate of DSWI following CABG surgery without giving up too much in the sense of the long-term survival benefits derived from the use of BITA grafts. Dr. Gatti does not presume to assign rigid rules; his intention was to suggest humbly a simple way to perform the selection of the patients.

References

1. Authors/Task Force members, Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, Falk V, Filipptos G, Hamm C, Head SJ, Jüni P, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann FJ, Richter DJ, Schauerte P, Sousa Uva M, Stefanini GG, Taggart DP, Torracca L, Valgimigli M, Wijns W, Witkowski A (2014) 2014 ESC/EACTS Guidelines on myocardial revascularization: the Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS) Developed with the special contribution of the European Association of Percutaneous Cardiovascular Interventions (EAPCI). Eur Heart J 35(37):2541–2619
2. Fuchs U, Zittermann A, Stuettgen B, Groening A, Minami K, Koerfer R (2005) Clinical outcome of patients with deep sternal wound infection managed by vacuum-assisted closure compared to conventional therapy with open packing: a retrospective analysis. Ann Thorac Surg 79:526–531
3. Paul M, Raz A, Leibovici L, Madar H, Holinger R, Rubinovitch B (2007) Sternal wound infection after coronary artery bypass graft surgery: validation of existing risk scores. J Thorac Cardiovasc Surg 133:397–403
4. Gatti G, Perrotti A, Reichart D, Maschietto L, Onorati F, Chocron S, Dalén M, Svenarud P, Faggian G, Santarpino G, Fischlein T, Pappalardo A, Maselli D, Dominici C, Nardella S, Rubino AS, De Feo M, Santini F, Nicolini F, Gherli R, Mariscalco G, Tauriainen T, Kinnunen EM, Ruggieri VG, Saccocci M, Biancari F (2016a) Glycated hemoglobin and risk of sternal wound infection after isolated coronary surgery. Circ J 81(1):36–43
5. Rubens FD, Chen L, Bourke M (2016) Assessment of the association of bilateral internal thoracic artery skeletonization and sternal wound infection after coronary artery bypass grafting. Ann Thorac Surg 101(5):1677–1682
6. Lindblom RP, Lytsy B, Sandström C, Ligata N, Larsson B, Ransjö U, Swenne CL (2015) Outcomes following the implementation of a quality control campaign to decrease sternal wound infections after coronary artery by-pass grafting. BMC Cardiovasc Disord 15:154
7. Lemaignen A, Birgand G, Ghodhbane W, Alkhoder S, Lolom I, Belorgey S, Lescure FX, Armand-Lefevre L, Raffoul R, Dilly MP, Nataf P, Lucet JC (2015) Sternal wound infection after cardiac surgery: incidence and risk factors according to clinical presentation. Clin Microbiol Infect 21(7):674.e11–674.e18
8. Kieser TM, Rose MS, Aluthman U, Montgomery M, Louie T, Belenkie I (2014) Toward zero: deep sternal wound infection after 1001 consecutive coronary artery bypass procedures using arterial grafts: implications for diabetic patients. J Thorac Cardiovasc Surg 148(5):1887–1895
9. Kubota H, Miyata H, Motomura N, Ono M, Takamoto S, Harii K, Oura N, Hirabayashi S, Kyo S (2013) Deep sternal wound infection after cardiac surgery. J Cardiothorac Surg 8:132
10. Taggart DP, Altman DG, Gray AM, Lees B, Gerry S, Benedetto U, Flather M, ART Investigators

(2016) Randomized trial of bilateral versus single internal-thoracic-artery grafts. N Engl J Med 375(26):2540–2549
11. Sá MP, Ferraz PE, Escobar RR, Nunes EO, Lustosa P, Vasconcelos FP, Lima RC (2014) Patency of skeletonized versus pedicled internal thoracic artery in coronary bypass graft surgery: a systematic review, meta-analysis and meta-regression. Int J Surg 12(7):666–672
12. Deo SV, Shah IK, Dunlay SM, Erwin PJ, Locker C, Altarabsheh SE, Boilson BA, Park SJ, Joyce LD (2013) Bilateral internal thoracic artery harvest and deep sternal wound infection in diabetic patients. Ann Thorac Surg 95(3):862–869
13. Mastrobuoni S, Gawad N, Price J, Chan V, Ruel M, Mesana TG, Rubens FD (2012) Use of bilateral internal thoracic artery during coronary artery bypass graft surgery in Canada: the bilateral internal thoracic artery survey. J Thorac Cardiovasc Surg 144(4):874–879
14. Ariyaratnam P, Bland M, Loubani M (2010) Risk factors and mortality associated with deep sternal wound infections following coronary bypass surgery with or without concomitant procedures in a UK population: a basis for a new risk model? Interact Cardiovasc Thorac Surg 11(5):543–546
15. Hemo E, Mohr R, Uretzky G, Katz G, Popovits N, Pevni D, Medalion B (2013) Long-term outcomes of patients with diabetes receiving bilateral internal thoracic artery grafts. J Thorac Cardiovasc Surg 146(3):586–592
16. Dorman MJ, Kurlansky PA, Traad EA, Galbut DL, Zucker M, Ebra G (2012) Bilateral internal mammary artery grafting enhances survival in diabetic patients: a 30-year follow-up of propensity score-matched cohorts. Circulation 126(25):2935–2942
17. Gatti G, Soso P, Dell'Angela L, Maschietto L, Dreas L, Benussi B, Luzzati R, Sinagra G, Pappalardo A (2015) Routine use of bilateral internal thoracic artery grafts for left-sided myocardial revascularization in insulin-dependent diabetic patients: early and long-term outcomes. Eur J Cardiothorac Surg 48(1):115–120
18. Gatti G, Dell'Angela L, Maschietto L, Luzzati R, Sinagra G, Pappalardo A (2016b) The impact of diabetes on early outcomes after routine bilateral internal thoracic artery grafting. Heart Lung Circ 25(8):862–869
19. Gatti G, Benussi B, Bollini M, Forti G, Poletti A, Rauber E, Gabrielli M, De Monte A, Sinagra G, Pappalardo A (2017a) Liberal bilateral internal thoracic artery use in people with diabetes neutralizes the negative impact of insulin-requiring status. J Cardiovasc Med (Hagerstown) 18(8):596–604
20. Gatti G, Maschietto L, Morosin M, Russo M, Benussi B, Forti G, Dreas L, Sinagra G, Pappalardo A (2017b) Routine use of bilateral internal thoracic artery grafting in women: a risk factor analysis for poor outcomes. Cardiovasc Revasc Med 18(1):40–46
21. O'Connor GT, Plume SK, Olmstead EM, Coffin LH, Morton JR, Maloney CT, Nowicki ER, Levy DG, Tryzelaar JF, Hernandez F (1992) Multivariate prediction of in-hospital mortality associated with coronary artery bypass graft surgery. Northern New England Cardiovascular Disease Study Group. Circulation 85(6):2110–2118
22. Hussey LC, Leeper B, Hynan LS (1998) Development of the sternal wound infection prediction scale. Heart Lung 27:326–336
23. Russo PL, Spelman DW (2002) A new surgical-site infection risk index using risk factors identified by multivariate analysis for patients undergoing coronary artery bypass graft surgery. Infect Control Hosp Epidemiol 23:372–376
24. Fowler VG Jr, O'Brien SM, Muhlbaier LH, Corey GR, Ferguson TB, Peterson ED (2005) Clinical predictors of major infections after cardiac surgery. Circulation 112:I358–I365
25. Friedman ND, Bull AL, Russo PL, Leder K, Reid C, Billah B, Marasco S, McBryde E, Richards MJ (2007) An alternative scoring system to predict risk for surgical site infection complicating coronary artery bypass graft surgery. Infect Control Hosp Epidemiol 28:1162–1168
26. Nashef SA, Roques F, Michel P, Gauducheau E, Lemeshow S, Salamon R (1999) European system for cardiac operative risk evaluation (EuroSCORE). Eur J Cardiothorac Surg 16:9–13
27. Gatti G, Dell'Angela L, Barbati G, Benussi B, Forti G, Gabrielli M, Rauber E, Luzzati R, Sinagra G, Pappalardo A (2016c) A predictive scoring system for deep sternal wound infection after bilateral internal thoracic artery grafting. Eur J Cardiothorac Surg 49(3):910–917
28. Nashef SA, Roques F, Sharples LD, Nilsson J, Smith C, Goldstone AR, Lockowandt U (2012) EuroSCORE II. Eur J Cardiothorac Surg 41(4):734–744
29. Gatti G, Barbati G, Luzzati R, Sinagra G, Pappalardo A (2016d) Prospective validation of a predictive scoring system for deep sternal wound infection after routine bilateral internal thoracic artery grafting. Interact Cardiovasc Thorac Surg 22(5):606–611
30. Perrotti A, Gatti G, Dorigo E, Sinagra G, Pappalardo A, Chocron S (2017) Validation of a predictive scoring system for deep sternal wound infection after bilateral internal thoracic artery grafting in a cohort of French patients. Surg Infect 18(2):18–18
31. Gatti G, Ledwon M, Gazdag L, Cuomo F, Pappalardo A, Fischlein T, Santarpino G (2018) Management of closed sternal incision after bilateral internal thoracic artery grafting with a single-use negative pressure system. Surg. doi: 10.1007/s13304-018-0515-7
32. Sauvage LR, Wu HD, Kowalsky TE, Davis CC, Smith JC, Rittenhouse EA, Hall DG, Mansfield PB, Mathisen SR, Usui Y (1986) Healing basis and surgical techniques for complete revascularization of the left ventricle using only the internal mammary arteries. Ann Thorac Surg 42(4):449–465
33. Zingone B, Rauber E, Gatti G, Pappalardo A, Benussi B, Dreas L, Lattuada L (2006) The impact of epiaortic ultrasonographic scanning on the risk of perioperative stroke. Eur J Cardiothorac Surg 29(5):720–728

34. Erdinc M, Ocal A, Sezer H, Kuzgun A, Ozturk C (1996) Double-loop sternal wiring technique. Ann Thorac Surg 62(1):320–321
35. Sutherland RD, Martinez HE, Guynes WA (1981) A rapid, secure method of sternal closure. Cardiovasc Dis 8(1):54–55
36. Atkins BZ, Wooten MK, Kistler J, Hurley K, Hughes GC, Wolfe WG (2009) Does negative pressure wound therapy have a role in preventing poststernotomy wound complications? Surg Innov 16(2):140–146
37. Furnary AP, Zerr KJ, Grunkemeier GL, Starr A (1999) Continuous intravenous insulin infusion reduces the incidence of deep sternal wound infection in diabetic patients after cardiac surgical procedures. Ann Thorac Surg 67(2):352–362
38. Gatti G, Pappalardo A, Gon L, Zingone B (2006) Protecting the crossover right internal thoracic artery bypass graft with a pedicled thymus flap. Ann Thorac Surg 82(5):1919–1921
39. Berríos-Torres SI, Umscheid CA, Bratzler DW, Leas B, Stone EC, Kelz RR, Reinke CE, Morgan S, Solomkin JS, Mazuski JE, Dellinger EP, Itani KMF, Berbari EF, Segreti J, Parvizi J, Blanchard J, Allen G, Kluytmans JAJW, Donlan R, Schecter WP; Healthcare Infection Control Practices Advisory Committee (2017) Centers for Disease Control and Prevention Guideline for the Prevention of Surgical Site Infection. http:www.amanetwork.com/journals/jamasurgery/fullarticle/2623725 Accessed 23 Jul 2017
40. Harrell FE (2001) Regression modeling strategies: with applications to linear models, logistic regression, and survival analysis. Springer, New York, pp 1–522
41. Haley R (1993) Measuring the intrinsic risk of wound infection in surgical patients. Probl Gen Surg 10:396–417
42. Swets JA (1988) Measuring the accuracy of diagnostic systems. Science 240(4857):1285–1293
43. DeLong ER, DeLong DM, Clarke-Pearson DL (1988) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44(3):837–845

Negative-Pressure Wound Therapy in Vascular Surgery

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1 Introduction

Atherosclerosis is one of the major problems in modern medicine. Mostly revealed in older population however, patients with positive genetic background, heavy smokers and drinkers, and diabetic patients are some of the most susceptible groups presenting the disease earlier. Besides coronary disease, other main clinical presentations are aortic, carotid, and peripheral vascular pathology; insidious, progressive diseases eventually causing invalidity; limb or tissue loss; and death. Final therapy after prevention and medical intervention is surgical, weighted due to tissue fragility and disrupted by patient general condition, older age, obesity, and smoking consumption. By virtue of technological improvements, modern vascular surgery becomes less invasive; however, invasive procedures are still not eradicated. A significant number of vascular patients are treated with invasive, extensive procedures, facilitated with various implants. Occlusive vascular disease, diabetes, but also immobility are inducing occurrence of peripheral ulcers.

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Extensive surgery and peripheral ulcers are the main cause of tissue damage and wound appearances in vascular patients, while comorbid conditions are supporting slow and insufficient healing. For these reasons vascular surgeons, and other physicians treating vascular patients, are looking forward to any improvements in wound healing, and negative-pressure wound therapy (NPWT) is one of the methods frequently used. Besides complex wounds caused by vascular disease, we are frequently facing complex problems caused by surgical site infection (SSI) after vascular procedures. Prolonged preoperative hospital stay, obesity, female sex, malnutrition, and malignant, renal, and peripheral occlusive disease are some of the risk factors for SSI [1–4]. In this chapter we would like to describe different, most frequent, indications for NPWT in vascular surgery or vascular patients. We are discussing challenges of graft infection, abdominal compartment syndrome (ACS), pedal ulcers, amputation stump complications, and some other complex wounds. Besides the literature data, experience and results from our own practice are presented [5].

2 Vascular Graft Infection

"God may very well be a surgeon, but one would be wise to remember that the converse cannot be true."

In what now would be regarded as the early days of vascular surgery, Shaw [6] said that arterial

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graft infections would "lead to loss of the patient or a large part of him." Historically, when aortic grafts were first introduced into common clinical in the 1960s, conservative treatment was the primary therapy for aortic graft infection, but it soon became evident that it was difficult, if not impossible, to eradicate infection with the graft material and antibiotics available at that time. Resection of the infected graft, followed by either extra-anatomic bypass or in-line replacement, became the mainstay of therapy. The incidence of vascular prosthetic graft infection now varies from 0.6% to 5%[2], with a limb amputation rate from 5% to 25% and a mortality rate from 25% to 88% [7–10].

In the last period of the twenty-first century, significant advances have been made in managing patients who have problems associated with graft infections, in the field of diagnosis and treatment.

Successful diagnosis depends upon a high index of clinical suspicion and aggressive workup of the problem when graft infection is suspected. The clinical picture is often rather unimpressive, reflecting the indolent nature of many late infections that may exist in virtual symbiosis with the patient and present many months to years after graft implantation. Current imaging modalities to diagnose prosthetic graft infection are ultrasound, computed tomography (CT) scanning, and magnetic resonance imaging (MRI). Relatively new techniques are fluorodeoxyglucose-positron emission tomography (FDG-PET) and single positron emission computer tomography (SPECT) with accurate visualization of increased metabolic activity, especially when combined with CT or MRI [6]. Some relatively new nuclear diagnostic developments may have added value in the detection of vascular prosthetic graft infection. More specific tracers are currently being developed, for example, 18F–folate, 18F–choline, 99 m–Technetium-labeled interleukin-8 or interleukin-2, and gallium-68, which act on the local immune response and can all be used in PET or SPECT imaging [11–13]. However, the precise added value in the detection of vascular prosthetic graft infection is also still unclear [14].

The surgical treatment of an infected aortic graft is challenging and involves a major intervention in patients who are often critically ill. Surgical treatment is based on two major principles: The first focuses on eradication of the infection and its sequel, which means an extensive debridement of all infected tissue, including part of the graft. The second principle focuses on maintaining or restoring perfusion to the lower limbs. Traditionally, the lower limb is reperfused by placing an extra-anatomic bypass through a non-infected part of the body. An alternative approach reported earlier or the so-called NAIS (neoaortic iliac system operation) is that of in situ replacement of infected aortic prosthesis with venous grafts (deep and superficial lower extremity veins) [15].

Still, an unanswered question that remains involves decision when to proceed with conservative treatment. As the population ages and the number of aortic grafts placed increases, even with prophylactic antibiotics and low infection rates, there will always be a small percentage of patients who develop aortic graft infections. Antibiotic therapy for established graft infection should always include parenteral, culture-specific drug therapy bactericidal to cultured or suspected organisms. Because the preferred bacteria mode of growth is as a biofilm, antibiotics capable of biofilm penetration and eradicating of slow-growing organisms are recommended. Local delivery of antibiotics to the surgical site in the form of antibiotic-impregnated beads or an antibiotic-impregnated vascular prosthesis has also been shown to be of value [16, 17].

Groin is the most frequent surgical approach in both open and endovascular surgery. Consequently the vast majority of vascular SSI are in this region. The classification of SSI in vascular surgery was made by Szilagyi [18]. In the presence of synthetic graft groin infection, especially early and deep groin (Szilagy grade III), may be a limb- and/or life-threatening wound infection. Since general condition of vascular patients does not always allow extensive radical procedure, described above, NPWT came out as a "second best primary used" solution. There are numerous literature data reporting experience in vascular SSI treated with VAC therapy including one RCT [19–28]. Outcome measures used were graft preservation (>80%), wound healing (20–50 days), and cost-

effectiveness (more than alginate dressing), while the most important side effect reported was major bleeding (<10%). Low rate of side effect and high rate of success support nowadays practice to use NPWT as a first option in patient with groin early and deep SSI. Still one should be aware of treatment failure that might occur probably even more frequently since reported experience might be biased by positive reporting. In patients who have acceptable risk for aggressive therapy on one side and their groin infection has negative predictors for conservative therapy (anastomosis is not intact, systemic signs of sepsis with aggressive offending organism like *Pseudomonas aeruginosa*), then NPWT might be loss of time. In all other circumstances, NPWT should be attempted.

Treatment of early deep groin SSI primarily demands aggressive debridement. Due to its off-label use, application of VAC system, in groin wound with exposed synthetic graft, is not standardized. The main problems are ingrowth, adherence to wound, and major bleeding from infected graft and anastomosis. Prevention may be achieved by an application of a nonadhesive silicone-based dressing before putting on polyurethane foam. Another described technique is the usage of two layers (polyvinyl alcohol and polyurethane) foams. In our experience major bleeding occurred in patients with severe groin infection where vein to graft anastomosis was exposed [5]. Negative pressure mostly used is continuous regime 125 mmHg; however, there is no data to prevent using lower intensity. NPWT is even more frequently used in superficial groin infection with more success. It provides faster wound healing and less hospitalization stay and saves time and costs for frequent dressings. In the last 7 years at our institution, 35 patients with early deep groin infection with exposed synthetic graft were treated. Major bleeding occurred in one patient; it was not possible to save graft in seven (20%) patients, while all other wounds healed until secondary suture. During follow-up two patients had reinfection and were treated with radical treatment. In Fig. 1 one of the patients is presented.

Improvements in treatment of such complex condition with NPWT might be reached with usage of new devices that are providing instillation of antibiotics and other antiseptic solution used to rinse the wound during NPWT. Another new procedure is named as Endovac [29]. It considers implantation of the stent graft inside the infected graft and then NPWT upon removal of infected prosthesis. Advantages are no need for clamping, scar tissue dissection, and no fragile anastomosis is left in infection zone. Disadvantage is that it is equivalent of "in situ" reconstruction.

3 Complex Wounds in Vascular Surgery (Laparotomy and Thoracolumbotomy)

In the era of development of aortic endovascular techniques, open repair still has its place in modern vascular surgery. Due to certain anatomical limits

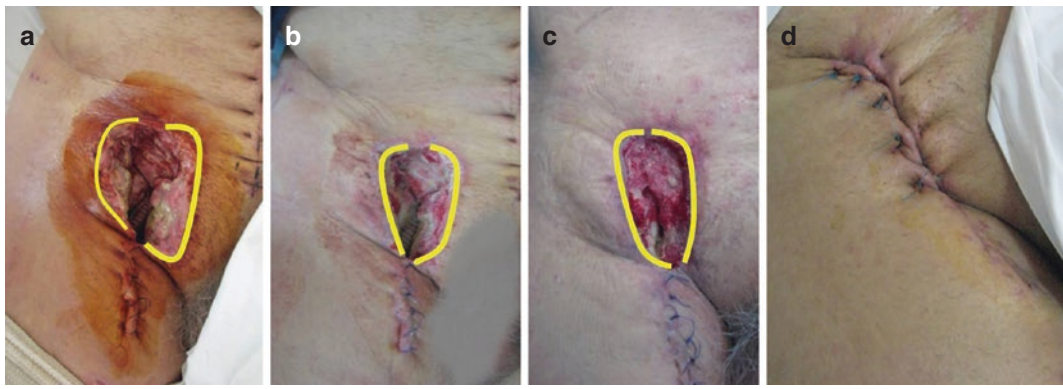

Fig. 1 (**a–c**) Treatment of synthetic graft infection in the groin. PTFE graft is exposed and wound is retracted with five dressings (15 days). (**d**) Finally secondary suture was possible [5]

that are preventing more extensive usage of endovascular techniques, open repair is nowadays used in more complex pathology requiring extensive procedures. Some of them are extending beyond abdominal cavity demanding opening thoracic department as well. Complex and long procedure and extensive incisions in fragile devastating atherosclerotic patients are contributing to wound dehiscence and infection in such patients. Even worse their general condition might prevent radical treatment of these complications, and NPWT has emerged as a savior in such a situations.

3.1 Laparotomy Wounds

In the published literature there are a lot of articles pertaining to the method of access during open abdominal aortic surgery, advising midline laparotomy comparing to the left retroperitoneal approach for infrarenal AAA repair, that although with fewer cardiopulmonary complications and shorter hospital stay, has more unsatisfactory postoperative wound complications. In high-experience centers, transabdominal approach because of its advantages is the method of choice, while retroperitoneal approach is reserved for specific pathological entities [30–32].

The classification system presented by the Centers for Disease Control and Prevention (CDC) broadly categorizes surgical site infection (SSI) into incisional or organ-space infections, with incisional infections further subdivided into superficial and deep [33]. Data on the rate of complications such as infection laparotomy wounds after aortic surgery are described in the literature up to 5% [34]. SSI after arterial intervention is a common nosocomial vascular complication and an important cause of postoperative morbidity and mortality. SSI leads to a significant increase in hospital stay, ICU admission, long-term surgical site complications, patient suffering, readmission, cost, and death [1, 35]. The traditional approach to treating SSI involves daily or more frequent dressing changes, sometimes involving packing of a wound cavity. There are a number of different dressing options, from simple dressings—such as nonadherent dressings—to more modern options such as foam, hydrocolloid, and alginate dressings. A Cochrane systematic review found 13 randomized controlled trials comparing dressings and topical treatments for SSI [36]. All of the trials were over 10 years old, small, and of poor quality. There was no evidence that the choice of dressing or topical treatment had any impact on healing rates. Given the potential complexity, size, and longevity of SSI, interest in alternative treatment options grows [37]. Even though we can see very big progress in the area of technique of closing laparotomy wounds lately, the risk of infections, dehiscence, and ventral hernia after an aortic surgery still remains high. Some of the risk factors contributing to the occurrence of wound infection are obesity, inadequate care, hematoma, lymphorea, and reoperations [38].

NPWT is an established mode of therapy after vascular procedures and in many centers is the method of choice [39]. It has been used successfully to manage many different high-risk surgical incisions including complex abdominal closures with good results [40]. Negative pressure at the incision surely decreases the likelihood of fluid accumulation; however, it is just one of several mechanisms of action of NPWT. In addition, it improves wound base perfusion and oxygenation, which may decrease SSI risk. The target value of negative pressure in laparotomy wound is 125 mmHg, while the time interval between the dressings is 3–5 days. Further replacement depends on condition of wound and its edges (Figs. 1 and 2). With adequate debridement and local processing before the application of the system for negative pressure, average duration of therapy can be significantly reduced.

The first time that NPWT was used in treatment of laparotomy wounds in our clinic was in 2009. Since then, 3500 patients were treated with an open aortic surgery, and 175 (2.5%) had wound infection of any levels. In 55 patients we applied NPWT, and the success and secondary suture was 100%. The infection rate of 2.5% can be explained with substantial number (750) of emergency procedures which was an independent predictor of wound infection. The average length of treatment was 2 weeks since we used NPWT in more extensive wound infection frequently localized in both groin and laparotomy wound. Most frequently indication for NPWT

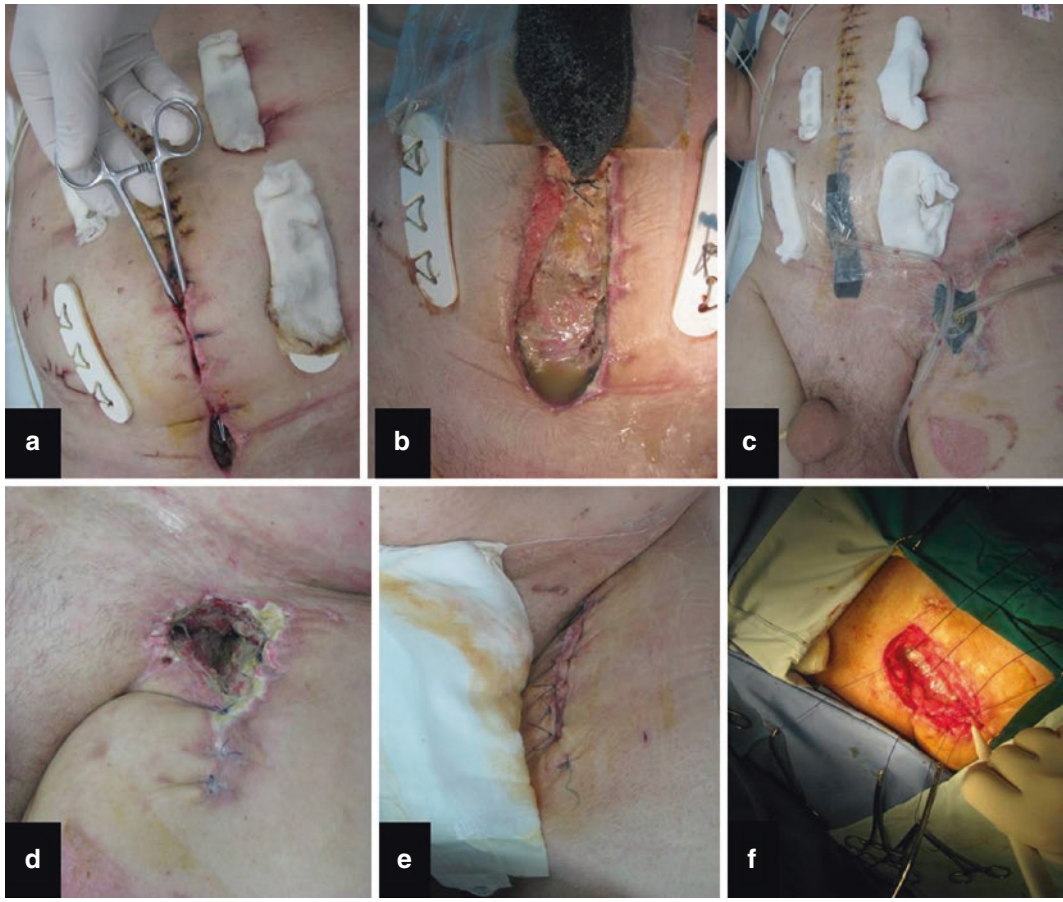

Fig. 2 (**a–c**) Concomitant infections of the laparotomy and groin wound in a 125 kg weight patient treated due to ruptured abdominal aortic aneurysm. Extensive secretion from both wounds required multiple daily dressings (more than 15 per day); patient was unmotivated in the ICU. (**d**) VAC dressing was applied to both wounds. (**e, f**) With only three dressings per week, patient was motivated, verticalized, and transmitted to semi-intensive care unit [2]

was extensive wound secretion. Once infection in other remote parts of the same laparotomy wound is suspected, we considered removing all sutures and openly connecting the two infected segments avoiding any tunneling in the wound. In our initial experience, tunneling rarely provides adequate wound healing and usually ends with complete wound opening (especially in patients with thick layer of adipose tissue). In Fig. 2 a patient with concomitant infection of the laparotomy and groin wound is presented.

3.2 Thoracolumbotomy Wounds

Standard approach for open thoracoabdominal aneurysm repair is thoracolumbotomy by partially opening the diaphragm as well. Such an extensive approach makes the early postoperative recovery potentially complicated. Long surgical incision, partially located at the patients' back side, resection, or damage of one or more ribs, sternum, and intercostal muscles and diaphragm are compromising breading, coughing, and exercise. Similar is if complications related to this surgery (prolonged mechanical ventilation, acute or delayed paraplegia, and cerebral ischemia) occur. In obese or in female patients, fat subcutaneous adipose tissue is prone to necrosis, while exclusion of most intercostal arteries is compromising perfusion of this region. Once infection and dehiscence occurs, they deteriorate pulmonary function and induce pleural effusion, atelectasis, pleural empyema, or even mediastinitis.

On the other side, primary pleural effusion or empyema might be the reason for wound infection. Both pathways are possible with the same eventual outcome leading to patient state deterioration and finally sepsis when multi-organ failure is experienced. Therefore, any patient with a thoracolumbotomy wound infection should undergo chest CT examination.

The closure and care of these wounds is very important for adequate postoperative recovery and survival. The literature data related to this topic is scarce. The experience of our clinic shows that aggressive treatment of infections at the beginning is extremely important. In addition to surgical treatment in the form of debridement, incision, and curettage, adequate local treatment is of great importance for the complete healing of the wound. Considering the size of the wound after open thoracoabdominal aneurysm's surgery and its very potential secretion, classic local treatment such as dressing and application of hydrocolloid solution usually remains without success. In addition it requires dressing several times a day that is time-consuming, costly, and discomforting for patient. The use of NPWT has been very useful in our experience.

As in the treatment of laparotomy wound, we used the same principle of pressure with a slightly longer duration of therapy. Sometimes we have a partial wound infection in two parts that are not connected, and then application system for NPWT often is not easy. In such wounds, it is advised to use a "bridging" technique or separate application using ""Y" connection. Of the 95 patients who have been subjected to an open surgical treatment of thoracoabdominal aneurysm in our clinic in the period 2009–2015, 7 of them (7.36%) were treated with NPWT, and 6 of these 7 were successfully secondary closed. The average duration of the therapy was 14 days (7–24 days).

In recent years, the indication for NPWT has been extended to include treatment of closed surgical incisions. Two simplified NPWT devices became commercially available in 2010 (Prevena™; KCI) and 2011 (PICO™; Smith & Nephew, Hull, UK). These NPWT devices consist of a single-use battery-powered negative-pressure therapy device, an easy-to-place dressing, and either a very small and easily portable canister or no canister at all. In the latter case, the liquid is removed by evaporation through a semipermeable dressing. The mechanisms of action of this closed incision management have been supported by biomechanical studies: increased blood flow, decreased lateral and shear stress at the suture lines with decreased risk of wound dehiscence, and increased lymph clearance with reduced formation of hematoma or seroma [41]. Also, given the significant percentage of complications of wounds after an open thoracoabdominal aneurysm repair, obvious conclusion is the more liberal use of NPWT for closed surgical incision**.**

Negative pressure of 125 mmHg is also recommended for treatment of closed surgical incisions. Initial application is immediately intraoperatively, after placement of skin sutures, and interval time to redressing is 7 days. Given that the average duration of treatment of these wounds with NPWT is relatively long, especially in thoracolumbotomy wounds where it is about 20 days, preventive use of these systems for treatment of closed surgical incisions (e.g., Prevena™, KCI, or PICO™, Smith & Nephew, Hull) could be very effective—future studies will show (Fig. 3).

3.3 Fasciotomy Wounds

Decompressive fasciotomy is frequently used in vascular surgery especially in vascular trauma or after late reperfusion. NPWT is rarely reported in such patients; however, it was shown that NPWT improves wound reduction, reduces dressing changes, and shortens wound closure time and hospital stay [42]. In our experience fasciotomy wound reduction is accomplished in a short period of time, while treatment of one young patient with combined vascular and orthopedic injury complicated with knee edema and infection usage of NPWT in both wounds reduced severe edema and improved leg mobility, patient comfort, and recovery that finally reduced hospital stay and antibiotic therapy of this patient bringing him faster to physical therapy (Figs. 4 and 5).

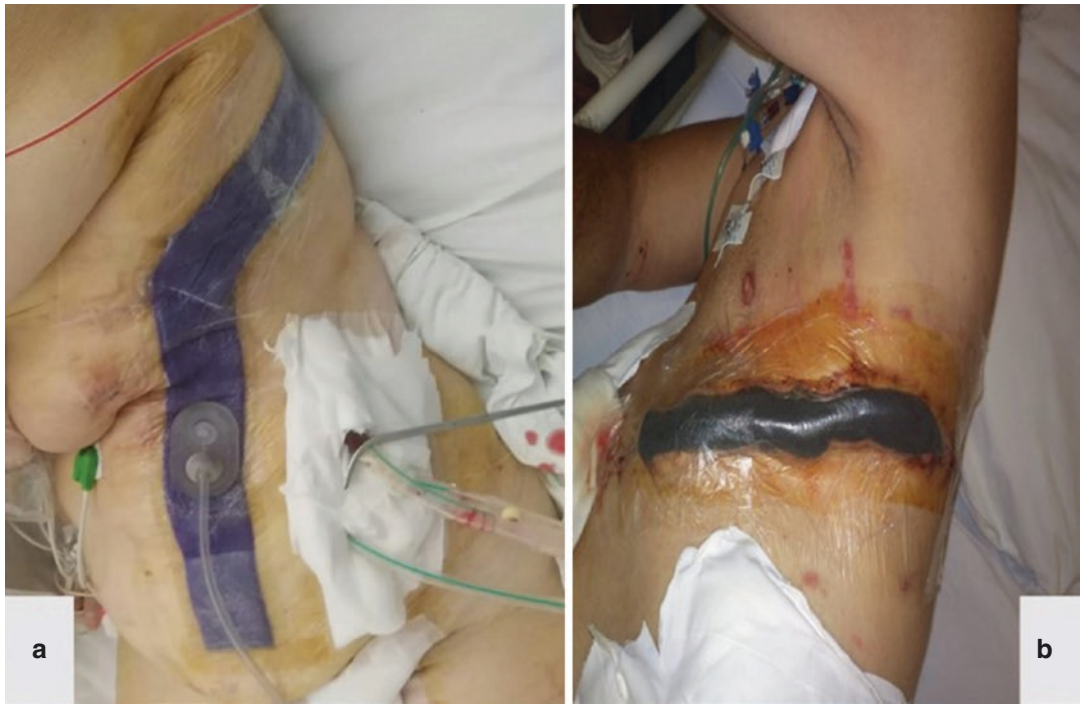

Fig. 3 (**a**) Prevention of SSI in patient with thoracolumbotomy with Prevena system (**b**) Treatment of SSI of thoracolumbotomy with VAC system

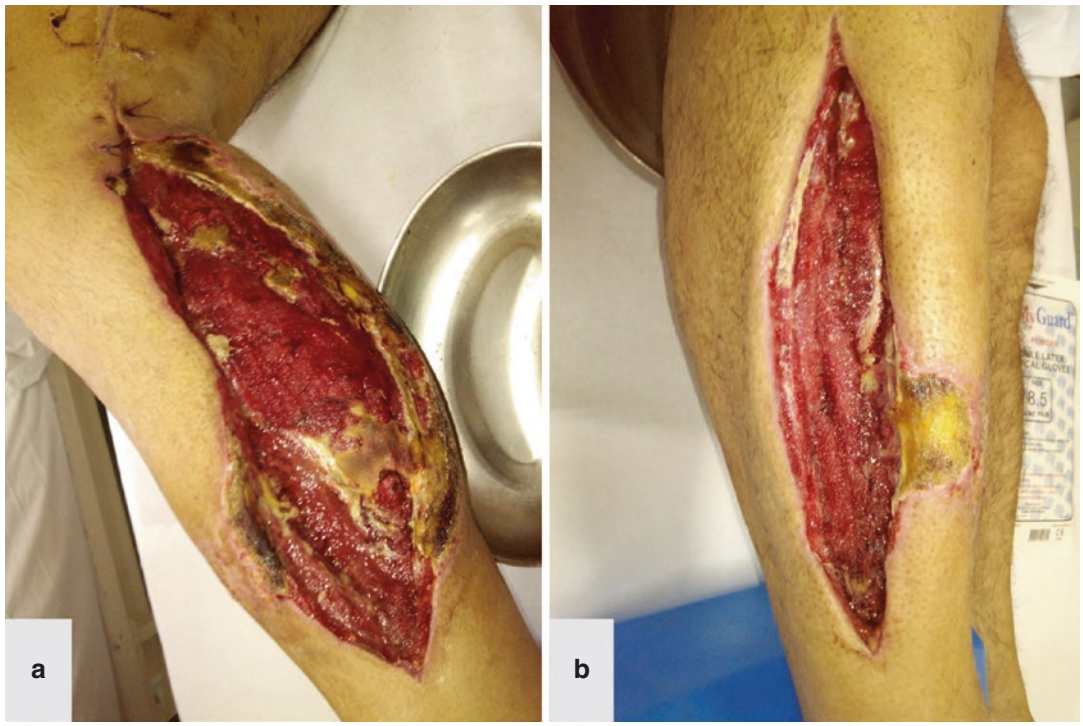

Fig. 4 (**a**) Fasciotomy wound after late revascularization. (**b**) Reduced wound after 15 days (five dressings) of NPWT

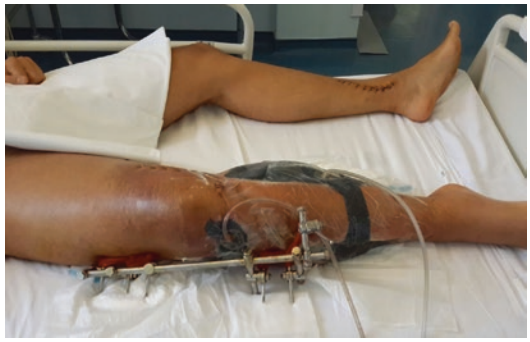

Fig. 5 Young patient with complex trauma and both vascular and bone injury. Medial and lateral fasciotomy were infected, and there were knee edema and signs of infection. NPWT with VAC system was applied in all three wounds and connected into one system

3.4 Wound after Amputation in Vascular Patient

3.4.1 Pedal Ulcers

Pedal/ft ulcers may have different etiologies. They can be caused by electrical, chemical, or radiation burns, or they can be due to malignancy, collagen vascular diseases, untreated osteomyelitis, as well as active Charcot disease [43]. Nevertheless, the most common cause of foot ulcer in "modern era" is diabetes mellitus (DM). The increase in the worldwide prevalence of DM has resulted in a dramatic rise in DM-related complications, such as diabetic foot ulcers. Between 45 and 60% of diabetic foot ulcers are caused by neuropathy, while around 10% have ischemic etiology. These two mechanisms in different ratio are the main initiating factors of infection [44, 45]. Around 25% of diabetic patients have a foot ulcer which leads to the infection development in 50% of patients. Consequently, infected ulcer results in amputation at around 20% of patients. The presence of infection is defined by ≥2 classic findings of inflammation or purulence. Infections are then classified into mild (superficial and limited in size and depth), moderate (deeper or more extensive), or severe (accompanied by systemic signs or metabolic perturbations) [46]. Vascular patients with peripheral arterial or vein insufficiency are frequently diagnosed with already developed chronic wounds. With an exception of DM, ulcers might be caused by ischemia and infection alone. Some of these wounds are requiring amputation after successful revascularization in the field of already damaged tissue.

Unfortunately, it is often the case that the patients reports on medical examination with very advanced local findings, such as infected foot ulcers and different types of phlegmonous/gangrenous processes those requiring amputations on different levels (debridement, finger amputation, metatarsal bone disarticulations, etc.). Upon removal of necrotic tissue, different kinds of wound treatments have been described in the literature: advanced moist wound therapy (AMWT), bioengineered tissue or skin substitutes, growth factors, electric stimulation, and NPWT [46–49].

NPWT is used to accelerate healing and to simplify local ulcer therapy. This kind of therapy promotes wound healing by delayed primary or secondary closure or as a preparation for skin transplant. The prerequisite for optimal effect for foot ulcer healing is oxygen/blood perfusion. In this regard in patient with foot ulcer, blood perfusion should be assessed by ankle-brachial pressure index and MDCT angiography, and if necessary revascularization should be performed. In a multicenter, randomized trial published from Armstrong and Lavery in 2005 [50], inclusion criteria for VAC therapy were partial foot amputation wounds up to the transmetatarsal level and evidence of adequate perfusion (TcPO2 ≥50 mmHg or a toe pressure ≥30 mmHg). In this trial they concluded that treatment with NPWT resulted in a higher proportion of wounds that healed, faster healing rates, and potentially fewer re-amputations than with standard treatment, in comparison with standard moist wound care.

There are few postulates of proper application of VAC system on foot ulcers. First of all, before the application it is necessary to remove all necrotic masses and to open all potentially infected compartments ensuring their drainage. If this fails, there is a high risk for infection progression along the muscle fascias and tendons. Further, a target value of negative pressure of 125 mmHg can be reduced to 50–75 mmHg in

case of pain development or worsening of wound perfusion. After first application, the initial cycle is 24–48 h, and further dynamics of system replacement and wound evaluation depends on local wound condition (no less than three times/week). Because of anatomical foot characteristics and ulcer localization, NPWT system application is not always easy. The biggest "technical" problem represents ulcers on plantar side of foot, interdigital spaces, toes, metatarsal bends, and a curvature of heel region (Fig. 1). Because of that, for enumerated types of wounds, it is recommended to use special dedicated foams for these particular locations or to use a "bridging" technique to ensure that additional pressure is not applied as a consequence of the placement of the tubing and/or SensaT.R.A.C.™/SensaT.R.A.C.™ pad. This involves using foam to allow placement of the SensaT.R.A.C.™/SensaT.R.A.C.™ pad or tubing on the dorsum of the foot [51].

Regardless of the revolutionary breakthrough in the treatment of foot ulcerations thanks to implementation of NPWT, for its success, the key is good patient selection, maximal revascularization if it is possible, and combining with other treatment modalities. Furthermore, by the gradual introduction into clinical practice, every clinician should striving to the individual defining of indication area in accordance with the group of patients under the own circumstances, including and financial constraints. Special benefit of the VAC therapy is the possibility of treatment at home with regular checks and wound evaluation on an outpatient basis. The last mentioned, together with mobility and evident better patient motivation because of reduced number of daily dressings and improved hygienic conditions, contributes to improving patients' quality of life.

3.4.2 Amputation Stump

Literature data regarding the NPWT in patients with major amputations, including below and above knee, are scarce. Almost, all until now published papers represent individual case reports or small case series. However, NPWT provided to be effective in stump healing due to residual hematomas, infections, dehiscence caused by chronic venous insufficiency, and residual wounds incurred during the prosthetic rehabilitation [51, 52].

In real circumstances of everyday vascular practice, infection and malperfusion after limb amputation are common findings in patients with occluded aortoiliac segment, as well as in patients with previous vascular surgery. These patients are frequently found after synthetic graft infection in the groin when extirpation is necessary without an option to restore flow. Also, in such patients, there is often no place for proximal amputations, and because of that the first next option is hip disarticulation.

In all of these situations, NPWT might be used to reduce the number of weekly dressings, improve perfusion, provide wound healing at the most distal level, and consequently improve survival. With VAC therapy, patients avoid uncomfortable and annoying everyday dressings.

Some of the extensive wounds after major or pedal amputation treated with NPWT cannot be reconstructed by conventional surgical methods such as secondary suture. In these situations, after adequate conditioning of wounds thanks to NPWT, it is necessary to perform a different kind of definitive plastic reconstruction using skin and muscle flaps (Fig. 6).

4 Compartment Syndrome After Aortic Surgery

Abdominal compartment syndrome (ACS) originates as consequence of increased intra-abdominal pressure (IAP) and causes potentially lethal multiple organ failure. Although it is known for more than a century that elevated IAP might lead to renal, respiratory, or cardiovascular impairment, the term ACS was first introduced in 1984 by Kron [53] who described that entity in patients that underwent to the aortoiliac surgery. ACS is a well-known complication of the aortic surgery, most frequently seen after open (OR) and endovascular repair (EVAR) of ruptured abdominal aortic aneurysm (RAAA). Retroperitoneal hematoma and intestinal edema are the main causes of raised IAP in these cases. Having in mind very high mortality, of up to

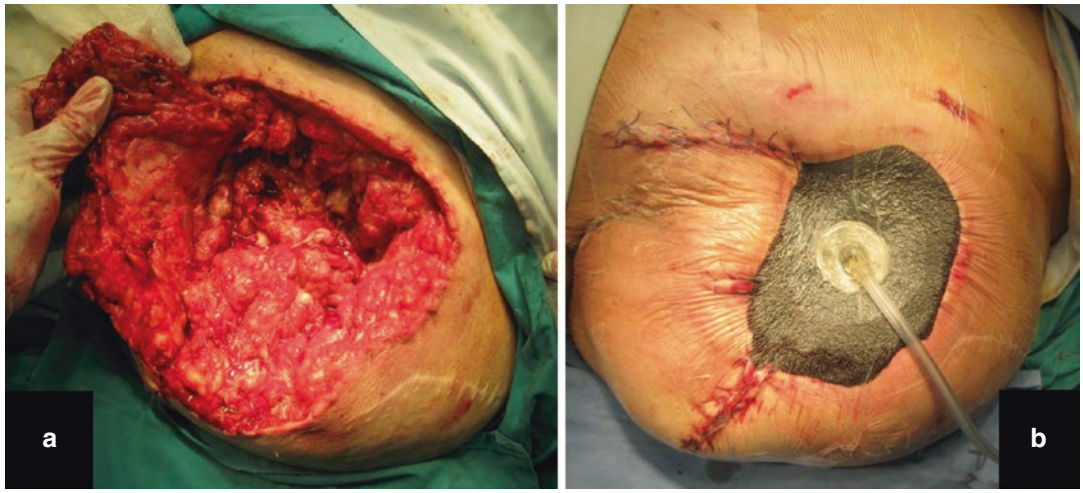

Fig. 6 (**a**) In aggressive and radical hip disarticulation on the ischemic field, VAC dressings are used in order to evacuate seroma from the operative wound and to increase perfusion of ischemic tissue. (**b**) After a week with VAC treatment, secondary suture could be safer to perform [5]

82%, among patients treated due to RAAA and underwent reoperation for ACS, education about management and treatment of ACS is of utmost importance.

The updated definition of ACS was published by World Society of the Abdominal Compartment Syndrome (WSACS) in 2013: intra-abdominal hypertension (IAH) is defined as a sustained or repeated pathological elevation in IAP >12 mmHg [54]. Also, ACS is defined as a sustained IAP >20 mmHg (with or without an abdominal perfusion pressure <60 mmHg) that is associated with new organ dysfunction or failure. Abdominal perfusion pressure (APP) represents the difference between the mean arterial pressure (MAP) and IAP. The previous subdefinition of ACS as an APP lesser than 60 mmHg was changed in the updated WSACS document because the evidence for this subdefinition was too weak. However, this subdefinition of ACS shouldn't be ruled out definitely because some patients with hypotension (MAP is lesser) and an intermediate IAH (15–19 mmHg) might develop organ dysfunction or failure and may have benefit from abdominal decompression. Based on values of intra-abdominal pressure, intra-abdominal hypertension is divided to grade I (IAP 12–15 mHg), grade II (IAP 16–20 mmHg), grade III (21–25 mmHg), and grade IV (greater than 25 mmHg).

ACS can cause a failure of different organ systems. The increased IAP reduces venous flow of the various intraperitoneal organs causing bowel ischemia and thus gut bacterial translocation. Furthermore, venous stasis increases the risk of deep vein thrombosis and pulmonary embolism. IAH can cause complications in distal organs and systems: it reduces total lung capacity and functional residual capacity leading to compressive basal atelectasis, ventilation dysfunction, hypoxemia, and hypercapnia; increases risk of ventilator-induced lung injuries, encephalopathy, oliguria, and elevation of serum creatinine level; and reduces cardiac output.

Bjorck et al. [55] reported that the incidence of IAH >20 mmHg after RAAA was about 50% after OR and approximately 20% after EVAR on stable patients. The same incidence of ACS after rEVAR was published in a study from the Zürich group [39, 56]. Also, Mehta published that endovascular repair of RAAA in 20% of cases was complicated with ACS requiring decompression. Intraoperative risk factors for developing of grade III or IV IAH during OR of RAAA include a longer cross-clamping time, increased intraoperative blood loss, and increased operative time [57]. On the other side, the Albany group documented risk factors during endovascular repair of RAAA: the need for an aortic balloon occlusion, the presence

of severe coagulopathy, massive transfusion requirements, and the emergent conversion of modular bifurcated stent grafts to aorto-uni-iliac devices. The latter was due to ongoing hemodynamic instability and inability to expeditiously cannulate the contralateral limb of the endograft. However, a systematic review, published by Karkos et al. [58], did not find significant association between ACS and age, local anesthesia, bifurcated approach, hemodynamic instability, balloon occlusion, and conversion to open repair. Other universal risk factors for developing ACS in vascular cases are massive fluid resuscitation (>5 L/24 h), sepsis or bacteremia, mechanical ventilation and use of positive end expiratory pressure, polytransfusion (>10 U packed red blood cells/24 h), and acidosis.

Physical examination is inadequate for establishing the diagnosis of IAH and ACS; hence, different IAP measurement techniques were introduced in clinical practice. Direct measurement of IAP is unsuitable. The first surrogate method was described by Kron [53]. Originally, after clamping a urinary catheter and filling the bladder with 50 mL of 0.9% saline, intermittent measuring of the IAP via the urinary drainage port and a needle connected to a pressure transducer can be performed. Balogh [59] introduced technique of continuous intra-abdominal pressure measurement utilizing a three-way catheter and connecting the irrigation port to a pressure transducer. Other methods of continuous IAP measurement are intragastric or via intraperitoneal drain. WSACS recommended trans-bladder measurement as a simple and low-cost technique. It should be taken at end-expiration with the patient supine and the transducer zeroed at the midaxillary line after an instillation of no greater than 25 mL of saline into the bladder. Measurement is performed 30–60 s after instillation to allow bladder detrusor muscle relaxation. IAP should be measured every 4 h (during first 48 h and more frequently if IAH appears) in patients with RAAA treated by means of open or endovascular method. There are also MDCT signs which point to the presence of IAH, and AC—"round belly sign" and "bowel wall thickening with enhancement"—could be noted in patients with IAH. These findings could be used as a predictive measure, but they are not sufficient for diagnosis or prognosis.

ACS is usually refractory to conservative treatment, and surgical abdominal decompression is frequently indicated. The main principles of open abdomen technique are prevention of adhesions between the bowel and abdominal wall, contamination of abdominal cavity, and lateralization of the abdominal wall ("lateralization of the abdominal wall is the phenomenon where the musculature and fascia of the abdominal wall, most exemplified by the rectus abdominal muscles and their enveloping fascia, move laterally away from the midline with time"). Open abdomen treatment requires a temporary abdominal closure which is the most important factor to avoid complications of ACS. The most commonly used techniques are Bogota bag, Wittmann patch, Barker technique (vacuum pack), and vacuum-assisted closure (VAC) therapy [60].

Bogota bag consists of a sterilized (by gas) plastic bag (3 L genitourinary irrigation bag) that is sewn to the skin or fascia of the anterior abdominal wall. Several modifications of the technique have been reported, including the use of double sheets and suction tubes. It is a cheap technique, but it does not prevent bowel adhesion to abdominal wall. Also, sutures can generate lacerations of the fascia or skin which can compromise fascia closure.

Wittmann patch consists of two opposite nonpermeable Velcro patches sutured to the fascia and connected on the middle [61]. Unlike Bogota bag technique, Wittmann patch enables continuous tension on the fascial edges. It prevents abdominal wall lateralization, but does not permit an effective drainage and does not prevent adhesions between the bowel and the abdominal wall.

Vacuum pack implies a plastic sheet which covers the viscera [62]. Afterward, moist sterile surgical towels are placed in the wound, and a surgical drain connected with a continuous negative pressure is placed on the towels and all is covered by an adhesive drape including a wide margin on the surrounding skin. The drains are then connected to wall suction, providing 100–

150 mmHg continuous negative pressure. The dressing should be changed every 2–3 days in operative room, but also it might be done in the ICU.

VAC therapy uses commercially available V.A.C. Abdominal Dressing System (KCI, USA). This technique drains excess peritoneal fluid better than other described techniques. In the first studies, polyurethane sponge (fabricated by KCI Medical) over a nonadhesive polyethylene sheet was used [63, 64]. A polyethylene sheet with a central thin polyurethane sponge is placed around the viscera in order to prevent adhesion formation between the viscera and abdominal wall. The system is then connected to a special portable pump as the vacuum source instead of wall suction. The pressure is usually adjusted to 125 mmHg (100–150 mmHg), and it is distributed evenly throughout the wound through sponge pores. VAC system applied in this manner does not prevent lateral retraction of the fascial edges. In order to minimize lateralization of abdominal wall, Björck et al. [65] described a combined technique using VAC system with a polypropylene mesh sutured on the fascia edge sustaining the fascia traction. A polypropylene mesh is applied at the first redressing after 2–3 days. It is divided in two halves and sutured to the fascial edges on each side. During redressing the mesh is opened in the midline, and, after changing of the inner layer of the VAC dressing, the mesh is tightened in order to approximate the mesh toward the midline. Redressing is performed under general anesthesia every 2–3 days. Finally, the entire mesh is removed, and the fascia is then closed with a running 0 polydioxanone suture. This technique can achieve a primary delayed fascial closure rate of approximately 90–100% of those surviving treatment of open abdomen [66, 67]. The median time to closure of the open abdomen was 10.5 and 17 days. Other authors used dynamic retention sutures instead of a mesh in order to prevent lateralization. This technique provides lesser fascial closure (only 67%), indicating that the mesh may be more effective in preventing lateralization during prolonged treatment of the open abdomen. In order to accelerate abdomen closure, some authors use abdominal reapproximation anchor abdominal wall closure system (ABRA system) in combination with VAC therapy. In a randomized study, Long compared the closure rate between study group where open abdomen was treated by VAC system in combination with ABRA system and VAC alone in control group [68]. Differences between the two groups regarding primary closure rate were not significant, but ABRA system enabled faster closure. Zurich group described VAC-over ETHIZIP technique as an efficient method of open abdomen treatment [39]. Nonsutured zipper drape (ETHIZIP) is set up beneath the abdominal wall, and the VAC system is then applied over the ETHIZIP using a subatmospheric pressure of 50 mmHg. In cases in whom primary fascial closure was not possible, the abdomen was closed using polypropylene mesh or a bilateral anterior rectus abdominis sheath turnover flap and direct skin closure.

Complications of open abdomen treatment using the abovementioned techniques such as graft infections and intestinal fistula are seldom reported mostly in cases with longer duration of open abdomen treatment. The largest study which included 30 patients with VAC treatment of open abdomen after AAA repair reported 17% of infectious complications (2 cases of graft infection, 1 case of aortoenteric fistula, 2 cases of intestinal fistula) [69]. These patients were treated due to postoperative intestinal ischemia, requiring relaparotomy and intestinal resection, when open abdomen therapy was initiated. In other studies, in which intestinal ischemia was not preceded by decompressive laparotomy, no patient developed intestinal fistula. In recently published systematic review of temporary abdominal closure after AAA repair, early graft infection was not reported [67].

ABThera is another implementation of the VAC system. It consists of spiderlike sponge which allows a better fluid drainage and a better wound contraction. Primary fascia closure rate was 89%. Identification of patients with IAH and incoming ACS are of utmost important because early recognition may optimize treatment and

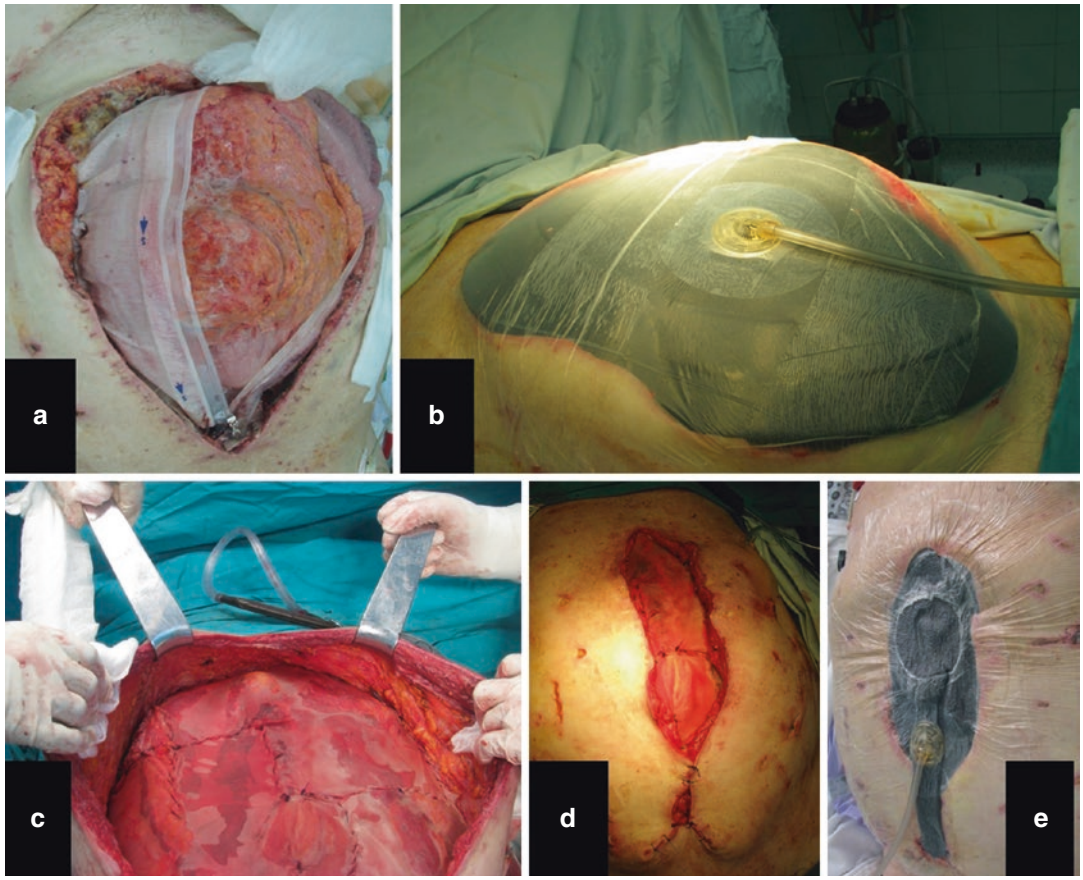

Fig. 7 (**a**) Abdominal compartment syndrome after repair of ruptured abdominal aortic repair was treated initially with "zip" abdominal suture. (**b**) Due to dehiscence of the "zip," VAC abdominal set was applied for 15 days when (**c, d**) synthetic mash was used to reconstruct abdominal wall. (**e**) Later smaller VAC dressings were used to support healing of the skin and subcutaneous tissue [5]

patient outcome. Despite many studies favor VAC therapy in treatment of open abdomen, there is a need for randomized controlled trials to determine the most efficient and safe method of temporary abdominal closure.

Besides complete midline laparotomy, abdominal decompression after endovascular repair of RAAA could be performed through a lumbotomy providing drainage of retroperitoneal hematoma; however, assessment of all intestines is insufficient, and bowel ischemia may be missed. Hörer [70] published a novel option in the treatment of ACS using tissue plasminogen activator (tPA)-assisted evacuation of the hematoma. 20F catheter drain was inserted into the retroperitoneal hematoma under computed tomography guidance, and then 20 mg of tPA solution was injected down through the drain to facilitate evacuation of the coagulated hematoma and decrease the abdominal pressure. However, since the full effect of tPA-assisted decompression might take hours or days, this approach is unsuitable for rapid decompression, while on the other side it does not allow inspection of the bowel (Fig. 7). Finally, it is contraindicated in patients with type I endoleak.

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References

1. Inui T, Bandyk D (2015) Vascular surgical site infection: risk factors and preventive measures. Semin Vasc Surg 28:201–207
2. Ozaki K, Hamdan A, Barshes N, Wyers M, Hevelone N, Belkin M, Nguyen LL (2015) Prospective, randomized, multi-institutional clinical trial of a silver alginate dressing to reduce lower extremity vascular surgery wound complications. J Vasc Surg 61:419–427
3. Wiseman J, Fernandes-Taylor S, Barnes M, Saunders RS, Saha S, Havlena J, Rathouz PJ, Kent KC (2015) Predictors of surgical site infection after hospital discharge in patients undergoing major vascular surgery. J Vasc Surg 62:1023–1031
4. Slegt J, Kluytmans J, Mulder P, Veen E, Gwan H, van der Laan L (2014) Surgical site infection after multiple groin incisions in peripheral vascular surgery. Surg Infect 15:752–756
5. Koncar I, Cvetković S, Dragas M, Pejkić S, Lazović G, Banzić I, Zuvela M, Marković M, Davidović L (2016) Vacuum-assisted wound closure in vascular surgery—clinical and cost benefits in a developing country. Vojnosanit Pregl 73(1):9–15
6. Shaw RS, Baue AE (1962) Management of sepsis complicating arterial reconstructive surgery. Surgery 53:75–86
7. Valentine RJ (2001) Diagnosis and management of aortic graft infection. Semin Vasc Surg 14:292–301
8. Bandyk DF (2002) Antibiotics—why so many and when should we use them? Semin Vasc Surg 15:268–274
9. Keidar Z, Nitecki S (2009) FDG-PET for the detection of infected vascular grafts. Q J Nucl Med Mol Imaging 53:35–40
10. Saleem BR, Meerwaldt R, Tielliu IF, Verhoeven EL, van den Dungen JJ, Zeebregts CJ (2010) Conservative treatment of vascular prosthetic graft infection is associated with high mortality. Am J Surg 200:47–52
11. van der Vaart MG, Meerwaldt R, Slart RH, van Dam GM, Tio RA, Zeebregts CJ (2008) Application of PET/SPECT imaging in vascular disease. Eur J Vasc Endovasc Surg 35:507–513
12. Annovazzi A, D'Alessandria C, Bonanno E, Mather SJ, Cornelissen B, van de Wiele C, Dierckx RA, Mattei M, Palmieri G, Scopinaro F, Signore A (2006) Synthesis of 99mTc- HYNIC-interleukin-12, a new specific radiopharmaceutical for imaging T lymphocytes. Eur J Nucl Med Mol Imaging 33:474–482
13. Signore A, Chianelli M, D'Alessandria C, Annovazzi A (2006) Receptor targeting agents for imaging inflammation/infection: where are we now? Q J Nucl Med Mol Imaging 50:236–242
14. Wyss MT, Weber B, Honer M, Späth N, Ametamey SM, Westera G, Bode B, Kaim AH, Buck A (2004) 18F-choline in experimental soft tissue infection assessed with autoradiography and high-resolution PET. Eur J Nucl Med Mol Imaging 31:312–316
15. Clagett GP, Bowers BL, Lopez-Viego MA, Rossi MB, Valentine RJ, Myers SI, Chervu A (1993) Creation of a neo-aortoiliac system from lower extremity deep and superficial veins. Ann Surg 218(3):239–249
16. Stone PA, Armstrong PA, Bandyk DF, Brumberg RS, Flaherty SK, Back MR, Johnson BL, Shames ML (2006) Use of antibiotic-loaded polymethylmethacrylate beads for the treatment of extracavitary prosthetic graft infections. J Vasc Surg 44:757–761
17. Armstrong PA, Back MR, Bandyk DF, Johnson BL, Shames ML (2007) Selective application of sartorius muscle flap and aggressive staged surgical debridement can influence long-term outcomes of complex graft infections. J Vasc Surg 46:71–78
18. Szilagyi DE, Smith RF, Elliott JP, Vrandecic MP (1972) Infection in arterial reconstruction with synthetic grafts. Am Surg 176:321–333
19. Colwell AS, Donaldson MC, Belkin M, Orgill DP (2004) Management of early groin vascular bypass graft infections with sartorius and rectus femoris flaps. Ann Plast Surg 52:49–53
20. Domingos Hadamitzky C, Schulte S, Horsch S (2007) Vacuum assisted wound closure in postoperative periprosthetic groin infections: a new gold standard? J Cardiovasc Surg 48:477–483
21. Demaria RG, Giovannini UM, Teot L, Frapier JM, Albat B (2003) Topical negative pressure therapy. A very useful new method to treat severe infected vascular approaches in the groin. J Cardiovasc Surg 44:757–761
22. Pinocy J, Albes JM, Wicke C, Ruck P, Ziemer G (2003) Treatment of periprosthetic soft tissue infection of the groin following vascular surgical procedures by means of a polyvinyl alcohol-vacuum sponge system. Wound Repair Regen 11:104–109
23. Giovanni UM, Demaria RG, Chaptal PA, Teot L (2001) Negative pressure for the management of an exposed vascular Dacron polyester patch. Ann Plast Surg 47:577–578
24. Kotsis T, Lioupis C (2007) Use of vacuum assisted closure in vascular graft infection confined to the groin. Acta Chir Belg 107:37–44
25. Svensson S, Monsen C, Kölbel T, Acosta S (2008) Predictors for outcome after vacuum assisted closure therapy of peri-vascular surgical site infections in the groin. Eur J Vasc Endovasc Surg 36:84–89
26. Dosluoglu HH, Schimpf DK, Schultz R, Cherr GS (2005) Preservation of infected and exposed vascular grafts using vacuum assisted closure without muscle flap coverage. J Vasc Surg 42:989–992
27. Brehm V, Steenvoorde P, Oskam J (2006) Regarding "preservation of infected and exposed vascular grafts using vacuum assisted closure without muscle flap coverage". J Vasc Surg 44:226
28. Monsen C, Wann-Hansson C, Wictorsson C, Acosta S (2014) Vacuum-assisted wound closure versus alginate for the treatment of deep perivascular wound infections in the groin after vascular surgery. J Vasc Surg 59:145–151
29. Thorbjørnsen K, DjivaniGidlund K, Björk M, Kragterman B, Wanhainen A (2016) Long-term out-

come after EndoVAC hybrid repair of infected vascular reconstructions. Eur J Vasc Endovasc Surg 51:724–732
30. Buck DB, Ultee KH, Zettervall SL, Soden PA, Darling J, Wyers M, van Herwaarden JA, Schermerhorn ML (2016) Transperitoneal versus retroperitoneal approach for open abdominal aortic aneurysm repair in the targeted vascular national surgical quality improvement program. J Vasc Surg 64(3):585–591
31. Ma B, Wang YN, Chen KY, Zhang Y, Pan H, Yang K (2016) Transperitoneal versus retroperitoneal approach for elective open abdominal aortic aneurysm repair. Cochrane Database Syst Rev 2:CD010373
32. Laohapensang K, Rerkasem K, Chotirosniramit N (2005) Left retroperitoneal versus midline transperitoneal approach for abdominal aortic aneurysms (AAAs) repair. J Med Assoc Thail 88(5):601–606
33. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR (1999) Guideline for prevention of surgical site infection, 1999. Hospital infection control practices advisory committee. Infect Control Hosp Epidemiol 20:250–278
34. Chiesa R, Melissano G, Castellano R (2001) Postoperative arterial infection: epidemiology, bacteriology and pathogenesis. In: Branchereau A, Jacobs M (eds) Complications in vascular and endovascular surgery. Futura, Armonk, NY, pp 61–73
35. Coomer NM, Kandilov AM (2016) Impact of hospital-acquired conditions on financial liabilities for Medicare patients. Am J Infect Control 44(11):1326–1334
36. Vermeulen H, Ubbink DT, Goossens A, de Vos R, Legemate DA, Westerbos SJ (2004) Dressings and topical agents for surgical wounds healing by secondary intention. Cochrane Database of Syst Rev 2:CD003554
37. Dumville JC, Owens GL, Crosbie EJ, Peinemann F, Liu Z (2015) Negative pressure wound therapy for treating surgical wounds healing by secondary intention. Cochrane Database Syst Rev 6:CD011278
38. Bandyk DF, Back MR (2005) Infection in prosthetic vascular grafts. In: Rutherford RB (ed) Vascular surgery. Saunders Elsevier, Philadelphia, pp 875–894
39. Mayer D, Rancic Z, Meier C, Pfammatter T, Veith FJ, Lachat M (2009) Open abdomen treatment following endovascular repair of ruptured abdominal aortic aneurysms. J Vasc Surg 50(1):1–7
40. Kugler NW, Carver TW, Paul JS (2016) Negative pressure therapy is effective in abdominal incision closure. J Surg Res 203(2):491–494
41. Hyldig N, Birke-Sorensen H, Kruse M, Vinter C, Joergensen JS, Sorensen JA, Mogensen O, Lamont RF, Bille C (2016) Meta-analysis of negative-pressure wound therapy for closed surgical incisions. Br J Surg 103(5):477–486
42. Saziye K, Mustafa C, Ilker U, Afksendyios K (2011) Comparison of vacuum-assisted closure device and conservative treatment for fasciotomy wound healing in ischemia reperfusion syndrome: preliminary results. Int Wound J 8:229–236
43. Blume PA, Walters J, Payne W, Ayala J, Lantis J (2008) Comparison of negative pressure wound therapy using vacuum-assisted closure with advanced moist wound therapy in the treatment of diabetic foot ulcers: a multicenter randomized controlled trial. Diabetes Care 31(4):631–636
44. Lepantalo M, Apelqvist J, Setacci C, Ricco JB, de Donato G, Becker F, Robert-Ebadi H, Cao P, Eckstein HH, De Rango P, Diehm N, Schmidli J, Teraa M, Moll FL, Dick F, Davies AH (2011) Management of critical limb ischaemia and diabetic foot. Clinical practice guidelines of the European Society for Vascular Surgery. Eur J Vasc Endovasc Surg 42:56074
45. Apelqvist J (2008) The foot in perspective. Diabetes Metab Res Rev 24(Suppl 1):S110–S115
46. Mulder G, Armstrong D, Seaman S (2003) Standard, appropriate, and advanced care and medical-legal considerations: part one-diabetic foot ulcerations. Wounds 15:92–106
47. Martson WA, Hanft J, Norwood P, Pollak R (2003) The efficacy and safety of Dermagraft in improving the healing of chronic diabetic foot ulcers: results of prospective randomized trial. Diabetes Care 26:1701–1705
48. Mannari RJ, Payne WG, Ochs DE, Walusimbi M, Blue M, Robson MC (2002) Successful treatment of recalcitrant, diabetic heel ulcers with topical becaplermin (rh-PDGF-BB) gel. Wounds 14:116–121
49. Peters EJ, Lavery LA, Armstrong DG, Fleischli JG (2001) Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial. Arch Phys Med Rehabil 82:721–725
50. Armstrong DG, Lavery LA (2005) Diabetic Foot Study Consortium. Negative pressure wound therapy after partial diabetic foot amputation: a multicentre, randomized controlled trial. Lancet 366:1704–1710
51. Sumpio B, Thakor P, Mahler D, Blume P (2011) Negative pressure wound therapy as postoperative dressing in below knee amputation stump closure of patients with chronic venous insufficiency. Wounds 23(10):301–308
52. Fuller M, Luff R, Van Den Boom M (2011) A novel approach to wound management and prosthetic use with concurrent vacuum-assisted closure therapy. Prosthetics Orthot Int 35(2):246–249
53. Kron IL, Harman PK, Nolan SP (1984) The measurement of intra-abdominal pressure as a criterion for abdominal re-exploration. Ann Surg 199:28–30
54. Kirkpatrick A, Roberts DJ, De Waele J, Jaeschke R, Malbrain MLNG, De Kuelenaer B, Duchesne J, Bjorck M, Leppaniemi A, Ejike JC, Sugrue M, Cheatham M et al (2013) Intra-abdominal hypertension and the abdominal compartment syndrome: updated consensus definitions and clinical practice guidelines from the World Society of the Abdominal Compartment Syndrome. Intensive Care Med 39:1190–1206
55. Acosta S, Björck M, Wanhainen A (2016) Negative-pressure wound therapy for prevention and treatment of surgical-site infections after vascular surgery. Br J Surg 104(2):e75–e84

56. Björck M, Wanhainen A (2014) Management of abdominal compartment syndrome and the open abdomen. Eur J Vasc Endovasc Surg 47(3):279–287
57. Mehta M, Taggert J, Darling R, Chang P, Kreienberg P, Paty P, Roddy SP, Sternbach Y, Ozsvath KJ, Shah DM (2006) Establishing a protocol for endovascular treatment for ruptures abdominal aortic aneurysms: outcomes of a prospective analysis. J Vasc Surg 44:1–8
58. Karkos CD, Menexes GC, Patelis N, Kalogirou TE, Giagtzidis IT, Harkin DW (2014) A systematic review and meta-analysis of abdominal compartment syndrome after endovascular repair of ruptured abdominal aortic aneurysms. J Vasc Surg 59(3):829–842
59. Balogh Z, Jones F, D'Amors S, Surgue M (2004) Continuous intra-abdominal pressure measurement technique. Am J Surg 188:679–684
60. Boele van Hensbroek P, Wind J, Dijkgraaf MG, Busch OR, Goslings JC (2009) Temporary closure of the open abdomen: a systematic review on delayed primary fascial closure in patients with an open abdomen. World J Surg 33(2):199–207
61. Wittmann DH, Aprahamian C, Bergstein JM, Edmiston CE, Frantzides CT, Quebbeman EJ, Condon RE (1993) A burr-like device to facilitate temporary abdominal closure in planned multiple laparotomies. Eur J Surg 159(2):75–79
62. Brock WB, Barker DE, Burns RP (1995) Temporary closure of open abdominal wounds: the vacuum pack. Am Surg 61(1):30–35
63. Miller PR, Thompson JT, Faler BJ, Meredith JW, Chang MC (2002) Late fascial closure in lieu of ventral hernia: the next step in open abdomen management. J Trauma 53(5):843–849
64. Garner GB, Ware DN, Cocanour CS, Duke JH, McKinley BA, Kozar RA, Moore FA (2001) Vacuum-assisted wound closure provides early fascial reapproximation in trauma patients with open abdomens. Am J Surg 182:630–638
65. Petersson U, Acosta S, Björck M (2007) Vacuum-assisted wound closure and mesh-mediated fascial traction–a novel technique for late closure of the open abdomen. World J Surg 31(11):2133–2137
66. Seternes A, Myhre HO, Dahl T (2010) Early results after treatment of open abdomen after aortic surgery with mesh traction and vacuum-assisted wound closure. Eur J Vasc Endovasc Surg 40:60–64
67. Acosta S, Wanhainen A, Björck M (2016) Temporary abdominal closure after abdominal aortic aneurysm repair: a systematic review of contemporary observational studies. Eur J Vasc Endovasc Surg 51(3):371–378
68. Long KL, Hamilton DA, Davenport DL, Bernard AC, Kearney PA, Chang PK (2014) A prospective, controlled evaluation of the abdominal reapproximation anchor abdominal wall closure system in combination with VAC therapy compared with VAC alone in the management of an open abdomen. Am Surg 80(6):567–571
69. Sörelius K, Wanhainen A, Acosta S, Svensson M, Djavani-Gidlund K, Björck M (2013) Open abdomen treatment after aortic aneurysm repair with vacuum-assisted wound closure and mesh-mediated fascial traction. Eur J Vasc Endovasc Surg 45(6):588–594
70. Hörer T, Skoog P, Pirouzram A, Larzon T (2012) Tissue plasminogen activator-assisted hematoma evacuation to relieve abdominal compartment syndrome after endovascular repair of ruptured abdominal aortic aneurysm. J Endovasc Ther 19(2):144–148

Deep Sternal Infection Following Bilateral Internal Thoracic Artery Grafting

Andrea Perrotti, Giuseppe Gatti, Fiona Ecarnot, and Sidney Chocron

1 Introduction

Deep sternal wound infection (DSWI) is a serious and potentially devastating complication after cardiac surgery that is associated with longer hospital stays, repeat surgical procedures, and mortality [1, 2]. In the infectious disease literature for all types of surgery, three categories of surgical site infection are defined: type 1, corresponding to superficial incisional; type 2, deep incisional (muscle and fascia); and type 3, deep organ space (bone and/or mediastinum) [3]. The most common definition is that of the US Centers for Disease Control and Prevention (CDC) including at least one of the following criteria:

1. An organism is isolated from mediastinal tissue or fluid obtained during the surgical procedure or needle aspiration.
2. Evidence of mediastinitis seen during surgery or by histopathological examination.
3. At least one of the following: fever (>38°), chest pain, or sternal instability, associated with either purulent discharge from the mediastinal area or organism cultured from blood or mediastinal widening on X-ray [3–6].

Despite many advances in prevention, DSWI remains significant, with an incidence ranging between 0.5 and 6.8% [7, 8]. Numerous studies have identified patient-related and procedure-related factors that may contribute to the occurrence of DSWI. Patient factors include obesity, female sex, age, chronic obstructive pulmonary disease (COPD), diabetes or hyperglycemia during the perioperative period, tobacco smoking, peripheral arterial occlusive disease, kidney dysfunction, recent treatment with antibiotics, skin infection anywhere on the body, and emergency or urgent surgery [5, 9–16]. The high incidence of DSWI in obese patients can be explained by the poor perfusion of subcutaneous adipose layers with low levels of prophylactic antibiotics in this tissue. In fact, Filsoufi showed that obesity was associated with a more than twofold increase in the risk of DSWI [7]. Patients with diabetes mellitus or hyperglycemia are also at increased risk of infection. The reason for the increased risk of DSWI in these patients is that hyperglycemia causes the formation of advanced glycation end products, which can affect host cell function by impairing humoral response, complement activation, chemotaxis, adhesions, and phagocytosis [17, 18]. In a large prospective study, Brown demonstrated that hyperglycemia was an independent risk factor for death, length of hospital stay, and infection rate [19]. Moreover, in a recent study, Gatti [20] reported the importance of HbA1c screening in all patients undergoing cardiac surgery because unrecognized diabetes and

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poor glycemic control may have immediate and long-term prognostic implications in both diabetic and nondiabetic patients. Patient carriage of *Staphylococcus aureus* on the skin and in the nostrils has been identified as an important risk factor for DSWI [21]. The Society of Thoracic Surgeons practice guidelines recommend routine nasal administration of 5-d mupirocin 2% for all patients undergoing cardiac surgery procedures in the absence of documented negative testing for staphylococcal colonization [22]. Surgical risk factors include inadvertent paramedian sternotomy [23], repeat exploration for bleeding [24], use of bone wax, extensive use of electrocauterization [13], prolonged duration of aortic cross clamp or cardiopulmonary bypass [11], postoperative bleeding [25], blood transfusions [7, 12, 26], prolonged mechanical ventilation [4, 7], and finally the use of both internal mammary arteries [4, 13, 27].

2 Why BITA?

In coronary artery bypass graft (CABG) surgery, the conventional strategy has been to utilize the left internal thoracic artery (LITA) for grafting to the left anterior descending artery and the radial artery or saphenous vein to bypass other coronary arteries [28]. The use of LITA grafts has been associated with improved survival at 10 years and reduced incidence of myocardial infarction, cardiac events, and reoperation compared with the use of vein graft alone [29, 30]. Prompted by this evidence, surgeons have attempted to use a bilateral internal thoracic artery (BITA) graft, which should further improve the long-term outcomes of coronary revascularization. Despite the increasing evidence that patients who receive BITA grafts have better long-term outcomes than those receiving single ITA grafts [31, 32], until now, BITA grafting has not been widely adopted. Indeed, BITA grafting is performed in only 4 and 12% of all CABG patients in North America and Europe, respectively [33]. The most salient, persistent, and serious objection to BITA grafting has been an increased risk of sternal wound complications [34, 35].

3 Pathophysiology

In coronary artery bypass grafting, it is clear from experimental and clinical evidence that the increased risk of wound complications appears to be an effect of sternal ischemia caused by the harvest of ITA [36]. Anatomic studies have reported that the harvesting of a single ITA causes significant devascularization of the ipsilateral hemisternum; thus bilateral ITA dissection brings on sternal wound complications [34, 35].

Arnold et al. [37] observed that sternal circulation depends only on a periosteal plexus that arises from segmental sternal branches of the ITA. When one ITA is mobilized, revascularization of that side of the sternum may be possible by crossover collaterals from the unharvested side with intact blood supply. Obviously, a tight sternal closure is necessary to ensure optimal and stable contact between the two sternal sides. According to the same study, complete sternal devascularization occurs with bilateral ITA harvesting. Seyfer [38] demonstrated no evidence for collateral flow to the sternum that could immediately substitute the missing ITA, with a 90% decrease in sternal blood flow after mobilization of one ITA. Moreover, inadequate blood flow impairs local immune response and reduces effectiveness of antibiotics when contamination occurs. These findings could explain the higher rate of sternal wound after BITA grafting.

4 Prevention

In most centers ITA is harvested as a pedicle, together with the vein, muscle, fat, and accompanying endothoracic fascia. The electrocautery that is used for harvesting damages the blood supply to the sternum, and this impairs sternal healing and exposes the sternum to the risk of infection. The anatomic study by Henriquez-Pino et al. [39] showed that some of the 4–6 sternal branches of the ITA and some intercostal branches may arise from the ITA as a common trunk. If that common trunk can be preserved during ITA dissection, then sternal collateralization may be improved. In a study using

technetium-99m methylene diphosphate bone scanning and single-photon emission computed tomography, Cohen et al. showed that a pedicled left internal thoracic artery graft to the left anterior descending artery reduces blood flow to the left side of the sternum during the acute postoperative period. This does not occur when the left internal thoracic artery is skeletonized [36]. This technique for ITA mobilization is associated with a low rate of deep sternal infection, probably associated with less devascularization of the sternum [40]. However, ITA skeletonization is not a universal remedy to avoid sternal complication, but contributes strongly, together with other preventive preoperative and intraoperative measures for avoiding wound problems. First of all, preoperative prevention is of paramount prevention; perioperative intranasal mupirocin is recommended for all cardiac surgery procedures to decrease sternal complications [41, 42], presurgical bathing with chlorhexidine is useful in reducing bacterial count [43], and preoperative identification of untreated diabetes and optimization of glycemic control is strongly recommended [20, 44]. Intraoperatively, meticulous wound opening and closing technique is mandatory. Bone wax should not be used [43], and tight sternal closure with a figure-of-eight technique or using the Robicsek weave technique may prevent sternum instability and infection [45, 46]. Lastly, in order to decrease the risk of wound infections, it is important to avoid bleeding complications that can require repeat surgery [47]. Rapid extubation and early removal of indwelling central venous and urinary catheters may contribute to avoiding complications [48, 49].

Conclusions

To conclude, it is clear that the problem of DSWI is multifactorial, and it is difficult to anticipate exactly which risks factors and triggering events may cause infection. The best results will be achieved through constant engagement and rigorous discipline of all the healthcare professionals in contact with the patient (anesthetist, surgeon, nurse) from the beginning of hospitalization to discharge. Regarding surgical treatment, there are many options for sternal wound closure depending on the degree of the wound and institutional policy. These include closed suction antibiotic catheter irrigation systems, bilateral pectoralis major muscle flap, omental transposition, the latissimus dorsi muscle flap, vacuum-assisted closure, and various combinations of the above [50].

References

1. Lepelletier D, Poupelin L, Corvec S, Bourigault C, Bizouarn P, Blanloeil Y, Reynaud A, Duveau D, Despins P (2009) Risk factors for mortality in patients with mediastinitis after cardiac surgery. Arch Cardiovasc Dis 102:119–125
2. Toumpoulis IK, Anagnostopoulos CE, Derose JJ Jr, Swistel DG (2005) The impact of deep sternal wound infection on long-term survival after coronary artery bypass grafting. Chest 127:464–471
3. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR (1999) Guideline for prevention of surgical site infection 1999. Infect Control Hosp Epidemiol 20:247–280
4. Ridderstolpe L, Gill H, Granfeldt H, Ahifeldt H, Rutberg H (2001) Superficial and deep sternal wound complications: incidence, risk factors and mortality. Eur J Cardiothorac Surg 20:1168–1175
5. Sakamoto H, Fukuda I, Oosaka M, Nakata H (2003) Risk factors and treatment of deep sternal wound infection after cardiac operation. Ann Thorac Cardiovasc Surg 9(4):226–232
6. Horan TC, Andrus M, Dudeck MA (2008) CDC/NHSN surveillance definition of health care-associated infection and criteria for specifics types of infections in the acute care setting. Am J Infect Control 36:309–332
7. Filsoufi F, Castillo JG, Rahmanian PB, Broumand SR, Silvay G, Carpentier A, Adams DH (2009) Epidemiology of deep sternal wound infection in cardiac surgery. J Cardiothorac Vasc Anesth 23:488–494
8. Kanafani ZA, Arduino JM, Muhlbaier LH, Kaye KS, Allen KB, Carmeli Y, Corey GR, Cosgrove SE, Fraser TG, Harris AD, Karchmer AW, Lautenbach E, Rupp ME, Peterson ED, Straus WL, Fowler VG (2009) Incidence of and preoperative risk factors for Staphylococcus aureus bacteremia and chest wound infection after cardiac surgery. Infect Control Hosp Epidemiol 30(3):242–248
9. Dodds Ashley ES, Carroll DN, Engemann JJ, Harris AD, Fowler VG, Sexton DJ, Kaye KS (2004) Risk factors for postoperative mediastinitis due to methicillin-resistant Staphylococcus aureus. Clin Infect Dis 38:1555–1560
10. Harrington G, Russo P, Spelman D, Borrell S, Watson K, Barr W, Martin R, Edmonds D, Cocks J,

Greenbough J, Lowe J, Randle L, Castell J, Browne E, Bellis K, Aberline M (2004) Surgical-site infection rates and risk factor analysis in coronary artery bypass graft surgery. Infect Control Hosp Epidemiol 25:472–476
11. Berrios-Torres SI, Mu Y, Edwards JR, Horan TC, Fridkin SK (2012) Improved risk adjustment in public reporting: coronary artery bypass surgical site infection. Infect Control Hosp Epidemiol 33:463–469
12. Lepelletier D, Perron S, Bizouarn P, Caillon J, Drugeon H, Michaud JL, Duveau D (2005) Surgical-site infection after cardiac surgery: incidence, microbiology, and risk factors. Infect Control Hosp Epidemiol 26:466–472
13. Robinson PJ, Billah B, Leder K, Reid CM, ASCTS Database Committee (2007) Factors associated with deep sternal wound infection and hemorrhage following cardiac surgery in Victoria. Interact Cardiovasc Thorac Surg 6:167–171
14. Risnes I, Abdelnoor M, Almdahl SM, Svennevig JL (2010) Mediastinitis after coronary artery bypass grafting : risk factors and long-term survival. Ann Thorac Surg 89:1502–1509
15. Gardlund B (2007) Postoperative surgical site infections in cardiac surgery; an overview of preventive measures. APMIS 115:989–995
16. Cayci C, Russo M, Cheema FH, Martens T, Ozcan V, Argenziano M, Oz MC, Ascherman J (2008) Risk analysis of deep sternal wound infections and their impact on long-term survival: a propensity analysis. Ann Plast Surg 61:294–301
17. Gan YH (2013) Host susceptibility factors to bacterial infections in type2 diabetes. PLoS Pathog 9:e1003794
18. Casqueiro J, Alves C (2012) Infections in patients with diabetes mellitus. A review of pathogenesis. Indian J Endocrinol Metab 16(Suppl 1):S27–S36
19. Brown JR, Edwards FH, O'Connor GT, Ross CS, Furnary AP (2006) The diabetic disadvantage: historical outcomes in diabetic patients undergoing cardiac surgery; the pre-intravenous insulin era. Semin Thorac Cardiovasc Surg 18:281–288
20. Gatti G, Perrotti A, Reichart D, Maschietto L, Onorati F, Chocron S, Dalén M, Svenarud P, Faggian G, Santarpino G, Fischlein T, Pappalardo A et al (2017) Glycated hemoglobin and risk of sternal wound infection after isolated coronary surgery. Circ J 81:36–43
21. von Eiff C, Becker K, Machka K, Stammer H, Peters G (2001) Nasal carriage as a source of Staphylococcus aureus bacteremia. Study group. N Engl J Med 344:11–16
22. Engelman R, Shahian D, Shemin R, Guy TS, Bratzler D, Edwards F, Jacobs M, Fernando H, Bridges C (2007) Workforce on Evidence Based Medicine, Society of Thoracic Surgeons. The Society of Thoracic Surgeons practice guidelines series: antibiotics prophylaxis in cardiac surgery, part II: antibiotic choice. Ann Thorac Surg 83:1569–1576
23. Zeitani J, Penta de Peppo A, Moscarelli M, Guerrieri Wolf L, Scafuri A, Nardi P, Nanni F, Di Marzio E, De Vico P, Chiariello L (2006) Influence of sternal size and inadvertent paramedian sternotomy on stability of the closure site: a clinical and mechanical study. J Thorac Cardiovasc Surg 132:38–42
24. Junoz P, Menasalvas A, Bernaldo de Quiros JC, Desco M, Vallejo JL, Bouza E (1997) Postsurgical mediastinitis: a case-control study. Clin Infect Dis 25:1060–1064
25. Bryan CS, Yarbrough WM (2013) Preventing deep wound infection after coronary artery bypass grafting: a review. Tex Heart Inst J 40:125–139
26. Banbury MK, Brizzio ME, Rajeswaran J, Lytle BW, Blackstone EH (2006) Transfusions increases the risk of postoperative infections after cardiovascular surgery. J Am Coll Surg 202:131–138
27. Diez C, Kock D, Kuss O, Silber RE, Friedrich I, Boergermann J (2007) Risk factors for mediastinitis after cardiac surgery – a retrospective analysis of 1700 patients. J Cardiothorac Surg 28:655–660
28. Aldea GS, Bakaeen FG, Pal J et al (2016) The Society of Thoracic Surgeons clinical practice guideline on arterial conduits for coronary artery bypass grafting. Ann Thorac Surg 101:801–809
29. Loop FD, Lytle BW, Cosgrove DM, Stewart RW, Goormastic M, Williams GW, Golding LA, Gill CC, Taylor PC, Sheldon WC et al (1986) Influence of the internal-mammary-artery graft on 10-years survival and other cardiac events. N Engl J Med 314:1–6
30. Cameron A, Davis KB, Green G, Schaff HV (1996) Coronary bypass surgery with internal-thoracic-artery graft: effects on survival over a 15-years period. N Engl J Med 334:216–219
31. Lytle BW, Blackstone EH, Loop FD, Houghtaling PL, Arnold JH, Akhrass R et al (1999) Two internal thoracic artery grafts are better than one. J Thorac Cardiovasc Surg 117:855–872
32. Buxton BF, Komeda M, Fuller JA, Gordon I (1998) Bilateral internal thoracic artery grafting may improve outcome of coronary artery surgery: risk-adjusted survival. Circulation 98(19 Suppl):III-1–III-6
33. Tabata M, Grab JD, Khalpey Z, Edwards FH, O'Brien SM, Cohn LH, Bolman RM 3rd (2009) Prevalence and variability of internal mammary artery graft use in contemporary multivessel coronary artery bypass graft surgery: analysis of the Society of Thoracic Surgeons National Cardiac Database. Circulation 120:935–940
34. Loop FD, Lytle BW, Cosgrove DM, Mahfood S, McHenry MC, Goormastic M, Stewart RW, Golding LA, Taylor PC (1990) J. Maxwell Chamberlain memorial paper. Sternal wound complications after isolated coronary artery bypass grafting: early and late mortality, morbidity, and cost of care. Ann Thorac Surg 49:179–187
35. Taggart DP, Altman DG, Gray AM, Lees B, Gerry S, Benedetto U, Flather M, Investigators ART (2016) Randomized trial of bilateral versus single internal-thoracic-artery grafts. N Engl J Med 375(26):2540–2549
36. Cohen AJ, Lockman J, Loberboym M, Bder O, Cohen N, Medalion B, Schachner A (1999) Assessment of

sternal vascularity with single photon emission computed tomography after harvesting of the internal thoracic artery. J Thorac Cardiovasc Surg 118:496–502
37. Arnold M (1972) The surgical anatomy of sternal blood supply. J Thorac Cardiovasc Surg 64:596–610
38. Seyfer AE, Shriver CD, Miller TR, Graeber GM (1988) Sternal blood flow after sternotomy and mobilization of internal mammary arteries. Surgery 104:899–904
39. Henriquez-Pino JA, Gomes WJ, Prates JC, Buffolo E (1997) Surgical anatomy of the internal thoracic artery. Ann Thorac Surg 64:1041–1045
40. Pevni D, Mohr R, Lev-Run O, Locer C, Paz Y, Kramer A, Shapira I (2003) Influence of bilateral skeletonized harvesting on occurrence of deep sternal wound infection in 1,000 consecutive patients undergoing bilateral internal thoracic artery grafting. Ann Surg 237:277–280
41. Cimochowski GE, Harostock MD, Brown R, Bernardi M, Alonzo N, Coyle K (2001) Intranasal mupirocin effectively reduces sternal wound infections after open heart surgery in diabetics and nondiabetics. Ann Thorac Surg 71:1572–1579
42. Bode LGM, Kloytmans JA, Werthiem HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, Troelstra A, Box AT, Voss A, van der Tweel I, van Belkum A, Verbrugh HA, Vos MC (2010) Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med 362:9–17
43. Lazar HL, Salm TV, Engelman R, Orgill D, Gordon S (2016) Prevention and management of sternal wound infections. J Thorac Cardiovasc Surg 152:962–972
44. Halkos ME, Puskas JD, Lattouf OM, Kilgo P, Kerendi F, Song HK, Guyton RA, Thourani VH (2008) Elevated preoperative hemoglobin A1c level is predictive of adverse events after coronary artery bypass surgery. J Thorac Cardiovasc Surg 136:631–640
45. Bottio T, Rizzoli G, Vida V, Casarotto D, Gerosa G (2003) Double crisscross sternal wiring and chest wound infections : a prospective randomized study. J Thorac Cardiovasc Surg 126:1352–1356
46. Robicsek F, Daugherty HK, Cook JW (1977) The prevention and treatment of sternum separation following open heart surgery. J Thorac Cardiovasc Surg 73:267–275
47. Boeken V, Eisner J, Feindt P, Petzold TH, Schulte HD, Game E (2001) Does the time of resternotomy for bleeding have any influence on the incidence of sternal infections, septic courses or further complications? J. Thorac Cardiovasc Surg 49:45–48
48. Graf K, Sohr D, Haverich A, Kuhn C, Gastmeier P, Chaberny IF (2009) Decrease of deep sternal surgical site infections rates after cardiac surgery by a comprehensive infection control program. Interact Cardiovasc Thorac Surg 9:282–286
49. LeGuillou V, Tavolacci MP, Baste JM, Hubscher C, Bedost E, Bessou JP, Litzler PY (2011) Surgical site infection after central venous catheter-related infection in cardiac surgery. Analysis of a cohort of 7557 patients. J Hosp Infect 79:236–241
50. Singh K, Anderson E, Garrett Harper J (2011) Overview and management of sternal wound infection. Semin Plast Surg 25(1):25–33

Deep Sternal Wound Infection After Cardiac Surgery

Hiroshi Kubota and Norihiko Ohura

1 Introduction

Deep sternal wound infection (DSWI) is a serious postoperative complication after cardiac surgery, because it dramatically increases rates of both postoperative morbidity and mortality. According to the literature, the incidence of deep sternal wound infection (DSWI) after cardiac surgery has been variously reported as between 0.8 and 5.0% [1–7]. Large retrospective and prospective outcome studies have identified epidemiologic factors associated with DSWI. Commonly cited risks of DSWI after cardiac surgery include advanced age, obesity, diabetes, smoking, prolonged operative time, use of internal thoracic artery conduits, hemodialysis, reoperative surgery, and prolonged cardiopulmonary bypass time [3, 7, 8]. Current management of DSWI after cardiac surgery involves adequate debridement of necrotic infected soft tissues and bone fragments, continuous administration of the antibiotics, and plombage of the defect with the omentum and/or well-vascularized flaps (Fig. 1). The recent introduction of negative-pressure wound therapy (NPWT), known as vacuum-assisted closure (VAC®), appears to be beneficial in the treatment of DSWI after cardiac surgery. In this chapter, relationship of the incidence of DSWI between each cardiac procedure (coronary artery disease, valvular disease, thoracic aortic disease), current clinical techniques to treat DSWI after cardiac surgery, and special technique to treat DSWI after thoracic aortic replacement using prosthetic graft are described and discussed.

2 Definition of DSWI

Generally, DSWI is defined, on the basis of the criteria of the US Centers for Disease Control and Prevention, as an infection involving deep soft tissues of the incision including the sternum or mediastinum and at least one of the following: (1) There is purulent drainage from the deep incision. (2) A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms—fever (>38 °C), localized pain, or tenderness, unless the site is culture-negative. (3) An abscess or another evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination. (4) There is diagnosis of a deep incisional surgical site infection by a surgeon or attending physician [9].

In addition, it is also necessary to evaluate DSWI from the viewpoint of blood flow and

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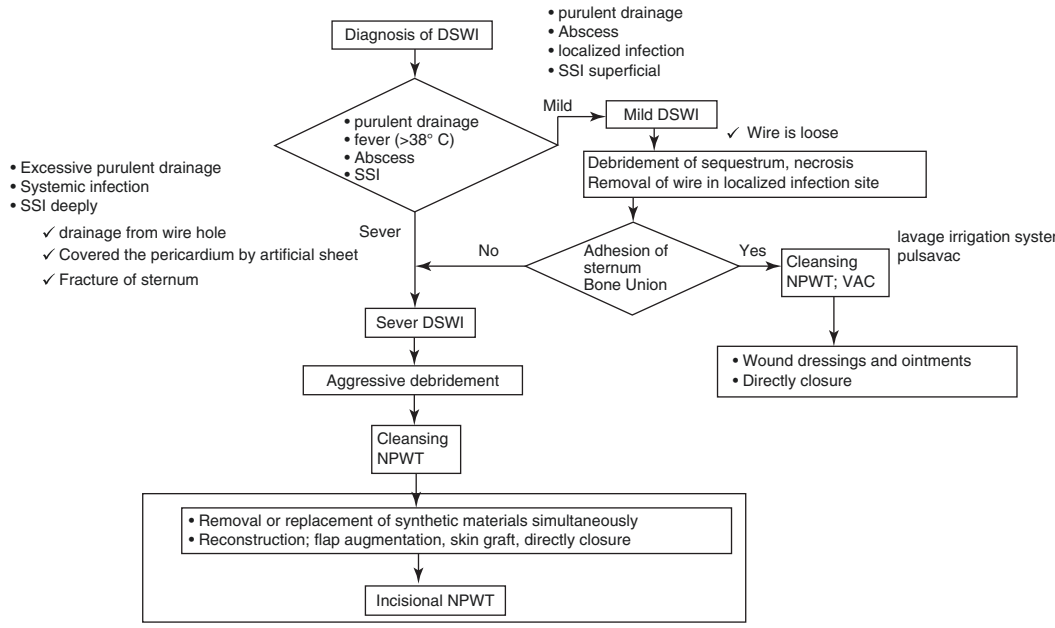

Fig. 1 Management of DSWI

infection site. In particular, one needs to consider whether the internal thoracic artery nourishing the sternum is used or not, whether infection extends to the mediastinum or not, and whether there is artificial graft or not. Because of these conditions, it is necessary to select reconstructive materials to be used.

Infection is deeply related to blood flow and artificial graft. In general, ischemic tissue/artifacts are susceptible to infection when microbial is loaded.

3 Recent Research on Incidence of DSWI After Each Cardiac Procedure

We reviewed the 73,700 adult cases recorded in the Japan Adult Cardiovascular Surgery Database (JACVSD) during the period from 2004 to 2009 and divided them into five groups: 26,597 isolated coronary artery bypass graft (CABG) cases, 23,136 valvular surgery cases, 17,441 thoracic aortic surgery cases, 4,726 valvular surgery plus CABG cases, and 1,800 thoracic aortic surgery plus CABG cases. We investigated the incidence of DSWI and effect of re-exploration for bleeding on DSWI mortality [10]. As results, the overall incidence of DSWI after cardiac surgery was 1.8%, as for each operative procedure, the incidence of postoperative DSWI was 1.8% in isolated CABG group, 1.3% in valve group, 2.8% in valve with CABG group, 1.9% in thoracic aorta group, and 3.4% in thoracic aorta with CABG group (Table 1). Overall incidence of re-exploration (OR) for bleeding in patients without/with postoperative DSWI was 3.6/11.1% ($p < 0.05$). Operative mortality is defined as inhospital or 30-day mortality (whichever is longer).

When operative procedure concomitant with CABG was done, the incidence of postoperative DSWI showed 1.5% of elevation compared with isolated original valve and thoracic aortic procedure. Internal thoracic artery use, prolonged operative time, and longer cardiopulmonary bypass time are possible mechanisms to explain these increased rates of DSWI [10].

The incidence of re-exploration for bleeding without/with DSWI according to operative procedure was 1.8/6.9%* in isolated CABG group, 3.8/11.8%* in valvular surgery, 4.9/6.1% (n.s.) in valvular surgery concomitant with CABG, 5.4/15.9%* in thoracic aortic surgery,

Table 1 Deep sternal wound infection after cardiac surgery

					CABG	Valve	Valve + CABG	TA	TA + CABG
Total 73,700 (cases)					26,597	23,136	4726	17,441	1800
DSWI	Incidence	1.8 (%)			1.8	1.3	2.8	1.9	3.4
	30 day mortality	Total 9.7 (%)			5.2	10.5	10.0	14.1	17.7
		Re-exploration for bleeding	−	8.1 (%)	4.3	8.8	10.7	11.6	13.0
			+	23.0 (%)	18.2	22.9	0	26.9	31.3
	Operative mortality	Total 25.8 (%)			19.0	23.0	22.3	34.9	50.0
		Re-exploration for bleeding	−	22.0 (%)	17.5	20.7	22.1	30.5	43.5
			+	48.0 (%)	39.4	40.0	25.0	57.7	68.8

DSWI deep sternal wound infection, *CABG* coronary artery bypass graft, *TA* thoracic aortic surgery

Table 2 Incidence of re-exploration for bleeding in patients with postoperative DSWI

	CABG	Valve	Valve + CABG	TA	TA + CABG
Overall incidence of re-exploration for bleeding 3.6 (%)	1.8	3.8	4.9	5.4	9.1
Incidence of re-exploration for bleeding in DSWI cases 11.1 (%)	6.9	11.8	6.1	15.9	25.8

and 9.1/25.8%* in thoracic aortic surgery concomitant with CABG (Table 2).

Overall 30-day mortality and operative mortality in patients with DSWI were 9.7 and 25.8%, respectively. The 30-day mortality in patients and operative mortality with DSWI were 5.2 and 19.0% in isolated CABG, 10.5 and 23.0% in valvular surgery, 10.0 and 22.3% in valvular surgery concomitant with CABG, 14.1 and 34.9% in thoracic aortic surgery, and 17.7 and 50.0% in thoracic aortic surgery concomitant with CABG, respectively.

Overall 30-day mortality and operative mortality in patients with DSWI with re-exploration for bleeding were 23.0 and 48.0%, respectively. It was significantly higher than 30-day mortality in patients with DSWI without re-exploration for postoperative bleeding (8.1 and 22.0%, $p < 0.05$).

The 30-day mortality and operative mortality in patients with DSWI of each operative procedure without/with re-exploration due to postoperative bleeding were 4.3/18.2% and 17.5/39.4% in isolated CABG, 8.8/22.9% and 20.7/40.0% in valvular surgery, 10.7/0% (n.s.) and 22.1/25.0% (n.s.) in valvular surgery concomitant with CABG, 11.6/26.9% and 30.5/57.7% in thoracic aortic surgery, and 13.0/31.3% and 43.5/68.8% (n.s.) in thoracic aortic surgery concomitant with CABG, respectively.

Patient's profile and OR of risk models for each operative procedure were also examined. Previous CABG history was a significant risk factor related to both re-exploration for bleeding and DSWI for all surgical groups. Age and cardiogenic shock were significant risk factors related to re-exploration for bleeding, and diabetes control was a significant risk factor related to DSWI for all surgical groups. Male gender, emergent or salvage operation, and operation combined with CABG were significant risk factors related to re-exploration for bleeding in two of three surgical groups. Body mass index (BMI), renal failure, chronic obstructive pulmonary disease (COPD), cardiogenic shock, administration of inotropic agents, and triple vessel disease were significant risk factors related to DSWI in two of three groups. Aortic rupture and aortic arch operation were significant risk factors related to both re-exploration for bleeding and DSWI in thoracic aortic surgery group.

Age and cardiogenic shock were significant risk factors related to re-exploration for bleeding, and diabetes control was a significant risk factor related to DSWI for all surgical groups. Previous CABG was a significant risk factor related to both re-exploration for bleeding and DSWI for all surgical groups [10].

These results suggest that, in addition to the above-described well-known risk factors that augment the risk of DSWI after cardiac surgery, deep hypothermia and usage of synthetic graft may worsen the clinical result of DSWI related to aortic

surgery. Because VAC system was approved for commercial use in 2010 in Japan, these presented data are from pre-approval VAC system era.

4 Clinical Techniques

Management of DSWI after cardiac surgery involves adequate debridement. Management is done according to the concept of wound bed preparation to control the infection. After removal of foreign matter and necrosis, the wound is cleansed by washing and irrigation, physically the bacterial count is decrease. After that, the soft tissue near the sternum with abundant blood flow is elevated as a flap and filled without gaps. Antibiotics and immune cells are recruited to the wound by the flap.

In the wound bed preparation concept, it is recommended that artificial products such as sternal wires, bone wax, hemostasis sheets, Teflon felt, PTFE sheet, and synthetic graft (if possible), and necrotic soft tissue including costal cartilage are removed as much as possible. Since cartilage is avascular tissue, the costal cartilage surrounded by necrotic tissue is removed by peeling from perichondrium (Fig. 1). It is desirable to replace the vascular graft at the time of reconstruction. However, when the patient is in poor systemic condition or when it is difficult to reconstruct and re-anastomose, it is impossible to remove it. In such a case, it is necessary to wash with a large amount of saline by the pulse lavage system such as Pulsavac®.

The key to success in the treatment of DSWI is to remove necrotic tissue and infected tissue and not to create space that is not filled with tissue. Because the remaining dead space is easily filled with exudate which is susceptible to reinfection and well-vascularized tissue delivers antibiotics and accelerates neutrophilic infiltration to the infection site that will be a strong barrier to the microorganisms, to fill the dead space with well-vascularized tissue is essential to treat DSWI after cardiac surgery. Reconstruction was used by the pedicled flaps (Table 3) (Fig. 2).

Table 3 Reconstruction material (flap) and arterial blood supply

Flap	Blood supply
(a) Latissimus dorsi muscle flap (LD)	Thoracodorsal artery
(b) Rectus abdominal muscle flap (RA)	Internal thoracic artery
(c) Pectoralis major muscle flap (PM)	Thoracoacromial artery
(d) Omental flap	Left and right gastroepiploic artery

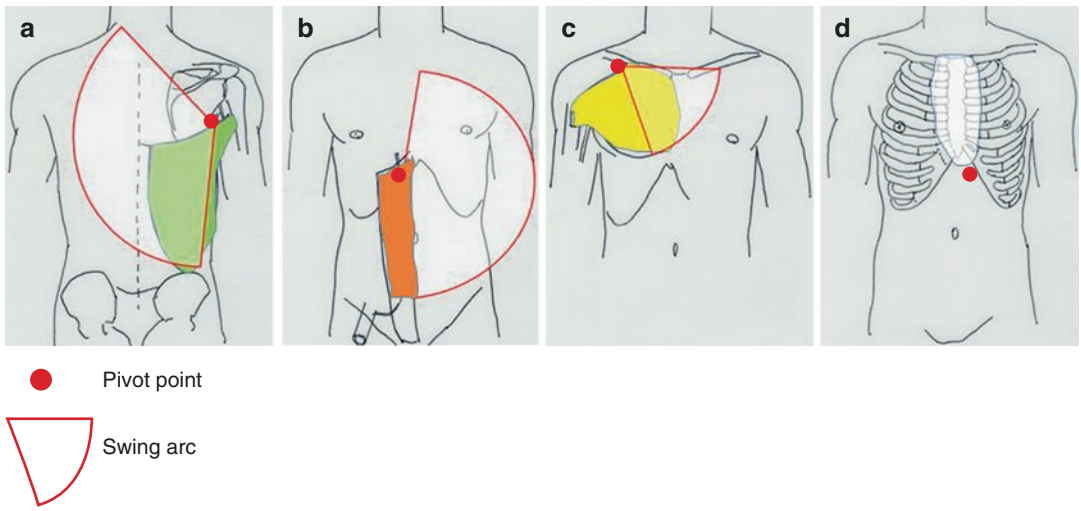

Fig. 2 Flaps used for reconstruction of sternal wounds. The red line dividing the sternum by upper and lower is made to match with the swing arc of the pectoralis major muscle flap (PM). Since the pivot point of the PM flap is fed by thoracoacromial artery, augmentation to the caudal site from the adhered site of the PM muscle is impossible

The application of NPWT for a short duration followed by definitive closure appears to be better than radical sternectomy with immediate flap closure [11].

Radical sternectomy including the entire sternum as well as the costal cartilages is recommended as opposed to conservative debridement alone. Current recommendation is to fill the defect is to use a composite flap technique that includes bilateral pectoralis major muscles with the overlying skin, latissimus dorsi muscle as well as the rectus abdominis muscle. The omental flap is a useful and effective flap, because of its resistance to infections. It is well-vascularized, has a large number of immunologically active cells, and absorbs wound secretions [12]. To realize less invasive omentopexy, as a new technique, usage of a laparoscopically harvested omental flap is considered [13].

In case of large skin defect that remains after tissue plombage to the dead space, reconstruction by skin graft or pedicled cutaneous flap is considered.

4.1 Major Pectoralis Muscle Flap

The pectoralis major muscle flap can be used in two ways. One is an advancement flap with a thoracoacromial pedicle released from the humerus, clavicle and external oblique. Another is a turnover flap based on perforators of the internal thoracic artery. Tri-pedicelled pectoralis major flap can be used to cover the entire sternum especially long and narrow defects [14]. The pectoralis major muscle flap is indicated for the upper part of the sternal defect. However, it is impossible for this flap to reach the back of the arch and to fill the deep sternal space. The great omental flap is selected to fill the periaortic arch space and the inter-mediastinum.

4.2 Rectus Abdominis Muscle Flap

The rectus abdominis muscle has two dominant pedicles. The superior pedicle is fed by the superior epigastric artery that is the terminal branch of the internal thoracic artery, and the inferior pedicle is fed by the inferior epigastric artery which is the branch of the external iliac artery. The rectus abdominis muscle can be used as a pedicle flap of the superior epigastric artery for the sternal wound. Therefore when the internal thoracic artery had already used as CABG, the rectus abdominis muscle flap cannot be used.

Furthermore, because the rectus abdominis muscle flap is used by the superior epigastric artery and sometimes the distal area (upper site of DSWI defect) of the flap undergoes necrosis caused by ischemia, the distal area of this flap is not usually used. Therefore this flap is indicated for the lower site of the sternal defect.

4.3 Latissimus Dorsi Muscle Flap

The primary vascular supply to the latissimus dorsi muscle arises from the thoracodorsal artery, which is a branch of the second part of the subscapular artery. The latissimus dorsi muscle flap is indicated for whole sternal defect. If it is necessary to fill the augmentation by large flap and muscle, the rectus abdominis muscles can be used. On the other hand, it requires body position change. LD flap is selected just when there is a large defect; moreover the rectus abdominis muscle cannot be used because ITA was already used.

4.4 Great Omental Flap: Right and Left Gastroepiploic Artery

The primary vascular supply to the greater omentum is derived from the right and left gastroepiploic artery.

It is possible for the greater omental flap to reach the back of the arch and to fill the deep sternal space. Furthermore, primary vascular supply to the greater omentum is derived from the right and left gastroepiploic artery. It is possible for the greater omental flap to reach the back of the arch and to fill the deep sternal space. Furthermore, the greater omental flap is very soft and flexible, following complicated shapes such as that of the aortic arch. As thin patients have small amount of this flap, enough augmentation is not done.

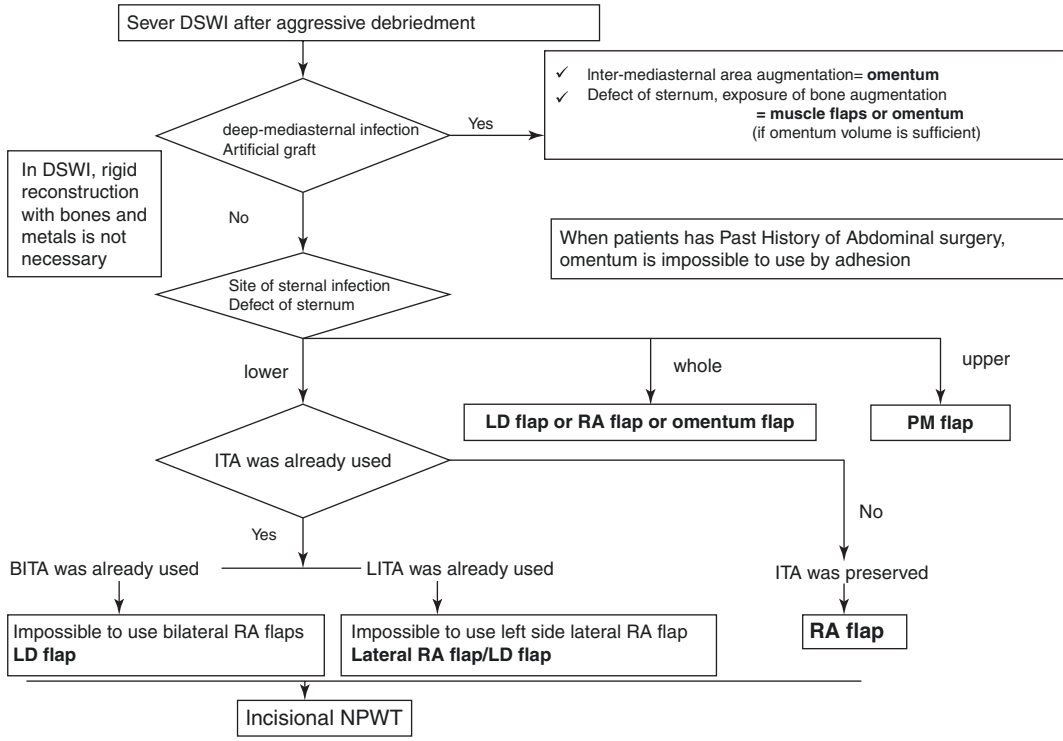

Fig. 3 Management of reconstruction using flaps

When the amount of fat in the great omental flap is large, it can be used for filling the sternal bone exposure near the body surface. When the amount of fat in the great omental flap is small, additional augmentation by the muscle flap such as pectoralis major muscle and rectus abdominis muscles is required. Omental Flap is the only flap that is capable of reaching the deep space of the mediastinum, so we consider that the great omental flap should not be used easily in shallow DSWI. When a deep DSWI is developed, there is no choice but to select that augmentation flap for filling later. Furthermore, it is impossible to use the great omental flap for adhesion when there is a past history of open abdominal surgery.

Reconstruction material was selected by the following algorithm (Fig. 3):

1. If there was deep mediastinal infection or not
2. Site of sternal infection: upper, whole, or lower
3. If ITA was intact or not

4.5 DSWI After Thoracic Aortic Surgery Using a Synthetic Prosthesis

Thoracic aortic operations using synthetic graft show high incidence of DSWI and high mortality, and aortic operation has significant risk factors related to both re-exploration for bleeding and DSWI in thoracic aortic surgery group. To treat DSWI involving synthetic graft infection, complete removal of the synthetic graft is theoretically essential (Fig. 4). However, not all patients are able to undergo re-replacement of the aorta because there is no ideal graft material that stands for infection, and re-replacement of the aorta using cardiopulmonary bypass is too invasive to treat compromised patients. Which graft material is the optimal graft material for the treatment of aortic graft infections is still a matter of controversy. Rifampicin-soaked Dacron grafts and cryopreserved arterial homografts are clinically

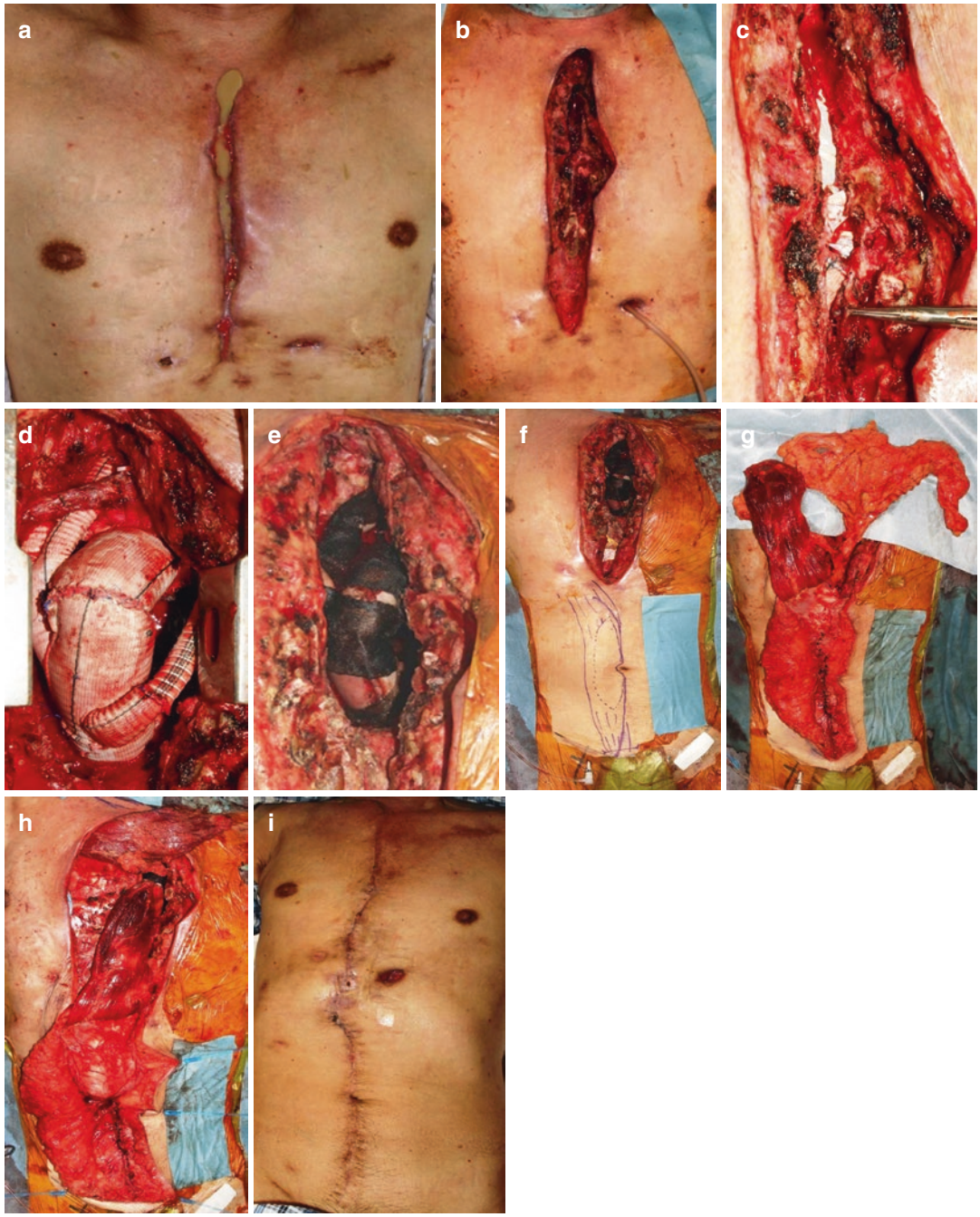

Fig. 4 (**a**) Deep sternal wound infection after replacement of the aortic arch using a synthetic graft. There was a large amount of pus that was discharged from the mediastinal space. (**b**) First stage of the radical partial sternectomy. (**c**) Partially exposed synthetic graft. (**d**) Second stage of the radical sternectomy. Large amount of purulent effusion was aspirated. Synthetic graft was fully exposed. Radical sternectomy including the entire sternum as well as the costal cartilages was performed. (**e**) Xeno-pericardial roll graft replacement. (**f**) Design of the rectus abdominis cutaneous muscle flap. Skin paddle was finally discarded. (**g**) Harvested rectus abdominis muscle and greater omental flap. Right gastroepiploic artery was pedicle of the omental flap. (**h**) Plombage of the rectus abdominis muscle and greater omental flap. (**i**) Muscle flap and omental flap without skin paddle were used, because skin defect was small and narrow. Wound was possible to suture directly without musculocutaneous flap

accepted. In our institute, orthotopic aortic reconstruction with intraoperatively prepared xenopericardial roll grafts with/without omentopexy and chest wall reconstruction with muscle flaps is performed as a first choice of rescue operation from 2009 [15]. Because of history of open abdominal surgery, the presence of an abdominal infection site, and hemodynamic instability, three patients did not undergo omentopexy. In the case that it is impossible to replace the synthetic graft, it is necessary to wash with a large amount of saline by the pulse lavage system such as Pulsavac®.

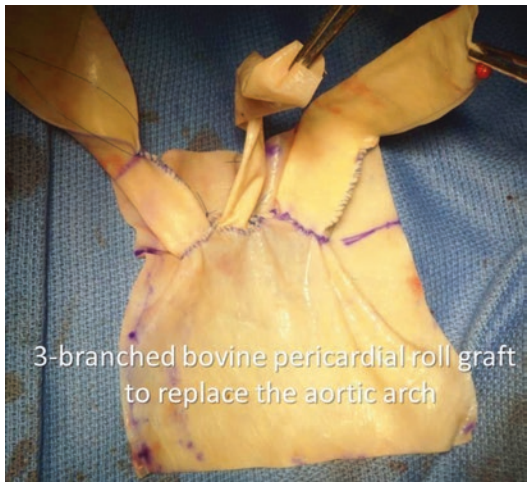

Fig. 5 Three-branched pericardial sheet

Figure 5 shows a three-branched pericardial sheet. Three holes in a row, two 10 mm holes and one 15 mm hole, were made 5 mm apart in a 10 × 10 cm equine pericardial sheet. Then three rectangular pericardial sheets were cut from another pericardial sheet, and each of them was sutured to the circumference of a hole and formed into a cylinder by continuous suturing with 5–0 polypropylene (Fig. 6). These branches were anastomosed to the native neck vessels.

Between 2009 and 2017, five patients underwent orthotopic xenopericardial roll graft replacements of the ascending aorta or aortic arch with an open distal anastomosis during deep hypothermic circulatory arrest with intermittent-pressure-augmented retrograde cerebral

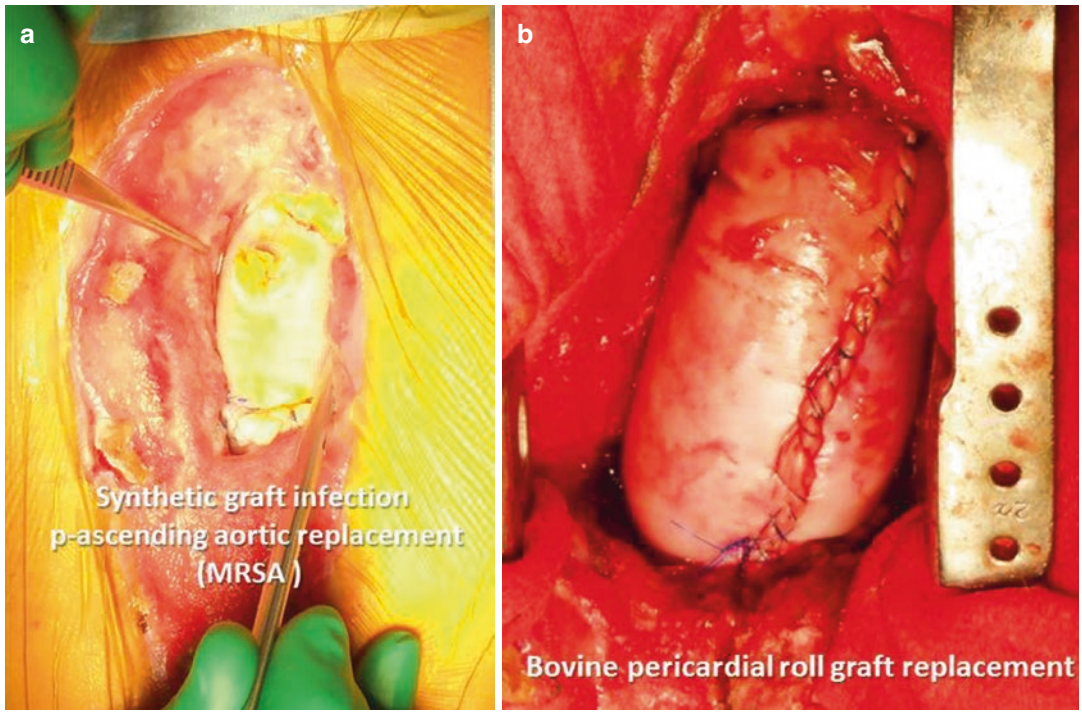

Fig. 6 Three rectangular pericardial sheets were cut from a pericardial sheet, and each of them was sutured to the circumference of a hole and formed into a cylinder by continuous suturing with 5–0 polypropylene

perfusion to treat DSWI involving graft infection [15–19]. In one patient, because of the postoperative dense adhesions after treating DSWI, the heart was not exposed at all. Graft material was equine or bovine pericardium.

Pathogens: Methicillin-resistant *Staphylococcus aureus* (MRSA) 2, methicillin-sensitive *Staphylococcus aureus* (MSSA) 2, and culture-negative 1

We followed the "Prevention and Treatment of Infective Endocarditis (Japanese Circulation Society 2008)" guidelines in regard to the antibiotic regimen. In accordance with the guidelines for active infective "native" valve endocarditis, the infection control team of our university administered the most appropriate antibiotic treatment to the patients in whom the causative organism had been identified. In culture-negative situations, we administered empiric antibiotic treatment. When inflammatory marker values had become normal and the diagnostic images no longer showed evidence of the infection, antibiotic treatment was stopped until 4–6 weeks. Otherwise, antibiotic treatment was continued.

None of the patients showed evidence of local recurrence of the infection or graft stenosis, calcification, or dilatation, including of the branches. No patients showed any evidence of a postoperative thromboembolic event. All patients are alive and well.

5 Discussion

Incidence and mortality of DSWI after cardiac surgery show that prevention of postoperative DSWI and establishment of the appropriate treatment for postoperative DSWI are the important factors to reduce the mortality after cardiac surgery.

In our study, previous CABG history was the most significant risk factor related to both re-exploration for bleeding and DSWI for all surgical groups. Age and cardiogenic shock were significant risk factors related to re-exploration for bleeding for all surgical groups, male gender, emergent or salvage operation, and operation combined with CABG were significant risk factors related to re-exploration for bleeding in two of three surgical groups. Diabetes control was a significant risk factor related to DSWI for all surgical groups. BMI, renal failure, COPD, cardiogenic shock, administration of inotropic agents, and triple vessel disease were significant risk factors related to DSWI in two of three groups. Aortic rupture and aortic arch operation were significant risk factors related to both re-exploration for bleeding and DSWI in thoracic aortic surgery group [10].

Thirty-day mortality in patients with DSWI with re-exploration for bleeding was significantly higher than DSWI without re-exploration in all operative procedure. It is known that patients who need re-exploration for bleeding after cardiac surgery are at higher risk of complications, morbidity, and mortality. Patients requiring resternotomy are at greater risk from the hazards of transfusion reactions, viral infections, and suppression of the immune system [20, 21]. Canadyova et al. [22] described that risk factors associated with higher inhospital mortality after re-exploration for bleeding and tamponade include delayed resternotomy, higher levels of lactate, lower levels of hematocrit before revision, older age, more complex cardiac procedures, redo operations, longer cardiopulmonary bypass, renal failure, and diabetes mellitus. If the time until re-exploration is prolonged, risk of complications, morbidity, and mortality will elevate. Kristensen et al. [23] reported that significant risk factors for reoperation for bleeding after cardiac surgery were low ejection fraction, high EuroSCORE, procedures other than isolated CABG, elongated time on cardiopulmonary bypass, low body mass index, diabetes mellitus, and preoperatively elevated s-creatinine. Surviving reoperated patients significantly had a lower EuroSCORE and a shorter time on cardiopulmonary bypass compared with non-survivors. Considering these results, to secure hemostasis to prevent re-exploration for bleeding is important to prevent DSWI, and when re-exploration for bleeding is required, prevention and earlier decision should be made to decrease DSWI after cardiac surgery. Kieser et al. [24] described that the use of chlorhexidine-alcohol and avoidance of BITA grafting in obese diabetic females

reduced DSWIs after BITA grafting in most diabetics.

Deniz et al. [25] studied the effectiveness of VAC therapy compared with conventional treatment outcomes at postoperative mediastinitis after cardiac surgery. Because the 90-day mortality was found significantly lower (8.5 vs. 23.2%) and overall survival at 1 year was significantly better (91.5 vs. 76.7%) in the VAC group than in the conventionally treated group, they concluded that VAC therapy was a safe and reliable option in DSWI after cardiac surgery.

Sachithanadan et al. [26] assessed the impact of DSWI on inhospital mortality and midterm survival following cardiac surgery in 4586 consecutive adult patients who underwent cardiac surgery via a median sternotomy. DSWI requiring revision surgery developed in 1.65% patients. Age, diabetes, a smoking history, and ventilation time were identified as independent predictors of a DSWI. DSWI patients were more likely to develop renal failure and require reventilation and a tracheostomy postoperatively. However, they detected that DSWI is not an independent predictor of higher inhospital mortality or reduced midterm survival compared with the patients without DSWI following cardiac surgery. They treated using VAC therapy in 81.5% patients. High-volume RBC transfusions and chronic infections are strongly associated with DSWI. RBC transfusion >4 units and the presence of chronic infection at the time of surgery represent important risk factors for DSWI after cardiac surgery.

Vacuum-assisted closure (VAC) therapy has shown promising results of wound healing process, postoperative hospital length of stay, and lower inhospital costs. Pericleous et al. [27] reported the outcome of patients with DSWI treated with VAC therapy and assessed the effect of contributory risk factors. Data of 52 patients who have been treated with VAC therapy from September 2003 to March 2012 were collected. Of the 52 patients (35 M:17 F), 88.5% (n = 46) were solely treated with VAC therapy, and 11.5% (n = 6) had additional plastic surgical intervention. Follow-up was complete (mean 33.8 months) with an overall mortality rate of 26.9% (n = 14), of whom 50% (n = 7) died in the hospital. No death was related to VAC complications, and logistic EUROscore, postoperative hospital length of stay, advanced age, chronic obstructive pulmonary disease (COPD), and long-term corticosteroid treatment appeared to be significant contributing factors in the long-term survival of patients treated with VAC therapy.

Tarzia et al. [28] retrospectively collected data from 7148 patients who underwent cardiac surgery between January 2002 and June 2012 and evaluated the results of the introduction of VAC therapy in the management of sternal wound dehiscence, compared with those of previous conventional treatments. Retrospectively collected data from 7148 patients who underwent cardiac surgery between January 2002 and June 2012 were examined. A total of 152 (2.1%) patients had a sternal wound dehiscence: 107 were treated with conventional treatments, and 45 were managed with VAC therapy. Patients were stratified according to preoperative risk factors and type of sternal wound dehiscence (superficial or deep; infected or not) and compared by means of a propensity-matched analysis. A cost analysis was also performed. Forty-five patients of each group matched for all preoperative risk factors and type of sternal wound dehiscence. SWD-related mortality rate was significantly lower in VAC therapy group (11 vs. 0%; $P = 0.05$). Incidences of mediastinitis, sepsis, delayed SWD infection, surgical sternal revision, and surgical superficial revision were all significantly lower in VAC group.

According to Japanese multicenter study, topical spraying of cefazolin and gentamicin reduces DSWI after cardiac surgery. Spraying was performed using spraying chip of fibrin glue and sprinkled widely over the operative field from the beginning to the end of the surgery intermittently [29].

Avoiding re-exploration for bleeding, earlier decision for re-exploration for bleeding, and development of the appropriate treatment including VAC therapy for DSWI may promise to improve the prognosis of cardiac surgery. The use of VAC has many possible advantages, like absorption of wound exudates, stimulation of

granulation-tissue formation, increase of blood flow in adjacent tissues, approximation of wound edges, and chest wall stabilization. Chang et al. [11] performed a retrospective chart review for all patients diagnosed with mediastinitis after open heart surgery, who had chest wall reconstruction. Although a wide range of potential flaps is available for sternal wound coverage, they found no significant difference in the development of postoperative complications caused by the type of flap closure on subset analysis. They also evaluated the morbidity and mortality associated with various approaches to treat mediastinitis and sternal wounds. The patients were divided into three groups who were treated with (1) conservative sternectomy, (2) radical sternectomy followed by immediate flap closure, or (3) radical sternectomy associated with a period of VAC therapy before definitive flap closure. In the third group treated with VAC therapy, an initial conservative sternectomy was performed at the first operation, with placement of the VAC over a layer of Xeroform (Covidien, Mansfield, MA) gauze covering the mediastinum. The VAC dressing was changed every 3 days until the patient was returned to the operating room for completion of the radical sternectomy followed by flap closure. As a result, 54 patients underwent chest wall reconstruction for poststernotomy mediastinitis. There were 15 patients in the conservative group and 8 patients in the radical sternectomy group who developed postoperative complications (62.5 vs. 33.3%, $P < 0.05$).

Tarzia et al. [28] also reported that because mean patient cost was 31,106€ in Group A and 24,383€ in Group B, thus achieving a mean savings of 6723€ per patient, the use of VAC therapy for the management of SWD was considerably effective in decreasing mortality (SWD related), incidence of complications, and need for surgical procedures, thus leading to a significant reduction of costs. Salles et al. [11] also reported that DSWIs triple the cost of a cardiac surgery and increase the length of hospital stay. Compared to a CABG operation without DSWI, the median cost of a single CABG with DSWI was $49.449 compared to $18.218 [30].

Recent advances in endoscopy have helped surgeons to find techniques to harvest the omentum laparoscopically. This has helped to minimize donor-site morbidity. Negative-pressure wound therapy, in combination with omentoplasty via diaphragm using laparoscopically harvested omentum and with the use of bilateral pectoral advancement flaps, is a valuable technique in the treatment of deep sternal wound infection because it produces good functional and aesthetic results [31].

Which graft material is most suitable for replacing an infected aorta is a matter of controversy. Although cryopreserved arterial homografts are excellent material for treat infected aortas, the supply is inadequate, and it is difficult to obtain one in time for an urgent operation. Autologous pericardium has been widely used to treat infective endocarditis, but its surface area is not great enough to reconstruct the great vessels, and bovine pericardium is great enough and twice as stiff as human pericardium [32]. Xenopericardium has been widely used to correct congenital cardiac defects, including as atrial and ventricular baffles and as a patches or valved conduits to enlarge the right ventricular outflow tract and main pulmonary artery. Bovine pericardium preserved in glutaraldehyde began to be used to enlarge the ascending aorta in 1979, and valved tubes were later used for total reconstruction of the ascending aorta [33, 34]. Salles et al. [35] listed the following as positive aspects of this bioprosthetic material: the softness of the biological tissue, which allows easy surgical handling, the good coaptation to suture lines resulting in a hemostatic anastomosis, and the lower thrombogenicity of glutaraldehyde-treated collagen tissue. Storage in bacteriostatic glutaraldehyde solution might provide long-term protection against graft infection. A lower incidence of infection in non-crimped bovine pericardial conduits used for replacement of the ascending aorta and arch has been reported in comparison with Dacron grafts [36], and the lower incidence may have been related to the antimicrobial activity of the glutaraldehyde absorbed into the pericardial tissue. Other researchers mention that residual glutaraldehyde maintains its antimicrobial prop-

erties during surgery and for several hours following conduit implantation [37, 38].

Czerny et al. [39]. reported the results of using bovine pericardial tube grafts to treat prosthetic graft infection or endovascular graft infection in 15 patients. They mentioned that this new concept, bovine pericardial tube grafts, may be superior to cryopreserved homografts because the likelihood of calcification seems to be less important and that another advantage of customized xenopericardial tissue is its availability, which turns out to be a problem with homografts. Pericardial sheets are soft and easy to handle. They can be formed into cylinders intraoperatively by rolling them up, and they provided us with a good operative field. It was easy to adjust the diameter of the roll graft to the diameter of the transected aorta. 10 cm of side length of the pericardium is ideal for construction of a neoaorta without trimming. Graft dilatation, mural thrombus formation, shrinkage, calcification, and recurrence of the infection are concerns during long-term follow-up.

The duration of postoperative antibiotic therapy is still a matter of controversy. We routinely prescribe antibiotics according to the Japanese guidelines for the treatment of infectious "native valve" endocarditis because the xenopericardium is a biomaterial.

Neck vessel reconstruction with xenopericardial branches in our patient has been challenging. A search of the literature retrieved no publications that described the same procedure. In view of the importance of adequate wide resection of the segment of aorta that contains the infected lesion, the arch vessels in our cases were reconstructed separately. Major concerns are stenosis due to shrinkage of the pericardium and stroke secondary to mural thrombi. Ho et al. [40] described the intermediate-term outcome of carotid endarterectomy with bovine pericardial patch closure in comparison with Dacron patch closure and primary closure. Although they used antiplatelet agents and Coumadin in only 13 and 0.2%, respectively, of their patients, stenosis had occurred in only 1.1% of the bovine pericardial patch angioplasty cases at 5 years, and the 5-year survival rate was significantly higher in the group who underwent bovine pericardial patch angioplasty than in the group who underwent Dacron patch angioplasty or primary closure. They concluded that bovine pericardial patch angioplasty following carotid endarterectomy was the best treatment. The need for treatment with anticoagulant or antiplatelet agents is also a matter of controversy. We recommend that patients who have undergone surgical individual reconstruction of aortic arch vessels be treated with an anticoagulant or antiplatelet drug to prevent strokes and graft stenosis secondary to mural thrombi.

In addition to the graft materials commonly used thus far, bioengineered tissue produced from pluripotent or multipotent stem cells, the so-called tissue-engineered vascular graft, may provide ideal vascular conduits [41].

Conclusions

Because DSWI is a serious complication after cardiac surgery, multidisciplinary treatment is required. To understand the incidence and risk factor of DSWI after cardiac surgery and to implement appropriate surgical treatment in combination with tissue debridement, reconstruction with muscle flaps; usage of omental flaps and VAC therapy according to each patient's anatomical, hemodynamic, and general condition; and improvement of substitutes for synthetic material are considered to be important to overcome DSWI after cardiac surgery.

References

1. Tang ATM, Ohri SK, Haw MP (2000) Novel application of vacuum assisted closure technique to the treatment of sternotomy wound infection. Eur J Cardiothorac Surg 17:482–484
2. Raudat CW, Pagel J, Woodhall D, Wojtanowski M, Van Bergen R (1997) Early intervention and aggressive management of infected median sternotomy incision: a review of 2242 open-heart procedures. Am Surg 63:238–241
3. Milano CA, Kesler K, Archibald N, Sexton DJ, Jones RH (1995) Mediastinitis after coronary artery bypass graft surgery. Circulation 92:2245–2251
4. Lu JC, Grayson AD, Jha P, Srinivasan AK, Fabri BM (2003) Risk factors for sternal wound infection and

midterm survival following coronary artery bypass surgery. Eur J Cardiothorac Surg 23:943–949
5. Gardlung B, Bitkover CY, Vaage J (2002) Postoperative mediastinitis in cardiac surgery-microbiology and pathogenesis. Eur J Cardiothorac Surg 21:825–830
6. Julian OC, Lopez-Belio M, Dye WS, Javid H, Grove WJ (1957) Appraisal of progress in surgical therapy. Surgery 42:753–761
7. Loop FD, Lytle BW, Cosgrove DM, Mahfood S, McHenry MC, Goormastic M, Stewart RW, Golding LAR, Taylor PC (1990) Sternal wound complications after isolated coronary artery bypass grafting: early and late mortality, morbidity and cost of care. Ann Thorac Surg 49:179–187
8. Milano CA, Kesler K, Archibald N, Sexton DJ, Jones RH (1995) Mediastinitis after coronary artery bypass graft surgery. Risk factors and long-term survival. Circulation 92:2245–2251
9. Mangram AJ, Horan TC, Peason ML, Silver LC, Jarvis WR (1999) Guideline for prevention of surgical site infection. Infect Control Hosp Epidemiol 20:247–278
10. Kubota H, Miyata H, Motomura N, Ono M, Takamoto S, Harii K, Oura N, Hirabayashi S, Kyo S (2013) Deep sternal wound infection after cardiac surgery. J Cardiothorac Surg 20(8):132
11. Chang EI, Festekjian JH, Miller TA, Ardehali A, Rudkin GH (2013) Chest wall reconstruction for sternal dehiscence after open heart surgery. Ann Plast Surg 71:84–87
12. Athanassiadi K, Theakos N, Benakis G, Kakaris S, Skottis I (2007) Omental transposition: the final solution for major sternal wound infection. Asian CardiovascThorac Ann 15(3):200–203
13. De Brabandere K, Jacobs-Tulleneers-Thevissen D, Czapla J, La Meir M, Delvaux G, Wellens F (2012) Negative-pressure wound therapy and laparoscopic omentoplasty for deep sternal wound infections after median sternotomy. Tex Heart Inst J 39(3): 367–371
14. Davison SP, Clemens MW, Armstrong D, Newton ED, Swartz W (2007) Sternotomy wounds: rectus flap versus modified pectoral reconstruction. Plast Reconstr Surg 120:929–934
15. Kubota H, Endo H, Noma M, Ishii H, Tsuchiya H, Yoshimoto A, Takahashi Y, Inaba Y, Nishino Y, Nunokawa M, Hosoi Y, Ikezoe T, Nemoto M, Makino Y, Nemoto Y, Matsukura M, Sugiyama M, Abe N, Takeuchi H, Nagao G, Kondo E, Yanagida O, Yoshino H, Sudo K (2015) Xenopericardial roll graft replacement for infectious pseudoaneurysms and graft infections of the aorta. J Cardiothorac Surg 10:133
16. Kubota H, Endo H, Noma M, Tsuchiya H, Yoshimoto A, Takahashi Y, Inaba Y, Matsukura M, Sudo K (2012) Equine pericardial roll graft replacement of infected pseudoaneurysm of the ascending aorta. J Cardiothorac Surg 7:54
17. Kubota H, Endo H, Noma M, Tsuchiya H, Yoshimoto A, Matsukura M, Takahashi Y, Inaba Y, Sudo K (2012) Equine pericardial roll graft replacement of infected pseudoaneurysm of the aortic arch. J Cardiothorac Surg 7:54
18. Kubota H, Tonari K, Endo H, Tsuchiya H, Yoshino H, Sudo K (2010) Total aortic arch replacement under intermittent pressure-augmented retrograde cerebral perfusion. J Cardiothorac Surg 5:97
19. Endo H, Kubota H, Tsuchiya H, Yoshimoto A, Takahashi Y, Inaba Y, Sudo K (2013) Clinical efficacy of intermittent pressure augmented-retrograde cerebral perfusion. J Thorac Cardiovasc Surg 145:768–773
20. Unsworth-White MJ, Herriot A, Valencia O, Poloniecki J, Smith EE, Murday AJ, Parker DJ, Treasure T (1995) Resternotomy for bleeding after cardiac operation: a marker for increased morbidity and mortality. Ann Thorac Surg 59:664–667
21. Miana LA, Atik FA, Moreira LF, Hueb AC, Jatene FB, Auler AO Jr, de Oliveira SA (2004) Risk factors for postoperative bleeding after adult cardiac surgery. Rev Bras Cir Cardiovasc 19:280–286
22. Čanádyová J, Zmeko D, Mokráček A (2012) Re-exploration for bleeding or tamponade after cardiac operation. Interact Cardiovasc Thorac Surg 14:704–708
23. Kristensen KL, Rauer LJ, Mortensen PE, Kjeldsen BJ (2012) Reoperation for bleeding in cardiac surgery. Interact Cardiovasc Thorac Surg 14:709–713
24. Kieser TM, Rose MS, Aluthman U, Montgomery M, Louie T, Belenkie I (2014) Toward zero: deep sternal wound infection after 1001 consecutive coronary artery bypass procedures using arterial grafts: implications for diabetic patients. J Thorac Cardiovasc Surg 148(5):1887–1895
25. Deniz H, Gokaslan G, Arslanoglu Y, Ozcaliskan O, Guzel G, Yasim A, Ustunsoy H (2012) Treatment outcomes of postoperative mediastinitis in cardiac surgery; negative pressure wound therapy versus conventional treatment. J Cardiothorac Surg 7:67
26. Sachithanandan A, Nanjaiah P, Nightingale P, Wilson IC, Graham TR, Rooney SJ, Keogh BE, Pagano D (2008) Deep sternal wound infection requiring revision surgery: impact on mid-term survival following cardiac surgery. Eur J Cardiothorac Surg 33:673–678
27. Pericleous A, Dimitrakakis G, Photiades R, von Oppell UO (2016) Assessment of vacuum-assisted closure therapy on the wound healing process in cardiac surgery. Int Wound J 13:1142–1149
28. Tarzia V, Carrozzini M, Bortolussi G, Buratto E, Bejko J, Comisso M, Mescola V, Penzo V, Guarino M, De Franceschi M, Pagnin C, Castoro M, Guglielmi C, Testolin L, Bottio T, Gerosa G (2014) Impact of vacuum-assisted closure therapy on outcomes of sternal wound dehiscence. Interact Cardiovasc Thorac Surg 19(1):70–75
29. Osawa H, Yoshii S, Abraham SJK, Okamoto Y, Hosaka S, Fukuda S, Tsuchiya K, Nakajima M, Honda Y, Takizawa K (2016) Topical spraying of cefazolin and gentamicin reduces deep sternal wound infections after heart surgery: a multicenter, large volume, retrospective study. Gen Thorac Cardiovasc Surg 64:197–202

30. Graf K, Ott E, Vonberg RP, Kuehn C, Haverich A, Chaberny IF (2010) Economic aspects of deep sternal wound infections. Eur J Cardiothorac Surg 37:893–896
31. Tebala GD, Ciani R, Fonsi GB, Hadjiamiri H, Barone P, Di Pietrantonio P, Zumbo A (2006) Laparoscopic harvest of an omental flap to reconstruct an infected sternotomy wound. J Laparoendosc Adv Surg Tech A 16(2):141–145
32. Miyamoto S, Hadama T, Tanaka H, Anai H, Sako H, Wada T, Iwata E, Hamamoto H (2004) MRSA aortic valve endocarditis treated by pericardium-lined Dacron patch and vancomycin-containing fibrin glue. Ann Thorac Cardiovasc Surg 10:252–254
33. Braile DM, Ardito RV, Greco OT (1983) Alargamento da raiz da aorta com 'patch' de paricardio bovino preservado pelo glutaraldehido. Arq Bras Cardiol 41:289–296
34. Ardito RV, Santos JLV, Mayorquim RC, Greco OT, Zaiantchic M, Soto HG, Jacob JLB, Braile DM (1987) Substituicao complete da aorta ascendente e da valve aortica com tubo valvulado de pericardio bovino. Rev Bras Cir Cardiovasc 2:129–138
35. Salles CA, Moreira MC, Borem PM, Gusmão JB, Teixeira VC, Silva RR, Prates HF, Araujo ES, Gelape CL, Silva SR (1998) Aortic reconstruction with crimped bovine pericardial conduits. J Heart Valve Dis 7(3):305–312
36. Salles CA, Puig LB, Casagrande IS, Vieira GL, Kalil RA, Souza LS, Andrade MA Jr (1991) Early experience with crimped bovine pericardial conduit for arterial reconstruction. Eur J Cardiothorac Surg 5:273–279
37. Gontijo B, Vrandecic M, Fantini F, Silva J, Barbosa JT, Ferrufino AB, Alcocer EO, Castro MF, Drumond LS, Barbosa MR et al (1995) Replacement of the ascending aorta and aortic arch with bovine pericardial grafts—a preliminary report. Eur J Cardiothorac Surg 9:127–132
38. Scott EM, Gorman SP (1991) Glutaraldehyde. In: Block SS (ed) Disinfection, sterilization, and prevention. Lea & Febiger, Philadelphia, pp 596–614
39. Czerny M, Allmen R, Opfermann P, Sodeck G, Dick F, Stellmes A, Makaloski V, Bühlmann R, Derungs U, Widmer MK, Carrel T, Schmidli J (2011) Self-made pericardial tube graft: a new surgical concept for treatment of graft infections after thoracic and abdominal aortic procedures. Ann Thorac Surg 92:1657–1662
40. Ho KJ, Nguyen LL, Menard MT (2012) Intermediate-term outcome of carotid endarterectomy with bovine pericardial patch closure compared with Dacron patch and primary closure. J Vasc Surg 55:708–714
41. Kurobe H, Maxfield MW, Breuer CK, Shinoka T (2012) Tissue-engineered vascular grafts for cardiac surgery: past, present, and future. Stem Cells Transl Med 1:566–567

Pedicled and Skeletonized Single and Bilateral Internal Thoracic Artery Grafts and the Incidence of Sternal Wound Complications

Andrew Bridgeman and Umberto Benedetto

1 Introduction

One of the major determinants of long-term outcomes in coronary artery bypass grafting (CABG) is the long-term patency of the conduit. Meanwhile the left internal thoracic artery (ITA) is unanimously acknowledged as the best coronary conduit [1]. The second best conduit is still widely debated [2]. Although the right ITA has equal function and patency rates as the left ITA, and despite mounting evidence on the long-term benefit of using bilateral ITAs (BITAs) over the past 20 years [3–5], the right ITA remains largely underused [6]. Concern about sternal wound complication is one of the main reasons limiting the use of more than one ITA. Post hoc analysis of the arterial revascularization trial (ART) demonstrated that, in the modern era of CABG surgery, sternal wound complications affect approximately 10% of patients. In particular, severe sternal wound infection requiring antibiotic therapy or sternal wound reconstruction still occurs in approximately 2 and 1% of the surgical population, respectively [7]. Given the detrimental effects of sternal wound complications, surgeons are cautious in employing techniques which may carry an increased risk of such complications. This is therefore an important consideration in the use of BITA.

There are two recognized techniques for harvesting the ITA: pedicled and skeletonized. Harvesting the ITA(s) with a pedicled technique may potentially lead to significant sternal devascularization [8, 9]. In contrast to the pedicled technique, the limited tissue mobilization associated with skeletonized ITA harvesting has been shown to preserve substantial collateral flow to the sternum by sparing sternal and intercostal branches that arise from the ITA as a common trunk [8, 9]. These results may have significant clinical implications with respect to reducing the risk of sternal wound complications by improving wound healing, particularly when both left and right ITAs are used [10]. The extent of such clinical benefit from skeletonized over pedicled ITA harvesting on sternal wound complications has been unclear [11, 12]. In addition, as skeletonized ITA harvesting is more technically demanding and time-consuming when compared to the pedicled technique, concerns have been raised over a perceived increased risk of injury to the ITAs during skeletonization that may affect early outcomes [13]. Consequently, in the absence of a general consensus, pedicled ITA harvesting remains the generally preferred approach worldwide.

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Recent evidence, however, has suggested that using a skeletonized technique with BITA grafting is associated with a similar risk of sternal wound complications to that of standard pedicled single ITA (P-SITA) harvesting [14].

2 Single Versus Bilateral Internal Thoracic Artery

The left ITA is widely accepted as the best coronary conduit [1] with a rate of over 90% angiographic patency at 10 years, compared to 50% for vein grafts [15–17]. Recent data from meta-analyses shows that the right ITA has angiographic superiority over saphenous vein graft (SVG) along with radial artery (RA) grafts [2]. This is in keeping with the long-term survival benefits observed with the use of BITA over SITA grafts [3, 4, 18–20]. However, despite increasing evidence of the long-term survival benefit of a second ITA, it remains largely underused, being used in only approximately 4% of CABG procedures in the United States [5] and 10% in the United Kingdom and Australia [21]. One of the major reasons for the low rates of BITA grafting is due to concerns over potential sternal wound complications, which are associated with substantially increased morbidity, inhospital mortality and cost [6]. Previous studies have found higher rates of sternal wound complications in BITA versus SITA grafting [7, 22]. Comparing 1-year costs of BITA and SITA grafts, ART found 9% higher costs for patients having undergone BITA compared to SITA grafts. This was due to longer theatre time and inhospital stays as well as slightly higher costs related to sternal wound complications during follow-up [23]. Data from the primary trial endpoint of 10-year follow-up is awaited. However, varying methods of harvesting the ITA exist, and these studies have not investigated the effect of harvesting technique on the rates of sternal wound complications. When these varying techniques have been analysed individually and compared in SITA and BITA grafting, the rates of sternal wound complications are found to be comparable [14], even in high-risk populations [24].

3 Pedicled Versus Skeletonized Harvesting

There are two widely established techniques for harvesting the internal thoracic artery. These are the pedicled and skeletonized techniques. The pedicled technique involves the dissection of the artery and accompanying vein, lymphatics and surrounding tissue away from the sternum, while the skeletonized technique involves the dissection of the artery away from the surrounding tissue, mobilizing only the artery itself.

Skeletonized harvesting has been proposed to minimize the risk of sternal wound complication by preserving sternal perfusion especially in the context of BITA use [6]. Research demonstrated better oxygen saturation and blood flow in the microcirculation of sternal tissue when using skeletonized rather than pedicled ITA harvesting [8]. Likewise, using radionuclide perfusion scanning, sternal perfusion was found to be greater after skeletonized rather than pedicled harvesting [9].

Whether skeletonized ITA harvesting should be considered the standard approach with BITA grafting and whether this approach also provides a significant benefit in SITA grafting are debated. The potential clinical superiority of skeletonized over pedicled harvesting on sternal wound complications has been addressed in only a few studies with conflicting results reported [11, 12]. Studies published to date are remarkably underpowered to detect any clinical benefit on low-rate events, such as sternal wound complications [12]. Moreover, skeletonized harvesting is more technically demanding and time-consuming, and, in the absence of general consensus, pedicled harvesting has been the preferred approach worldwide.

The ART is one of the largest studies of contemporary CABG with a high proportion of patients having undergone skeletonized ITA harvesting [7]. This study is one of the largest analyses of the impact of ITA harvesting performed to date. The trial found that skeletonized BITA (S-BITA) harvesting did not increase the risk of sternal wound complications when compared with pedicled SITA (P-SITA), and subgroup

analysis suggested a protective effect from S-BITA among high-risk subjects. On the other hand, pedicled BITA (P-BITA) grafts seemed to increase the risk of sternal wound complications among low-risk subgroups (i.e. those who are not obese or insulin-dependent diabetics). It found no evidence that skeletonized SITA (S-SITA) harvesting added any protective effect when compared with a P-SITA. It demonstrated that skeletonization while performing BITA was safe because it did not increase the risk of damage to the harvested ITA. In fact, the rate of an injured/unsatisfactory second ITA was 1.0% by using a skeletonized technique and 2.1% by using a pedicled technique, thus supporting previous reports [25]. Moreover, the mortality rate at 30 days and 1 year was comparable between the two techniques.

Conclusions

Evidence would suggest that BITA harvesting can be safely performed using the skeletonized technique without increasing the risk of sternal wound complications when compared with the standard approach using a P-SITA [14]. The most recent meta-analysis data demonstrates that not only is BITA grafting associated with more superior long-term survival but also, when adopting a skeletonized harvesting technique, there is an "almost negligible clinical difference in terms of deep sternal wound infection (DSWI) compared with SITA grafting" [26]. S-BITA harvesting does not seem to significantly increase the risk even in higher-risk groups, such as insulin-dependent diabetics, females and obese patients (BMI >30) who have been shown to benefit from the use of BITA grafting [24, 27, 28]. On the other hand, P-BITA is associated with an approximately twofold increased risk of any sternal wound complication [14]. The detrimental effect of P-BITA harvesting on sternal wound complication is relevant not only in high-risk cases but also in the lowest-risk CABG population (those who are not diabetic or obese), whereas S-BITA harvesting did not significantly increase the risk of sternal wound complications. Conversely, in the context of a SITA graft, there is no evidence of the superiority of S-SITA harvesting over P-SITA harvesting in reducing the risk of sternal wound complications.

References

1. Loop FD, Lytle BW, Cosgrove DM, Stewart RW, Goormastic M, Williams GW, Golding LA, Gill CC, Taylor PC, Sheldon WC et al (1986) Influence of the internal-mammary-artery graft on 10-year survival and other cardiac events. N Engl J Med 314:1–6
2. Benedetto U, Raja SG, Albanese A, Amrani M, Biondi-Zoccai G, Frati G (2015) Searching for the second best graft for coronary artery bypass surgery: a network meta-analysis of randomized controlled trials. Eur J Cardiothorac Surg 47:59–65
3. Taggart DP, D'Amico R, Altman DG (2001) Effect of arterial revascularisation on survival: a systematic review of studies comparing bilateral and single internal mammary arteries. Lancet 358(9285):870–875
4. Yi G, Shine B, Rehman SM, Altman DG, Taggart DP (2014) Effect of bilateral internal mammary artery grafts on long-term survival: a meta-analysis approach. Circulation 130:539–545
5. Tabata M, Grab JD, Khalpey Z, Edwards FH, O'Brien SM, Cohn LH, Bolman RM 3rd (2009) Prevalence and variability of internal mammary artery graft use in contemporary multivessel coronary artery bypass graft surgery. Circulation 120:935–940
6. Catarino PA, Black E, Taggart DP (2002) Why do UK cardiac surgeons not perform their first choice operation for coronary artery bypass graft? Heart 88:643–644
7. Taggart DP, Altman DG, Gray AM, Lees B, Gerry S, Benedetto U, Flather M, Investigators ART (2016) Randomized trial of bilateral versus single internal-thoracic-artery grafts. N Engl J Med 375:2540–2549
8. Kamiya H, Akhyari P, Martens A, Karck M, Haverich A, Lichtenberg A (2008) Sternal microcirculation after skeletonized versus pedicled harvesting of the internal thoracic artery: a randomized study. J Thorac Cardiovasc Surg 135:32–37
9. Boodhwani M, Lam BK, Nathan HJ, Mesana TG, Ruel M, Zeng W, Sellke FW, Rubens FD (2006) Skeletonized internal thoracic artery harvest reduces pain and dysesthesia and improves sternal perfusion after coronary artery bypass surgery: a randomized, double blind, within-patient comparison. Circulation 114:766–773
10. Sa MP, Cavalcanti PE, de Andrade Costa Santos HJ, Soares AF, Albuquerque Miranda RG, Araujo ML, Lima RC (2015) Skeletonized versus pedicled bilateral internal mammary artery grafting: outcomes and concerns analyzed through a meta-analytical approach. Int J Surg 16(Pt B):146–152

11. Sa MP, Ferraz PE, Escobar RR, Vasconcelos FP, Ferraz AA, Braile DM, Lima RC (2013) Skeletonized versus pedicled internal thoracic artery and risk of sternal wound infection after coronary bypass surgery: meta-analysis and meta-regression of 4817 patients. Interact Cardiovasc Thorac Surg 16:849–857
12. Onorati F, Esposito A, Pezzo F, di Virgilio A, Mastroroberto P, Renzulli A (2007) Hospital outcome analysis after different techniques of left internal mammary grafts harvesting. Ann Thorac Surg 84:1912–1919
13. Raja SG, Dreyfus GD (2005) Internal thoracic artery: to skeletonize or not to skeletonize? Ann Thorac Surg 79:1805–1811
14. Benedetto U, Altman DG, Gerry S, Gray A, Lees B, Pawlaczyk R, Flather M, Taggart DP, Arterial Revascularization Trial investigators (2016) Pedicled and skeletonized single and bilateral internal thoracic artery grafts and the incidence of sternal wound complications: insights from the Arterial Revascularization Trial. J Thorac Cardiovasc Surg 152:270–276
15. Fitzgibbon GM, Kafka HP, Leach AJ, Keon WJ, Hooper GD, Burton JR (1996) Coronary bypass graft fate and patient outcome: angiographic follow-up of 5,065 grafts related to survival and reoperation in 1,388 patients during 25 years. J Am Coll Cardiol 28:616–626
16. Weintraub WS, Jones EL, Craver JM, Guyton RA (1994) Frequency of repeat coronary bypass or coronary angioplasty after CABG using saphenous vein grafts. Am J Cardiol 73:103–112
17. Gansera B, Schmidtler F, Angelis I, Kiask T, Kemkes BM, Botzenhardt F (2007) Patency of internal thoracic artery compared to vein grafts — postoperative angiographic findings in 1189 symptomatic patients in 12 years. Thorac Cardiovasc Surg 55:412–417
18. Takagi H, Goto SN, Watanabe T, Mizuno Y, Kawai N, Umemoto T (2014) A meta-analysis of adjusted hazard ratios from 20 observational studies of bilateral versus single internal thoracic artery coronary artery bypass grafting. J Thorac Cardiovasc Surg 148:1282–1290
19. Rizzoli G, Schiavon L, Bellini P (2002) Does the use of bilateral internal mammary artery (IMA) grafts provide incremental benefit relative to the use of a single IMA graft? A meta-analysis approach. Eur J Cardiothorac Surg 22:781–786
20. Weiss AJ, Zhao S, Tian DH, Taggart DP, TD Y (2013) A meta-analysis comparing bilateral internal mammary artery with left internal mammary artery for coronary artery bypass grafting. Ann Cardiothorac Surg 2:390–400
21. Bridgewater B, Keogh B, Kinsman R, Walton P Sixth National Adult Cardiac Surgical Database Report 2008. https://www.scts.org/_userfiles/resources/SixthNACSDreport2008withcovers.pdf. Accessed 19 Sep 2017
22. Gray AM, Murphy J, Altman DG, Benedetto U, Campbell H, Flather M, Gerry S, Lees B, Taggart DP (2017) One-year costs of bilateral or single internal mammary grafts in the Arterial Revascularisation Trial. Heart 103(21):1719–1726
23. Itagaki S, Cavallaro P, Adams DH, Chikwe J (2013) Bilateral internal mammary artery grafts, mortality and morbidity: an analysis of 1 526 360 coronary bypass operations. Heart 99:849–853
24. Benedetto U, Montecalvo A, Kattach H, Amrani M, Raja SG, Harefield Cardiac Outcomes Research Group (2015) Impact of the second internal thoracic artery on short- and long-term outcomes in obese patients: a propensity score matched analysis. J Thorac Cardiovasc Surg 149:841–847
25. Athanasiou T, Crossman MC, Asimakopoulos G, Cherian A, Weerasinghe A, Glenville B, Casula R (2004) Should the internal thoracic artery be skeletonized? Ann Thorac Surg 77:2238–2246
26. Buttar SN, Yan TD, Taggart DP, Tian DH (2017) Long-term and short-term outcomes of using bilateral internal mammary artery grafting versus left internal mammary artery grafting: a meta-analysis. Heart 103:1419–1426
27. Cho KR, Kim JS, Choi JS, Kim KB (2006) Serial angiographic follow-up of grafts one year and five years after coronary artery bypass surgery. Eur J Cardiothorac Surg 29:511–516
28. Wee CC, Girotra S, Weinstein AR, Mittleman MA, Mukamal KJ (2008) The relationship between obesity and atherosclerotic progression and prognosis among patients with coronary artery bypass grafts the effect of aggressive statin therapy. J Am Coll Cardiol 52:620–625

Part II

Neurosurgery

Wound Management and Wound Infections in Neurosurgery

Stephanie Schipmann, Eric Suero Molina, Michael Schwake, and Walter Stummer

1 Introduction

The central nervous system (CNS) is known as a delicate and vulnerable system enjoying protection by multiple layers of covering, providing an anatomical barrier against microbial invasion. During surgery these barriers are breached, increasing the risk of infection. The CNS differs from other systems in having less immune activity and lower immunoglobulin levels [1]. Therefore, organisms are able to invade and multiply more quickly, harming the host with a higher mortality than in other parts of the body. Infections spreading to the CNS often have devastating complications increasing morbidity and mortality [2].

Therefore, a thorough understanding of factors associated with infections in neurosurgical patients is crucial, in order to avoid infections in the first place. This chapter focuses on such factors and offers strategies for the control of infectious complications.

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2 History of Wounds and Their Management in Neurosurgery

The first neurosurgical operations, trepanations, were described more than 10,000 years ago. Surgery in the past was primarily done for emergency and life-threatening cases, as wound infections were dreaded.

Hippocrates (460–377 BC), known as the "Father of Medicine," was the first to document on treating cranial wounds. His treatise "On Wounds in the Head" comprises a collection of accurate observation of head wounds and detailed description of anatomic aspects of the layers involving the cranium [3]. Hippocrates stated that the localization of the wound has an impact on outcome, as parts with thin layers of flesh, e.g., the bregma, are generally associated with a more unfavorable outcome: "the bone in this part of the skull is more readily crushed, fractured or crushed in, and (the injuries) are more often fatal, and medical cure and escape from death more difficult (with wounds) here than in any other part of the head" [3]. He was aware that primary closure of head injuries might result in infectious complications and recommended open healing so that the wound could drain and heal secondly by granulation [4]:

You should rather make the wound suppurate as quickly as possible [3].

In addition, Hippocrates depicted the importance of a dry wound for effective healing.

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In antique medicine the concept of the formation of pus was seen as a critical step in wound healing. This doctrine of suppuration was reiterated by Galen (130–216) and maintained until the nineteenth century [5]. Therefore, surgeons of the early nineteenth century welcomed the appearance of pus in their wounds according to the assumption of Galen [5, 6].

A milestone in the development of modern neurosurgery clearly was the establishment of methods for the control of wound infections. Prior to infection control, half of all patients died due to infection after major operations [6].

In the second half of the nineteenth century, Joseph Lister (1827–1912) developed aseptic principles and introduced them into the world of surgery, leading to a significant reduction in the manifestation of postoperative wound infections [7]. Together with the studies of Robert Koch on pyogenic organisms and sterilization and Louis Pasteur's germ theory, these methods allowed the results of elective surgery become acceptable [6]. William W. Keen (1837–1932) was the first neurosurgeon to implement the infection control principles of Lister, comprising the generation of a clean operating environment, shaving and scrubbing the patients head, and cleaning of surgical instruments and the surgeon's hands [5]. In 1887 Keen (Fig. 1) was the first American neurosurgeon operating on a meningioma *on an elective basis*. The patient, a young man, recovered from surgery without major deficit and lived for another 30 years after surgery [8].

Fig. 1 Portrait of William W. Keen (1837–1932) (Image courtesy of the National Library of Medicine)

During World War I, Harvey Cushing (1869–1939), probably the most influential surgeon on modern neurosurgery, introduced techniques for the reduction of infections of traumatic brain wounds. Before Cushing, most traumatic brain injuries were treated by cruciate scalp incision, small craniotomy, and drainage of the wound through an unclosed scalp. These open drainage techniques lead to a mortality rate of up to 60% [9].

The most challenging aspects of Cushing's treatment concept were primary wound closure, removing the bone around the site of cranial penetration, the use of antiseptics (Dakin's dichloramine-T), and debridement [10].

Certainly infections cannot be attributed to the intervention of the devil but must be laid at the surgeon's door [11].

Harvey Cushing's precise surgical techniques, attention to closure of wounds, and perception to details enabled him a breakthrough in the treatment of intracranial pathologies. In 1915, Cushing reported a mortality rate of 8.4% for his series of 130 surgically treated brain tumors. Only one of these deaths was attributed to a postoperative wound infection [11]. These results compare favorably to today's surgical site infection rates. Today, one hundred years later, infection rates for brain tumor surgery still range from 2.04% [12] to 17.5% [13] in comparison.

3 Anatomy of the Scalp

Managing cranial wounds, planning surgical approaches and flaps, and a good knowledge of the anatomy of the scalp and its layers are essential. The scalp comprises the soft tissue covering the cranial vault. It extends from the eyebrows to the superior nuchal line; laterally it reaches down the zygomatic arch and the ear [14, 15]. The scalp consists of five layers, which are now described in detail (Fig. 2).

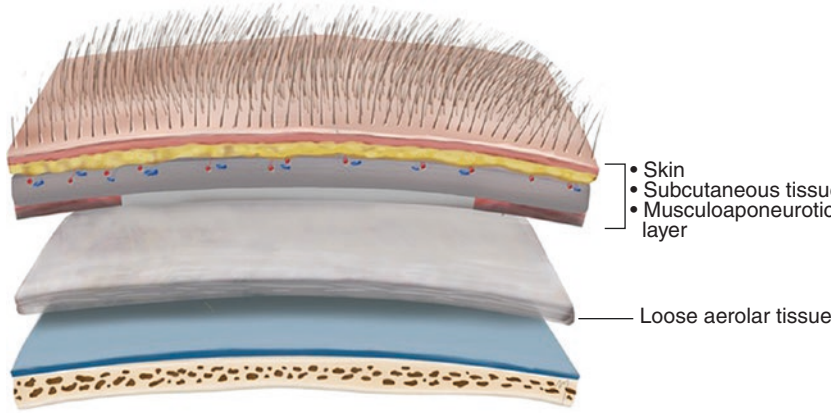

Fig. 2 Layers of the scalp (Reprinted with permission http://plasticsurgerykey.com/scalp-and-temple Accessed 2/17/17)

3.1 Skin

The scalp skin is thicker than the skin elsewhere on the body ranging from 3 mm to 8 mm. The skin is divided into an external epidermis and inner dermis, containing sebaceous and sweat glands and hair follicles [15, 16]. It is firmly attached to the deeper tissues [14].

3.2 Subcutaneous

The subcutaneous connective tissue comprises the principal blood vessels and nerves of the scalps, forming a rich vascular network [15]. The fat lobules are separated by multiple fibrous septa [16].

3.3 Galea Aponeurotica

The galea aponeurotica arises from the superior nuchal line and connects the bellies of the occipitofrontalis muscle [16]. Laterally, it extends as a thin sheet overlying the temporalis fascia [15]. It is made of 1–2-mm-thick dense fibrous tissue. Its surface is closely attached to the subcutaneous tissue [16]. The galea contains the vessels that ramify into a plexus in the subcutaneous layer as well as in the deeper subgaleal layer [17].

3.4 Subgaleal Layer

This layer consists of loose connective tissue allowing the mobility of the scalp on the underlying skull [15]. It becomes thicker in the temporoparietal regions [14]. The layer is less vascularized [16]. It is of great surgical importance as it is the level for the mobilization of scalp flaps.

3.5 Periosteum

The periosteum is a dense fibrous sheet that adheres tightly to the outer table of the skull, particularly along the suture lines [16].

4 Blood Supply of the Scalp

The blood supply of the scalp is provided by a system of anastomoses between arteries from the external and internal carotid artery (Fig. 3).

From the external carotid artery derive:

1. Occipital artery
2. Posterior auricular artery
3. Superficial temporal artery

From the internal carotid artery derive (branch: ophthalmic artery):

1. Supraorbital artery
2. Supratrochlear artery

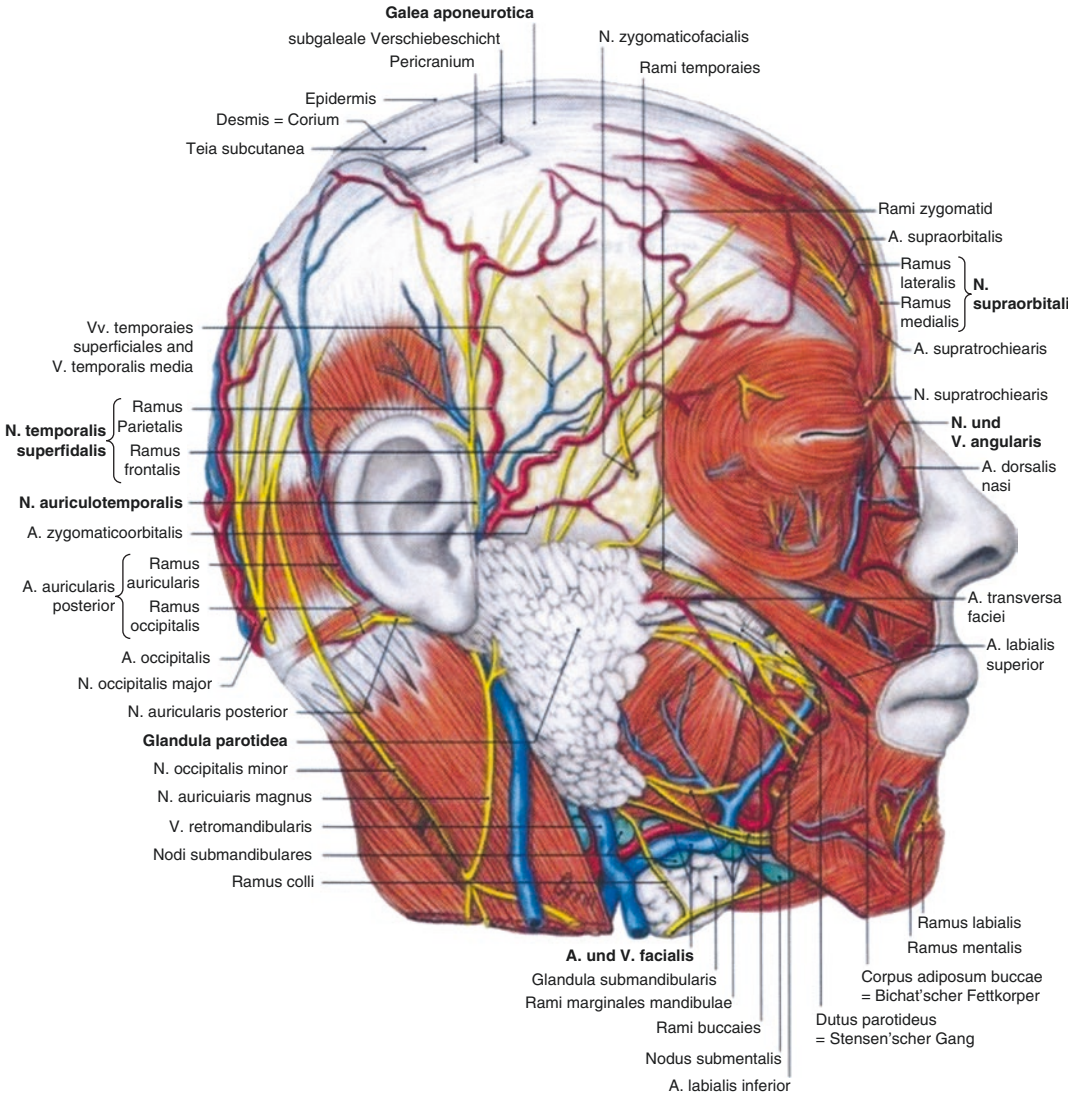

Fig. 3 Blood and nerve supply of the scalp [43] (Reprinted with permission)

Corresponding veins accompany the arteries. Additionally, veins have multiple connections to diploic veins and to the cerebral sinuses [18].

The main blood vessels run on the superficial surface of the galea aponeurotica, being closely attached to the subcutaneous fat tissue and sending penetrating branches superiorly to the subcutaneous tissue and to the less vascularized subgaleal layer [14, 16]. This anatomic principal is of great surgical importance as surgical dissection is usually performed within the subgaleal layer and the blood supply of the tissue is not at risk [16]. Due to the rich anastomoses, the viability of the scalp is not endangered even if only one major artery is preserved for an extensive scalp flap [15].

5 Surgical Techniques of Wound Closure and Approaches: Practical Implications in Neurosurgery

5.1 Skin Incision and Design of Surgical Flap

Planning a surgical approach, several factors have to be considered. Beside enabling a sufficiently large craniotomy to reach the underlying pathology, blood and nerve supply have to be considered when planning the skin incision. Due to the wide vascular network and several anastomoses, necrosis of the scalp flap is very uncommon. However, in patients with the history of irradiation or patients that are likely to receive radiotherapy after tumor resection or in case of reoperation through the same approach, blood supply might be compromised. In these cases, special attention should be paid to preserve the mayor supplying vessels. In addition, the sensory nerve supply has to be considered, and incisions damaging large neurovascular trunks should be avoided as this might result in extensive hypesthetic and poorly vascularized tissues [16].

When cutting the scalp, some arteries are held open by dense connective tissue leading to profusely bleeding [19]. To avoid hemorrhage, the use of self-retaining scalp clips has been established. These clips are placed on the upper three layers of the scalp and provide sufficient hemostasis due to their inherent closing pressure [20].

5.2 Shaving

For a long time preoperative shaving of the head has been a well-established practice among neurosurgeons. It was believed that hair removal led to a reduction of postoperative surgical site infections and was essential for the maintenance of a sterile operating field. In addition, shaving improves visualization of the incision line and facilitates wound closure and dressing application. However, several studies failed to show an increase in wound infections in patients being not shaved [21, 22]. Conversely, study results indicated even a higher risk for infection after shaving. This might be due to bacterial colonization caused by razor-induced epidermal injury or change in the protective skin flora [23].

During the last decade, there has been a trend toward less hair removal. Some surgeons just perform hair clipping without any hair removal [21]. Beside a possible impact on the incidence of surgical site infections, limited hair removal has a considerable cosmetic value for the patient [21]. Strictly speaking, however, prospective randomized controlled trails are needed to investigate the exact role of shaving before neurosurgical procedures.

5.3 Suture

Not only is the aim of sutures to close the wound effectively and restore the physical integrity but also to create a cosmetically adequate wound without complications. The scar has become one of the patient's measures to define a good standard of care. Given the anatomic features of the scalp and its layers, a two-layered wound closure is favored in neurosurgical practice [15]. The use of absorbable sutures like polyglycolic acid (Vicryl®) is recommended for closure of the deeper layers, including the galea aponeurotica. It is important to incorporate the periosteum in deep wound closure as it contributes to the generation of a fine linear scalp scar [16]. For skin closure, monofilament nylon can be used due to its properties such as reduced tissue trauma and low incidence of adherence and descending microorganisms. As an alternative, metal skin staples can be applied, which are timesaving while producing cosmetically acceptable results.

5.4 Use of Prophylactic Antibiotics

In the past, the use of prophylactic antibiotics was common under neurosurgeons due to the fear of life-threatening infectious complications. The

choice of the antibiotic regime was based mainly on individual experience. Many surgeons tended to use wide-spectral and prolonged antibiotic therapies.

Over the years, several trials have been conducted and showed the efficacy of prophylactic antibiotics in the reduction of postoperative surgical site infections and postoperative meningitis [24, 25]. In the face of increasing antimicrobial resistance and concerns about side effects, new guidelines recommending narrow-spectrum antibiotic prophylaxis have recently been implemented.

The major pathogen in clean neurosurgery is *Staphylococcus aureus*, arising from the skin flora. The main aim of antibiotic prophylaxis is to reduce the bacterial inoculum before skin incision and during surgery [26]. A prophylactic antibiotic regimen should cover the most likely organisms and be adapted to local hospital guidelines. Regular examinations of the main organism causing wound infections should be performed, and local stakeholders should include these results in their specific hospital guidelines [27].

For clean neurosurgical procedures, e.g., craniotomies, preoperative narrow-spectrum single-shot antibiotics, e.g., cefuroxime 30–60 min before incision, are recommended [28]. Depending on the length of surgery, the administration of the antibiotics should be repeated every 3–4 h. The prolonged use of antibiotic prophylaxis has failed to show benefits. However, there is a lack of randomized controlled trails addressing this issue.

6 Risk Factors for Wound Infections

Due to its rich vascular network, the scalp is an area with a high wound healing potential. However, about 2.47% of all neurosurgical patients develop wound infections [26]. Several risk factors for wound infections have been described. The number of previous surgeries was found to be an independent risk factor for wound infections after craniotomy [25, 26, 29]. Multiple operations lead to the formation of scar tissue and altered blood supply, possibly contributing to higher susceptibility to wound infections. In addition, the duration of surgery is another independent risk factor [12, 25, 30, 31]. Duration of surgery can reflect surgical difficulties, complexity of the operation, and the surgeon's experience, among others [26]. In long-lasting operations, the risk of contamination during surgery is increased making the surgical site vulnerable to pathogens for a longer time period [30, 32]. Hardy et al. [30] could show a 43% increase of the risk to develop wound infections with each additional hour in surgery in patients undergoing craniotomy due to a brain tumor. As a consequence, strategies for optimizing structural processes are necessary, e.g., minimizing delays once the patient is in the operating room.

Several authors reported postoperative CSF leak as a risk factor for neurosurgical wound infections [25, 33–35]. CSF leaks provide a portal of entry for organisms and lead to a higher risk for meningitis [36].

The patient's immune status might influence the manifestation of wound infection. Referring to this, risk factors associated with the development of wound infections are preoperative use of steroids, chemotherapy within 30 days preoperative, diabetes mellitus, postoperative sepsis, and recent systemic infection [12, 31, 37].

Furthermore, studies revealed the implantation of foreign material as a risk factor for wound infection. Other studies reported the use of surgical drains and external ventricular drains as being significantly associated with the occurrence of wound infections [29, 32, 34, 38].

In addition, length of stay on the intensive care unit (ICU) was shown to be associated with a higher risk for wound infections. This might be due to multifactorial reasons, comprising concomitant diseases, immunosuppression, and a higher likelihood of skin colonization in the ICU [26]. Complementarily, other studies revealed the factor length of stay as being associated with wound infections [25, 39].

The knowledge of potential risk factors is essential for risk stratification, planning, and timing of surgery. Based on the risk factors, a patient collective at high risk can be identified, requiring a close follow-up in order not to miss potentially devastating wound infections.

7 Diagnosis and Management of Wound Infections

Surgical site infections are classified by the Centers for Disease Control and Prevention (CDC) as superficial, deep incisional, and organ-space infections. Superficial infections comprise infections of the scalp [40]. Deep incisional infections affect the bone and epidural space, e.g., leading to an epidural empyema (Fig. 4). Organ-space infections in neurosurgery include brain abscess, subdural empyema, and meningitis.

7.1 Diagnosis of Wound Infections

Local signs of neurosurgical wound infections comprise swelling, warmth, pain, erythema, and purulent discharge. Systemic symptoms like high temperature, elevated infectious parameters in the blood, lassitude, and meningism can occur.

Assuming deeper wound and craniotomy flap infections, contrast-enhanced MRI or CT scan helps in defining the extent of the infection. In addition, diffusion-weighted MR imaging (DWI) and apparent diffusion coefficient (ADC) help in distinguishing abscess from other pathologies (Fig. 5) [41]. Lumbar puncture for cerebrospinal

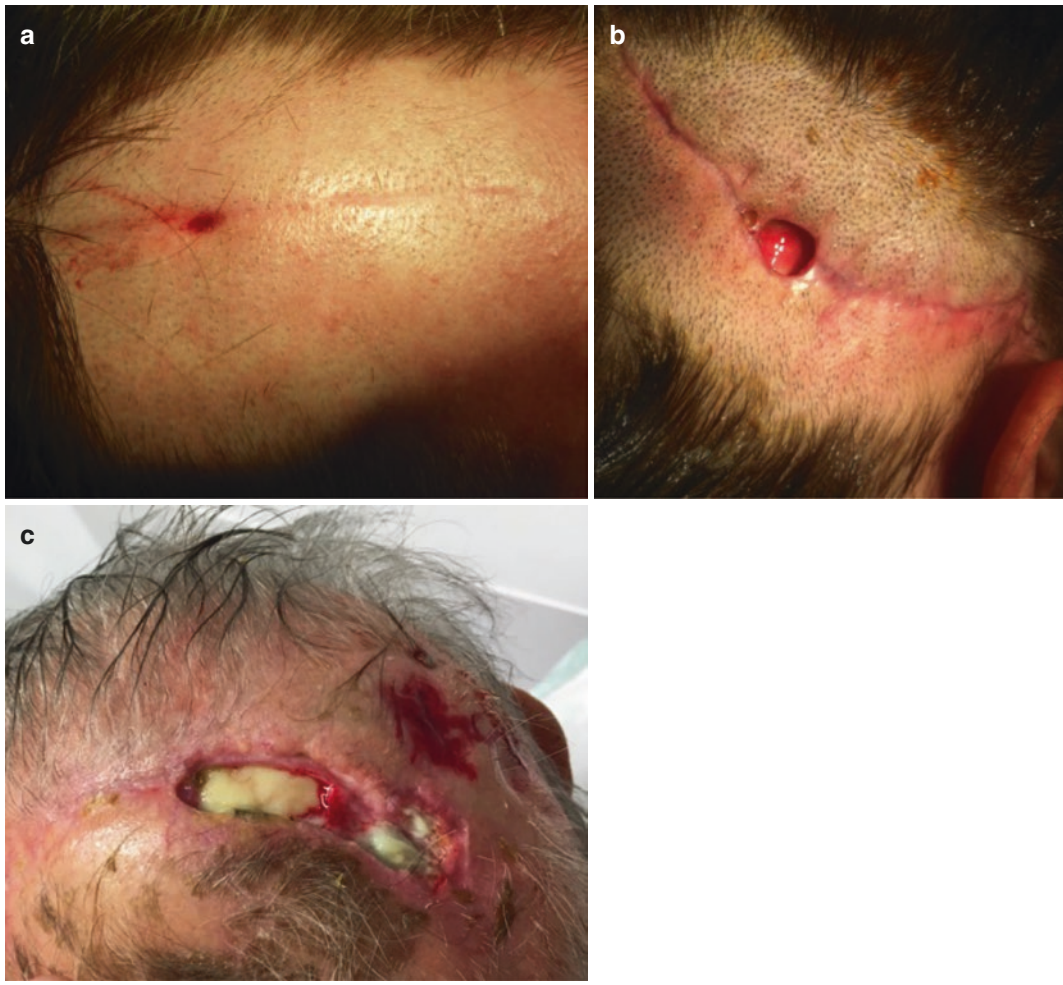

Fig. 4 Types of wound infections. (**a**) Superficial wound infection. (**b**) Deep wound infection. (**c**) Deep wound infection with exposure of cranioplasty

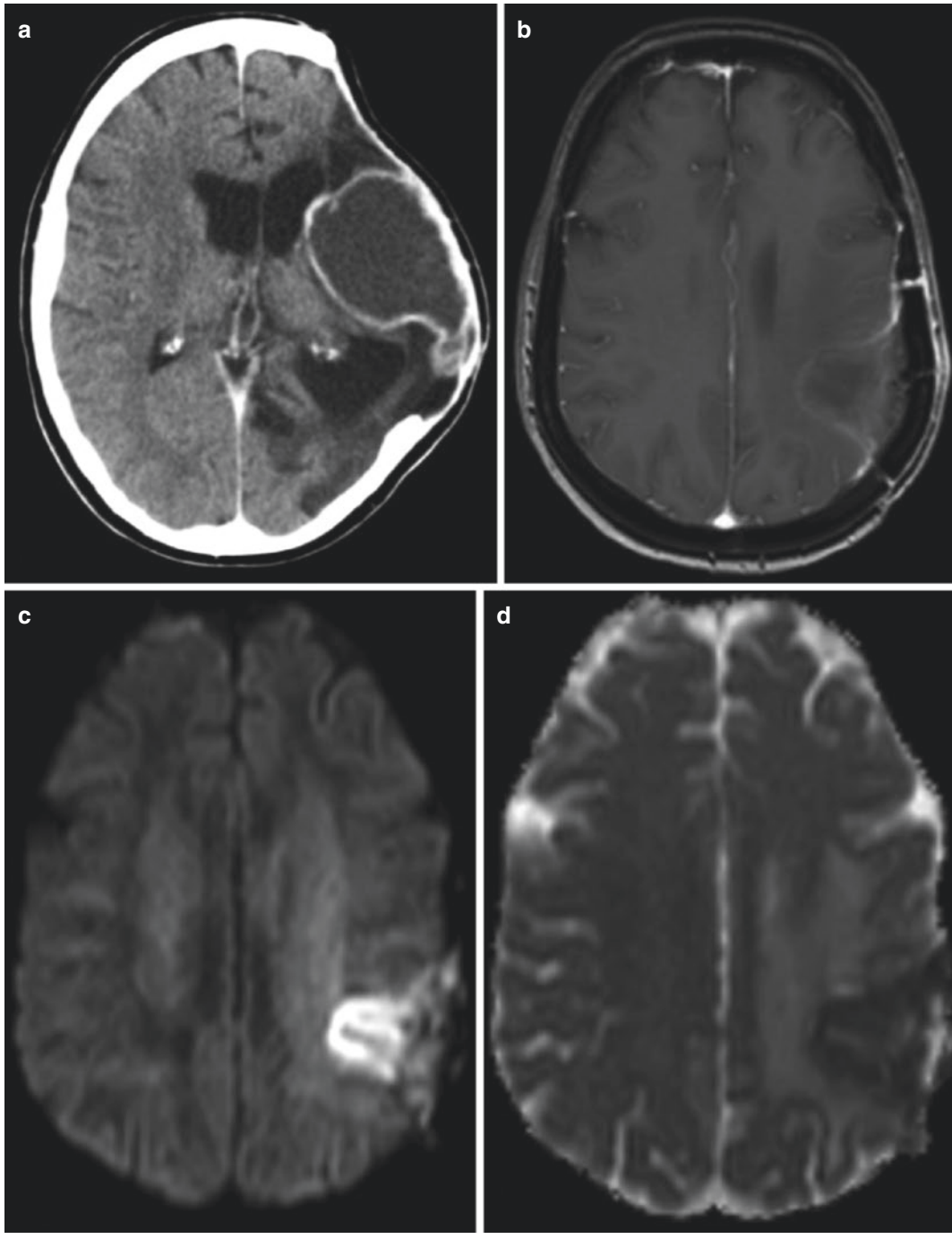

Fig. 5 (**a**) Contrast-enhanced CT scan of a patient after decompressive hemicraniectomy due to stroke with ring-enhancing lesion with central low attenuation, typical for a cerebral abscess. (**b**) Contrast-enhanced MRI scan of a patient after multimodal treatment of a left parietal glioblastoma showing a contrast-enhancing lesion, that has a high DWI signal centrally. (**c**) Shows low signal on ADC. (**d**) Representing true restricted diffusion, indicating the presence of a cerebral abscess (Reprinted with permission)

fluid (CSF) examination is necessary for the diagnosis of meningitis.

7.2 Treatment

Due to the potential spreading of the infection into the brain, an aggressive treatment should be advocated. Only minor superficial infections should be treated with local antiseptic means and wound dressings. Failure of local therapy and presence of deeper infections require treatment consisting of systemic antibiotics and surgical debridement.

Surgical wound revision comprises removal of all purulent material, visible sutures, and mechanical debridement. In case of infected bone flap, the bone should be removed preventing it from being a source for chronic infection. The dura mater provides a mechanical barrier and should not be opened unless subdural infection is assumed. In case of organ-space infection, thorough drainage and insertion of abscess and/or subdural drains are recommended. After bone flap removal, a cranioplasty with computer-assisted designed implants can be performed subsequently. There is no clear consensus at which time cranioplasty should be performed; intervals of 6–12 weeks have been advocated. Medical treatment of infection should be finished, and the wound should be closed at the time of cranioplasty [42].

Systemic antibiotic therapy should be adapted to the depth and extent of the infection according to the CDC classification. Prior to the administration of antibiotics, microbiological cultures should be obtained. The most likely organisms causing neurosurgical wound infections are gram-positive cocci, with *Staphylococcus aureus* being the most frequent organism found, followed by *Staphylococcus epidermidis*, both accounting for 40% of wound infections after craniotomy [26].

The choice for initial calculated antibiotic therapy should consider the most likely causative organisms and the ability of the antibiotics to penetrate into the infected region.

Duration of antibiotic therapy depends on the extent of the infection. In case of deep infections involving the bone and brain, treatment durations up to 6 weeks are required.

In case of superficial wound infections, second-generation cephalosporins like cefuroxime are recommended; in case of known allergies, clindamycin is favored. For deep wound infections, broad-spectrum ß-lactam antibiotics in combination with ß-lactam inhibitors are feasible. Infections extending into the CSF space and brain require broad antibiotic therapy covering anaerobic organisms, e.g., with a combination metronidazole, vancomycin, and third-generation cephalosporins like ceftriaxone. According to the microbiological results, the presence of multiresistant organisms and antibiogram antibiotic therapy should be adapted.

References

1. Simberkoff MS, Moldover NH, Rahal J Jr (1980) Absence of detectable bactericidal and opsonic activities in normal and infected human cerebrospinal fluids. A regional host defense deficiency. J Lab Clin Med 95(3):362–372
2. O'Keeffe AB, Lawrence T, Bojanic S (2012) Oxford craniotomy infections database: a cost analysis of craniotomy infection. Br J Neurosurg 26(2):265–269
3. Hanson M (1999) Hippocrates on head wounds. Walter de Gruyter GmBh, Berlin
4. Panourias IG, Skiadas PK, Sakas DE, Marketos SG (2005) Hippocrates: a pioneer in the treatment of head injuries. Neurosurgery 57(1):181–189
5. Miller JT, Rahimi SY, Lee M (2005) History of infection control and its contributions to the development and success of brain tumor operations. Neurosurg Focus 18(4):e4
6. Alexander JW (1985) The contributions of infection control to a century of surgical progress. Ann Surg 201(4):423–428
7. Lister J (1875) An address on the effect of the antiseptic treatment upon the general salubrity of surgical hospitals. Br Med J 25(782):769–771
8. Stone JL (1985) W. W. Keen: America's pioneer neurological surgeon. Neurosurgery 17(6):997–1010
9. Carey ME (2011) Cushing and the treatment of brain wounds during World War I. J Neurosurg 114(6):1495–1501
10. Cushing H (1918) Notes on penetrating wounds of the brain. Br Med J 1(2982):221–226

11. Cushing H (1915) Concerning the results of operation for brain tumor. JAMA 64:189–195
12. McCutcheon BA, Ubl DS, Babu M, Maloney P, Murphy M, Kerezoudis P, Bydon M, Habermann EB, Parney I (2016) Predictors of surgical site infection following craniotomy for intracranial neoplasms: an analysis of prospectively collected data in the American College of Surgeons National Surgical Quality Improvement Program Database. World Neurosurg 88:350–358
13. Kamme C, Soltesz V, Sundbarg G (1984) Aerobic and anaerobic bacteria in neurosurgical infections. Per-operative culture with flexible contact agar film. J Hosp Infect 5(2):147–154
14. Tolhurst DE, Carstens MH, Greco RJ, Hurwitz DJ (1991) The surgical anatomy of the scalp. Plast Reconstr Surg 87(4):603–612
15. Ellis H, Mahadevan V (2014) The surgical anatomy of the scalp. Surgery 32(Suppl 1):e1–e5
16. Seery GE (2002) Surgical anatomy of the scalp. Dermatol Surg 28(7):581–587
17. Hayman LA, Shukla V, Ly C, Taber KH (2003) Clinical and imaging anatomy of the scalp. J Comput Assist Tomogr 27(3):454–459
18. Sheldon JJ (1990) Blood vessels of the scalp and brain. Clin Symp 42(3):3–36
19. Lemos MJ, Clark DE (1988) Scalp lacerations resulting in hemorrhagic shock: case reports and recommended management. J Emerg Med 6(5):377–379
20. Langford P, Wolfe R, Danks RA (2009) Wound healing after craniotomy: a randomized trial comparing scalp clips to artery forceps for scalp hemostasis. J Neurosurg 111(6):1175–1178
21. Sebastian S (2012) Does preoperative scalp shaving result in fewer postoperative wound infections when compared with no scalp shaving? A systematic review. J Neurosci Nurs 44(3):149–156
22. Broekman ML, van Beijnum J, Peul WC, Regli L (2011) Neurosurgery and shaving: what's the evidence? J Neurosurg 115(4):670–678
23. Sheinberg MA, Ross DA (1999) Cranial procedures without hair removal. Neurosurgery 44(6):1263–1265
24. Liu W, Ni M, Zhang Y, Groen RJ (2014) Antibiotic prophylaxis in craniotomy: a review. Neurosurg Rev 37(3):407–414
25. Korinek AM, Golmard JL, Elcheick A, Bismuth R, van Effenterre R, Coriat P, Puybasset L (2005) Risk factors for neurosurgical site infections after craniotomy: a critical reappraisal of antibiotic prophylaxis on 4,578 patients. Br J Neurosurg 19(2):155–162
26. Schipmann S, Akalin E, Doods J, Ewelt C, Stummer W, Suero Molina E (2016) When the infection hits the wound: matched case-control study in a neurosurgical patient collective including systematic literature review and risk factors analysis. World Neurosurg (95):178–189
27. Alerany C, Campany D, Monterde J, Semeraro C (2005) Impact of local guidelines and an integrated dispensing system on antibiotic prophylaxis quality in a surgical centre. J Hosp Infect 60(2):111–117
28. Weber WP, Marti WR, Zwahlen M, Misteli H, Rosenthal R, Reck S, Fueglistaler P, Bolli M, Trampuz A, Oertli D, Widmer AF (2008) The timing of surgical antimicrobial prophylaxis. Ann Surg 247(6):918–926
29. Sneh-Arbib O, Shiferstein A, Dagan N, Fein S, Telem L, Muchtar E, Eliakim-Raz N, Rubinovitch B, Rubin G, Rappaport ZH, Paul M (2013) Surgical site infections following craniotomy focusing on possible post-operative acquisition of infection: prospective cohort study. Eur J Clin Microbiol Infect Dis 32(12):1511–1516
30. Hardy SJ, Nowacki AS, Bertin M, Weil RJ (2010) Absence of an association between glucose levels and surgical site infections in patients undergoing craniotomies for brain tumors. J Neurosurg 113(2): 161–166
31. Mracek J, Hommerova J, Mork J, Richtr P, Priban V (2015) Complications of cranioplasty using a bone flap sterilised by autoclaving following decompressive craniectomy. Acta Neurochir 157(3):501–506
32. Gaberel T, Borgey F, Thibon P, Lesteven C, Lecoutour X, Emery E (2011) Surgical site infection associated with the use of bovine serum albumine-glutaraldehyde surgical adhesive (BioGlue) in cranial surgery: a case-control study. Acta Neurochir 153(1):156–162
33. Blomstedt GC (1985) Infections in neurosurgery: a retrospective study of 1143 patients and 1517 operations. Acta Neurochir 78(3–4):81–90
34. Chiang HY, Kamath AS, Pottinger JM, Greenlee JD, Howard MA 3rd, Cavanaugh JE, Herwaldt LA (2014) Risk factors and outcomes associated with surgical site infections after craniotomy or craniectomy. J Neurosurg 120(2):509–521
35. Patir R, Mahapatra AK, Banerji AK (1992) Risk factors in postoperative neurosurgical infection. A prospective study. Acta Neurochir 119(1–4):80–84
36. Mollman HD, Haines SJ (1986) Risk factors for postoperative neurosurgical wound infection. A case-control study. J Neurosurg 64(6):902–906
37. Rosseto RS, Giannetti AV, de Souza Filho LD, Faleiro RM (2015) Risk factors for graft infection after Cranioplasty in patients with large hemicranial bony defects. World Neurosurg 84(2):431–437
38. Kim T, Han JH, Kim HB, Song KH, Kim ES, Kim YH, Bang JS, Kim CY, Oh CW (2013) Risk factors of surgical site infections after supratentorial elective surgery: a focus on the efficacy of the wound-drain-tip culture. Acta Neurochir 155(11):2165–2170
39. Buffet-Bataillon S, Haegelen C, Riffaud L, Bonnaure-Mallet M, Brassier G, Cormier M (2011) Impact of surgical site infection surveillance in a neurosurgical unit. J Hosp Infect 77(4):352–355
40. Centers for Disease Control and Prevention. The National Healthcare Safety Network (NHSN). Surgical site infection (SSI) event. https://www.cdc.gov/nhsn/pdfs/pscmanual/9pscssicurrent.pdf. Accessed 17 Feb 2017
41. Chang SC, Lai PH, Chen WL, Weng HH, Ho JT, Wang JS, Chang CY, Pan HB, Yang CF (2002) Diffusion-weighted MRI features of brain abscess and cystic or

necrotic brain tumors: comparison with conventional MRI. Clin Imaging 26(4):227–236
42. Baumeister S, Peek A, Friedman A, Levin LS, Marcus JR (2008) Management of postneurosurgical bone flap loss caused by infection. Plast Reconstr Surg 122(6):195e–208e
43. Tillmann BN (2016) Atlas der Anatomie des Menschen. Springer, Heidelberg, pp 19–145

Part III

Lower Extremity Ulcers

Venous Ulcers: General Information

Melvin A. Shiffman

1 Introduction

Venous leg ulcers affect about 500,000–2 million people annually in the USA [1]. Chronic venous ulcers remain the leading causes for nonhealing wounds in the lower extremities [2].

A leg ulcer has been defined as the loss of skin below the knee on the leg or foot, which takes more than 6 weeks to heal [3]. 80% of leg ulcers have a venous component [3].

Venous leg ulcers are a common, chronic, and recurring condition. Ulcers take longer to heal in lower socioeconomic groups. This may be due to difficulties in accessing evidence-based management, such as Doppler assessment and compression therapy [4].

2 Community and Environmental Assessment Process Categories of Venous Disorders [5]

1. Reticular and spider veins
2. Varicose veins
3. Varicose veins and leg swelling
4. Varicose veins and evidence of venous stasis skin changes
5. Varicose veins and a healed venous stasis ulceration
6. Varicose veins and an open venous ulceration. CEAP 2004 revised classification (Table 16.1)

3 Cause

Sustained venous hypertension that results from chronic venous insufficiency or an impaired calf muscle pump [3]. The blood tends to collect and pool because the valves in the larger veins become damaged by a previous thrombosis in the vein or varicose veins. Gravity causes blood to flow back through the damaged valves and pool in the lower veins.

4 Risk Factors

1. Obesity
2. Immobility
3. Varicose veins
4. Deep vein thrombosis
5. Congestive heart failure
6. Incompetent valves
7. Congenital absence of valves
8. Pregnancy

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Table 16.1 CEAP Classification Revision 2004

Clinical classification	
C0	No evidence of venous disease
C1	Superficial spider veins (telangiectasia or reticular veins)
C2	Simple varicose veins only
C3	Ankle edema of venous origin
C4a	Skin pigmentation in the ankle area, dermatitis, and/or eczema
C4b	Lipodermatosclerosis
C5	Healed venous ulcer
C6	Open venous ulcer
S	Symptomatic
A	Asymptomatic
Etiologic classification	
Ec	Congenital
Ep	Primary
Es	Secondary
En	No venous cause
Anatomic classification	
As	Superficial veins
Ap	Perforating veins
Ad	Deep veins
An	No venous anatomy identified
Pathophysiologic classification	
Pr	Reflux
Po	Obstruction
Pr, o	Reflux and obstruction
Pn	No venous pathology

Modified from Images for CEAP Classification Revision 2004

5 Appearance

Venous ulcers are usually large, irregular shaped, shallow, painless, and situated around the medial or lateral malleolus. Pain is related to edema, phlebitis, or infection. The ulcers may be associated with other signs of venous hypertension:

1. Varicose veins
2. Varicose eczema
3. Atrophie blanche (Fig. 16.1)
4. Hemosiderin pigmentation
5. Venous flare (spider veins) (Fig. 16.2)
6. Edema of the lower leg
7. Chronic venous stasis
   (a) Warty hyperplasia of the skin
   (b) Thickening of the subcutaneous tissues
8. Lipodermatosclerosis

6 Past History

The following history may suggest venous ulceration:

1. Preexisting varicose veins
2. Deep vein thrombosis
3. Phlebitis
4. Previous fracture
5. Trauma or surgery
6. Family history of venous disease

Symptoms of venous insufficiency such as pains or heaviness in the legs, aching, itching, swelling, breakdown of the skin surface, pigmentation, and eczema may indicate early pre-venous ulceration.

7 Differential Diagnosis

7.1 Arterial Ulcer

Check for reduced pulses in the foot and ankle and possibly the femoral artery. The most common areas of poor blood supply are the tip of the toes or over the tibia and are typically painful and

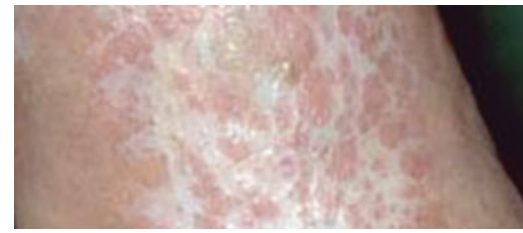

Fig. 16.1 Atrophie blanche

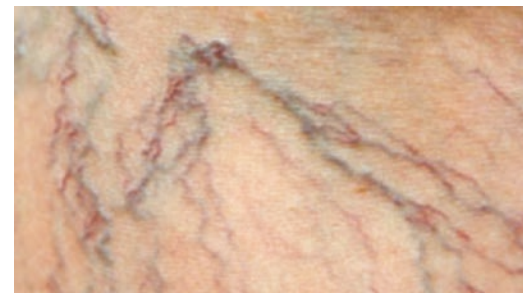

Fig. 16.2 Venous flare (spider veins)

deep. There may be peripheral cyanosis and claudication.

Measurement of ankle brachial pressure index using Doppler will determine the presence of arterial ulcer.

7.2 Neuropathic Ulcer

The neuropathic ulcer is painless and deep, often with overlying hyperkeratosis.

It occurs in sites of loss of nerve supply and recurrent trauma such as the heel and metatarsal heads.

7.3 Malignancy

Chronic venous ulcers can develop into malignant ones. Malignant ulcers in the area of the legs may be rare but attention should be paid to an ulcer with rolled edges and ulceration occurring in the area of scar tissue. Marjolin's ulcer should be considered [6].

7.4 Rheumatoid Ulcer

Rheumatoid ulcers are sharp, deep, and well demarcated with a punched-out appearance. They occur typically on the dorsum of the foot and calf and may be slow to heal. Venous ulcers also occur in rheumatoid patients, so the differentiation may be difficult. The ulcers often will respond to a disease-modifying antirheumatic drug (DMARDs).

8 Tests

Measurement of surface area of the ulcer will allow the physician to follow the rate of healing or failure to progress. Look for signs of infection or cellulitis. Culture any discharge for culture and sensitivity. Biopsy the ulcer that has an atypical appearance or fails to heal after 12 weeks of active treatment.

If there is an associated dermatitis in chronic ulcer patients, they should be referred for patch testing ("leg ulcer series") to determine allergens to which a leg ulcer patient may be exposed to, i.e., wound dressings.

Other tests can be performed if there is suspicion of an alternative or additional cause for the ulcer: tests such as complete blood count, erythrocyte sedimentation rate, C-reactive protein, albumin, HbAlc, autoantibody screen, and clotting and hemoglobinopathy screen.

9 Treatment

Control edema and reduce venous hypertension through compression therapy. Debride all dead tissue and control infection with antibiotics. Topical antibiotics can cause sensitivity and there is no place for them, except for metronidazole gel, which may be useful for malodorous ulcers.

Although multilayer compression dressing remains the gold standard treatment, there are various surgical procedures aimed at healing chronic venous ulcers (CVUs) with little or no evidence on the efficacy of these treatment methods. Adding superficial vein ligation and stripping to compression do not improve wound-healing rate [2] but may reduce the risk of recurrence [4].

Wounds that do not close at 4 weeks are unlikely to achieve complete wound healing and may benefit from surgery or other therapies. Autograft or allograft may be helpful. Pinched skin grafting may be indicated in patients with extensive areas of ulceration. Bioengineered skin substitutes may be considered.

Adjunctive therapies, such as ultrasound, pulsed electromagnetic fields, and electrical stimulation, may aid in treating chronic venous ulcers.

10 Complications

1. Immobility due to pain
2. Infection
3. Negative impacts on daily life and functioning
4. Osteomyelitis
5. Septicemia

6. Recurrence
7. Mortality
8. Loss of quality of life

11 Secondary Prevention of a Recurrent Ulcer

Correctly fitted compression hosiery should be worn for 5 years after an ulcer has healed. Underlying comorbidities, e.g., diabetes and rheumatoid arthritis, should be managed appropriately.

References

1. Margolis DJ, Bilker W, Santanna J, Baumgarten M (2002) Venous leg ulcer: incidence and prevalence in the elderly. J Am Acad Dermatol 46(3):381–386
2. Malas MB, Qazi U, Lazarus G, Valle MF, Wilson LM, Haberl EB, Bass EB, Zenilman J (2014) Comparative effectiveness of surgical interventions aimed at treating underlying venous pathology in patients with chronic venous ulcer. J Vasc Surg Venous Lymphat Disord 2(2):212–225
3. https://www.nice.org.uk/advice/esmpb2/chapter/full-evidence-summary-medicines-and-prescribing-briefing. Accessed 24 Nov 2016
4. Venous leg ulcers. Gravitational leg ulcers; causes, treatment. http://patient.info/doctor/venous-leg-ulcers-pro. Accessed 19 Mar 2017
5. CEAP classification for venous disease. http://www.mycvl.com/physician-resources/ceap. Accessed 23 Mar 2017
6. Chong AJ, Klein MB (2005) Images in clinical medicine. Marjolin's ulcer. N Engl J Med 352(10):e9

Part IV

Antimicrobial Therapy

Review of Clinical Pharmacokinetics of Levofloxacin with Special Emphasis in Burn Wound Patients

Ranjeet Prasad Dash, Rana Rais, and Nuggehally R. Srinivas

1 Introduction

Novel fluoroquinolones as a class have attained good prescription rate over the past two decades. The advantage of fluoroquinolones is that they provide options against gram-positive organisms and/or anaerobes while still maintaining impressive activity against gram-negative pathogens [1, 2]. Furthermore, it has been observed that newer fluoroquinolones provide distinctive pharmacokinetic profile with availability of higher drug concentrations in respiratory tract tissues and fluids relative to serum concentrations, following oral and intravenous administration [3, 4]. Amongst the novel fluoroquinolones, levofloxacin has gained significant importance in managing community-acquired pneumonia [5]. Levofloxacin (Fig. 1) offers broad-spectrum antibacterial activity especially against gram-negative organisms [5]. From the pharmacokinetic perspective, levofloxacin can be used both via oral and intravenous routes since it exhibits higher systemic and tissue concentrations after both oral and intravenous administration [5]. Levofloxacin is also a preferred drug for the management of burn-associated infections [6].

A major concern associated with the treatment in burn injury patients is the effect of pathological condition on the pharmacokinetic disposition of the drug being used in the treatment [5]. The pathological changes that occur in a burn injury patient have been suggested to have pharmacokinetic implications which may result in the alteration of either protein binding, volume of distribution, clearance or oral bioavailability [7]. The extent of impact on the pharmacokinetics of the drug is dependent on the type and degree of burn injury and the time that elapsed between burn injury and drug administration [7]. It should also be noted that burn/thermal injury would result in the enhanced intestinal permeability that will subsequently increase the bioavailability of large and hydrophilic molecules [8]. Because the levels of albumin and α1-acid glycoprotein decrease in burn injury patients, it may lead to increase in the free fraction of the drug in plasma [9]. The volume of distribution may change as a result of altered protein binding and/or enlarged extracellular volume [10]. The above mechanistic episodes with other related changes such as glomerular filtration rate, tubular secretion, hepatic blood flow and drug-metabolizing enzyme activity may in totality affect the drug clearance and overall drug exposures in burn injury patients [10].

The pathological condition of burn injury has a significant impact on the pharmacokinetic of antibacterial [6]. Several antimicrobials such as

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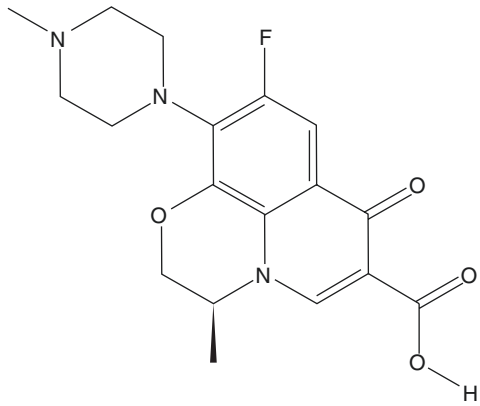

Fig. 1 Chemical structure of levofloxacin

ciprofloxacin, vancomycin, gentamicin and amikacin have demonstrated significant pharmacokinetic alterations in severely burned patients [11–14]. Burn injury may result in suboptimal dosing and impaired efficacy of the antimicrobial therapies and may result in development of resistance against bacterial pathogens [6]. This requires dose titration to achieve desired efficacy. Keeping in view the significance of antibacterial pharmacokinetics in burn injury patients, this article will focus on understanding the pharmacokinetic aspects of levofloxacin in healthy, diseased and burn injury patients.

2 Case Study of Levofloxacin

2.1 Levofloxacin Pharmacokinetics in Healthy Subjects

Chow et al. [15] carried out the pharmacokinetic study of levofloxacin administered as 750 mg intravenous infusion once daily for 7 days. The subjects were classified in two groups based on their renal clearance (≤ or ≥80 mL/min). The steady-state plasma concentration was attained in 2 days in all the subjects. However, the maximum plasma concentration (C_{max}) and area under the curve (AUC) values of subjects with clearance ≤80 mL/min were 1.6 and 1.8 times higher as compared to the subjects with clearance ≥80 mL/min, thus suggesting that dose adjustments were needed in subjects with higher degree of renal impairment [15]. Multiple-dose intravenous pharmacokinetic study of levofloxacin carried out for 7 days (200 mg single dose on days 1 and 7 and 200 mg twice daily doses from days 2 to 6) in healthy Chinese volunteers resulted in the achievement of steady-state concentration of levofloxacin in 3 days. The half-life was found to be 6 h, and cumulative urinary excretion rate was 88 ± 5%. No significant differences were observed in the AUCs measured on days 1 and 7, thus implying that levofloxacin did not accumulate even after multiple dosing [16]. Nakashima et al. [17] conducted a phase 1 pharmacokinetic study following single (200 mg) and multiple (200 mg given 3 times daily for 7 days) oral dose of levofloxacin under fed condition. The half-life was found to range between 4 and 6 h. Almost, 85–92% of levofloxacin was excreted in urine within 48 h, and a minimal amount of the intact levofloxacin (approximately 4%) was recovered in faeces in 72 h, thus confirming both complete oral absorption of levofloxacin and renal route as the primary path for the elimination of levofloxacin. Furthermore, as noted earlier levofloxacin did not show any accumulation; additionally, lack of chiral conversion of levofloxacin was documented in this study.

2.2 Levofloxacin Pharmacokinetics in Special Populations

Madhavi and Priyanka [18] studied the effect of menstrual cycles on the pharmacokinetics of levofloxacin. The findings suggested that the salivary concentration of levofloxacin decreased during the luteal phase owing to the fact that luteal phase exhibits high progesterone levels which in turn induced cytochrome P450 (CYP) enzymes and accelerated the drug metabolism of CYP substrate such as levofloxacin. By the same token, elevated oestrogen levels observed during the follicular stage inhibited the CYP enzymes and resulted in higher salivary levofloxacin concentration [18]. Based on these findings, it was suggested that female subjects may develop resistance towards bacterial infection during the luteal phase [18]. A formal gender effect study observed differences in

the pharmacokinetics of levofloxacin in male versus female subjects [19]. Following a single intravenous dose of levofloxacin (500 mg), the female subjects showed 43 and 23% higher C_{max} and AUC, respectively, as compared to the male counterparts. Although, no significant differences were observed in the half-life, the clearance and volume of distribution in males were 1.3 and 1.5 times higher in male subjects relative to female subjects [19]. Thee et al. [20] described the pharmacokinetic study of levofloxacin in paediatric subjects in three age groups such as 0–2, 2–6 and >6 years, who received body weight adjusted oral dosing of 15 mg/kg of levofloxacin. In this study, no significant differences in the systemic exposure and half-life of levofloxacin were observed amongst the stratified groups. However, the half-life of levofloxacin in the paediatric group was found to be 3 h almost two-fold lower relative to the adult subjects (6 h), conferring a somewhat faster clearance of levofloxacin in paediatric subjects. With respect to the infection status, no significant difference in the pharmacokinetic parameters of levofloxacin was observed between the human immunodeficiency virus (HIV)-infected and HIV-noninfected paediatric subjects [17, 20].

The concentrations of levofloxacin in skin blister fluid relative to those of serum have been reported after a 500 mg oral dose of levofloxacin [21]. The C_{max} of levofloxacin observed for serum samples was approximately two times higher as compared to blister fluid samples. The half-life of levofloxacin appeared to be comparable between the two (8.1 h for serum and 9.2 h for blister fluid). Therefore, this study demonstrated the accessibility of levofloxacin to the deeper tissues and body fluids. Chow et al. [22] observed that levofloxacin (750 mg once daily for 3 days, orally) achieved higher concentrations in the skin tissues as compared to plasma. The tissue/plasma ratio was 1.37 and 1.97 for C_{max} and AUC, respectively. Child et al. [23] conducted a crossover study in six healthy subjects where they received 500 mg of levofloxacin orally every 12 h for five doses in period 1 and 500 mg every 24 h for three doses. No significant difference was found for levofloxacin concentration in plasma and inflammatory fluid collected from the blisters. The overall penetration into inflammatory fluid ranged from 88 to 101% with the 12-h regimen and 83 to 112% with the 24-h regimen. As judged by the mean urinary recoveries of 87 and 86% over the corresponding interval of the 12- and 24-h regimens, respectively, there was no accumulation of levofloxacin.

2.3 Levofloxacin Pharmacokinetics in Patients

2.3.1 Respiratory Infection

Benko et al. [24] carried out a pharmacokinetic/pharmacodynamic study involving multiples doses of levofloxacin (500 mg, intravenous infusion) in 12 patients with respiratory infections caused by various pathogens. The maximum plasma levofloxacin concentration and the area under the free concentration-time curve for the free fraction of levofloxacin were 8.13 ± 1.64 mg/L and 49.63 ± 15.60 mg h/L, respectively [24].

Boselli et al. [25] conducted a pharmacokinetic study of levofloxacin (500 mg), administered once or twice daily in critically ill patients with severe community-acquired pneumonia. The concentrations of levofloxacin were monitored both in plasma and epithelial lining fluid of the patients. From a pharmacodynamic/clinical perspective, the data demonstrated that the concentrations of levofloxacin exceeded the required minimum inhibitory concentration values of <1 mg/L (serum) and >1 mg/L (epithelial lining fluid) for inhibiting the pathogens. The clearance in the patients dosed once daily was 45.3 mL/min as compared to 40.0 mL/min in patients dosed twice daily, suggesting there was no accumulation of levofloxacin in patients despite multiple dosing of the drug. Pharmacokinetic study following multiple intravenous administration of levofloxacin (1000 mg, once daily) in stable chronic lung disease patients showed significantly higher levofloxacin concentration in the alveolar cells (11.5 times) and epithelial lining fluid (2 times) as compared to the plasma tissue. No significant difference was observed in the half-life for levofloxacin in plasma (8.7 h) and epithelial lining fluid (7 h); however, the half-life of levofloxacin was found to be five to six times

higher for the alveolar cells (49.5 h). The findings of this study indicated that levofloxacin showed deep tissue penetration following a single intravenous dose of 1000 mg once daily and was well tolerated.

Furlanut et al. [26] described the pharmacokinetic study of oral/intravenous levofloxacin in 17 elderly patients suffering from the lower respiratory tract infection. The elderly patients showed slightly longer elimination half-life (9 h) and higher AUC (80 μg h/mL) as compared to healthy subjects who showed a half-life of 6.6 h and AUC of 55.3 μg h/mL [26, 27]. The probable reason for this altered pharmacokinetics of levofloxacin may be due to the declined renal function in the aged patients because 71% of levofloxacin is excreted via renal route [26]. The overall clinical success rate in this study was 94.1% [26]. Noreddin et al. [28] carried out a pharmacokinetic study at three intravenous dose levels; 500, 750 and 1000 mg of levofloxacin in young and elderly patients with community-acquired pneumonia. Younger patients showed higher clearance of levofloxacin (10.4 L/h) as compared to elderly patients (7.4 L/h), whereas the half-life of levofloxacin was longer in elderly patients (9.8 h as compared to younger patients with 7.2 h half-life). Regardless of the three dose levels, the AUC values for levofloxacin were 1.4 times higher in elderly patients (in comparison to younger patients. Based on the AUC/MIC data, 750 mg provided optimum efficacy. An interesting comparative pharmacokinetic study of levofloxacin (500 mg twice daily) in healthy and early-onset ventilator-associated pneumonia (VAP) patients showed a 20–40% lower exposure in patients as compared to healthy individuals [29]. Cumulative urinary excretion during the 12-h dosage interval confirmed the greater excretion of unchanged drug in these patients compared with healthy subjects (76% versus 68%) [29].

2.3.2 Miscellaneous

Bellmann et al. [30] observed that inflammatory condition slightly increased the tissue distribution of levofloxacin as compared to normal adipose tissue following single-dose intravenous administration. Interindividual variability in tissue penetration was high, as indicated by a coefficient of variation of approximately 82%. Geller et al. [31] evaluated the pharmacokinetics of levofloxacin (240 mg for 7 days) administered as an aerosol in the patients with cystic fibrosis. The sputum concentration of levofloxacin was 150-fold higher as compared to plasma concentration with no significant difference in time to reach maximum plasma concentration (T_{max}). The plasma half-life was 7.49 h as compared to sputum half-life of 4.58 h. Thus, it may be presumed that aerosol delivery system may be considered as a better alternative for treating respiratory infection as compared to oral and intravenous therapies. The patients with cystic fibrosis, although did not show any significant difference in the total clearance, volume of distribution, maximum serum concentration and elimination half-life for levofloxacin (500 mg daily, for 14 days, oral), displayed a rapid attainment of T_{max} as compared to non-cystic fibrotic patients [32]. Rebuck et al. [33] described the pharmacokinetic study of levofloxacin (500 mg once daily) following intravenous and oral administration in critically ill and healthy patients. A 1.2 times higher exposure and lower clearance were observed in patients as compared to healthy subjects. Levofloxacin showed higher penetration to the prostate tissues in acute prostatitis patients undergoing prostatectomies following administration of 500 mg of levofloxacin orally every 24 h for 2 days prior to surgery, and then on the day of surgery, 500 mg was administered as an hour-long, constant-rate intravenous (IV) infusion [34]. The $AUC_{prostate}$:AUC_{plasma} was found to be 2.96 suggesting the efficiency of the penetration of levofloxacin into prostrate tissue for combating infections [34]. Single- and multiple-dose (10 days once daily) oral pharmacokinetic study of levofloxacin (350 mg) in 10 HIV-infected patients did not show any significant difference in the pharmacokinetic parameters except for peak concentrations in plasma, which were 4.79 ± 1.00 and 6.92 ± 1.56 μg/mL for single- and multiple-dose data, respectively, suggesting no accumulation even after multiple dosing and infected condition had no effect on drug clearance [35]. Another study in 30 HIV-infected patients who received 750 mg of drug for 14 days followed with placebo for another

14 days showed that levofloxacin was rapidly absorbed with a maximum plasma concentration (T_{max}) of 1.5 h and elimination half-life ranging from 7.2 to 9.4 h [36]. Hutschala et al. [37] observed that the accessibility of levofloxacin (500 mg intravenous) to the cardiac tissues following cardiac surgery was low as observed from the AUC_{tissue}:AUC_{plasma} ratio of 0.6. Pharmacokinetic profile of levofloxacin (single dose 500 mg oral) did not change in typhoid patients when compared to that of healthy individuals except 1.3 higher volume of distribution in typhoid patients [38]. Weinrich et al. [39] observed significantly higher liver penetration for levofloxacin (500 mg intravenous) in patients for liver resection. The tissue/serum ratio of levofloxacin was found to be 3.72 at the time of liver resection, thus suggesting that levofloxacin is a good candidate for antibiotic prophylaxis before invasive hepatobiliary procedures [39].

2.3.3 Renal Impairment

Bellmann et al. [40] described the pharmacokinetic study of levofloxacin (500 mg intravenous) in 11 critically ill patients who were managed with continuous venovenous infiltration. Out of the 11 patients, 4 patients were on haemofiltration, 4 patients showed moderate renal impairment but were not put on haemofiltration, and 3 had normal renal function. The observed clearance of levofloxacin in patients with normal renal function was comparable to that of healthy subjects. The half-life in renal impaired patients who were not on haemofiltration was 20–25 h which was slightly higher as compared to 30 h as seen in patients on haemofiltration. Thus, levofloxacin dose adjustment was necessary in patients with renal failure without haemofiltration. It was noted that haemofiltration decreased the systemic exposure of levofloxacin and increased the volume of distribution. Malone et al. (2001) also observed that the clearance of levofloxacin was substantially increased during continuous venovenous haemofiltration (CVVH) and continuous venovenous haemodiafiltration (CVVHDF) [41].

Sowinski et al. [42] conducted pharmacokinetic study in noninfected patients with end-stage renal disease upon intravenous infusion (over 1 h) dosing of levofloxacin (250 mg), after a scheduled haemodialysis session [42]. The clearance of levofloxacin reduced to approximately 75% in renal impaired patients. The half-life value of levofloxacin was 35 h in the renal impaired patients and thus supported the need for dose adjustment. The condition of pyelonephritis in women, however, did not have any effect on the elimination of the intravenously administered levofloxacin since the half-life of levofloxacin was found to be 7 h which was comparable to the value observed in healthy male subjects [43]. Additionally, in this study it was confirmed that *E. coli* was completely eradicated from urine within 3–6 h duration following first dose of levofloxacin [43].

Tsaganos et al. [44] determined the effect of intermittent haemodialysis on pharmacokinetics of levofloxacin (500 mg single dose and for 3 days) in patients with end-stage renal disease. Although, the plasma concentration and half-life of levofloxacin was higher in the patients as compared to single dose, the clearance of levofloxacin was equivalent in both the cases, thus indicating no drug accumulation of levofloxacin in patients that were undergoing haemodialysis [44]. A study by Guenter et al. [45] in five renal impaired patients at a dose level of 500 mg/day as an intravenous infusion showed higher clearance of 154 L/h, thus suggesting dose adjustment in renal impaired patients.

2.3.4 Levofloxacin Pharmacokinetics in Burn Injury Patients

Kiser et al. [6] conducted a pharmacokinetic study in 11 severe burn injury patients following intravenous dosing of levofloxacin (750 mg, once daily for 4 days). The various pharmacokinetic parameters such as total body clearance, renal clearance, volume of distribution and elimination half-file for levofloxacin were similar between the single intravenous dose (i.e., day 1) and repeated daily intravenous doses (i.e., day 4) of levofloxacin. The mean values of the various pharmacokinetic parameters observed in burn injury patients were similar to the reported values either in healthy subjects or critically ill patients who received similar intravenous doses of levofloxacin. However, one key observation from the

study was the observation of high interindividual variability amongst the burn injury patients. The various pharmacokinetic/pharmacodynamic measures such as minimum inhibitory concentration (MIC)/C_{max} and/or MIC/AUC reported in this study suggested the effectiveness of levofloxacin either used alone and/or in combination with other antibiotics in burn injury patients [6]. Figure 2 shows a comparison of dose-normalized C_{max} and AUC of levofloxacin in healthy subjects with that of burn injury patients, whereas Fig. 3 shows the clearance of levofloxacin in healthy and burn patients. A summary of pharmacokinetic parameters in healthy subjects and patients is shown in Table 1.

3 Discussion

The popularity and continued success of levofloxacin as one of the leading fluoroquinolones can be attributed to its favourable safety and tolerability profiles. Furthermore, levofloxacin has demonstrated very impressive pharmacodynamic profile which is accompanied by a consistent and dependable pharmacokinetic behaviour.

On the basis of the review of the pharmacokinetic disposition of levofloxacin, the following deductions can be summarized:

(a) The clinical pharmacokinetics of levofloxacin remained unaltered when single-dose data was compared with multiple-dose data with key parameters such as half-life, clearance and volume of distribution almost indistinguishable suggesting the existence of stationary pharmacokinetics for levofloxacin [46].
(b) The lack of any noticeable disparity between intravenous and oral pharmacokinetics of levofloxacin with almost complete oral bioavailability of the drug was an advantage in making switch decisions between oral and intravenous therapy in the targeted patient population [46].
(c) To a large extent, the existence of similarity in the pharmacokinetics of levofloxacin has been confirmed between healthy human volunteers and intensive care patient population (e.g., respiratory infection), however, with

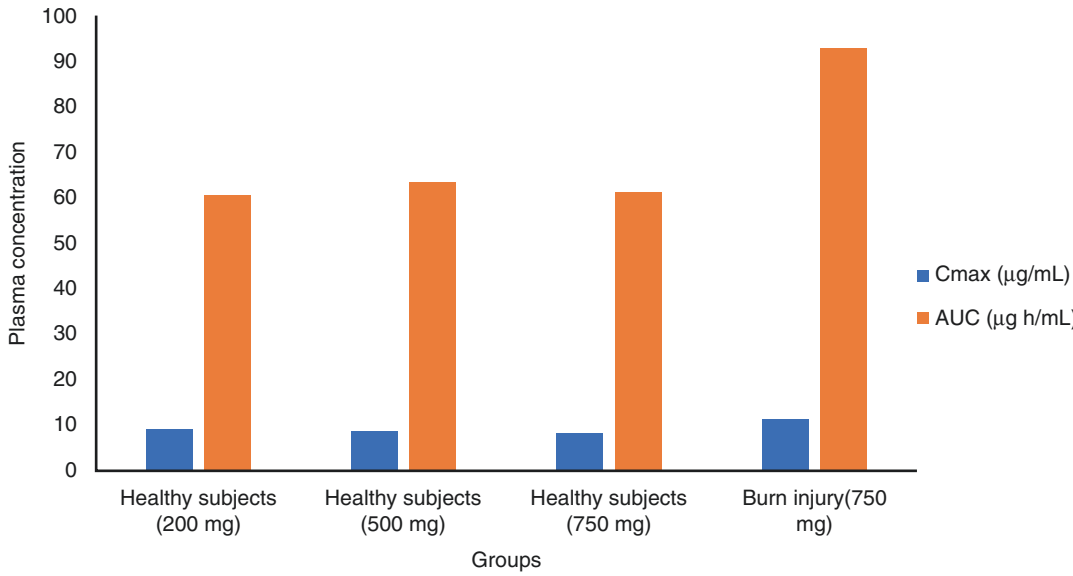

Fig. 2 Dose-normalized C_{max} and AUC values of levofloxacin in healthy subjects with respect to the dose administered in burn injury patients (750 mg). Data represented for dose levels of 750, 200 and 500 mg in healthy subjects and 750 mg in burn patients corresponds to Refs. [15, 16, 19, 45], respectively

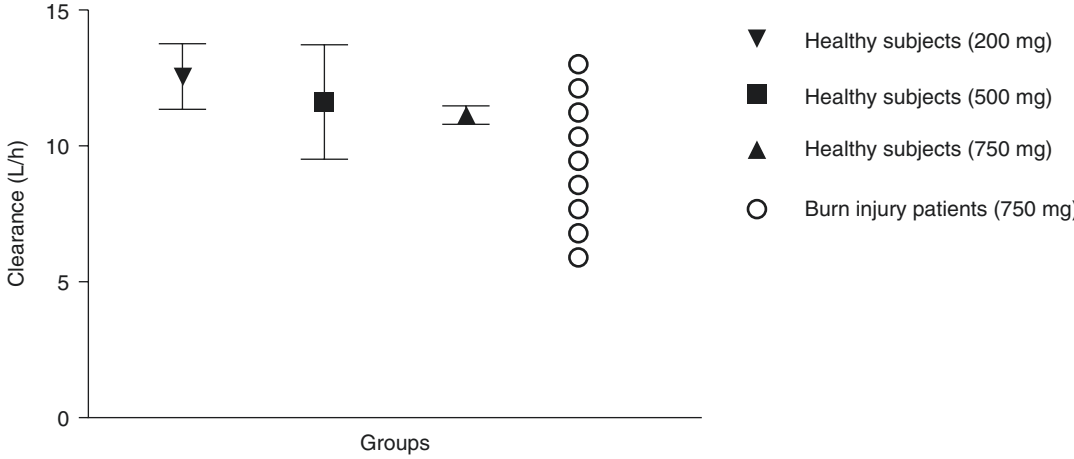

Fig. 3 Mean (± SD) clearance of levofloxacin in healthy subjects at different dose levels interspersed with the clearance in individual burn injury patients. Data represented for dose levels of 750, 200 and 500 mg in healthy subjects and 750 mg in burn patients corresponds to Refs. [15, 16, 19, 45], respectively

the caveat that any compromised renal function would result in the altered pharmacokinetics of levofloxacin which may have to be factored in proper dosing decisions [15, 33].

(d) The lack of the effect of either sex or age on the pharmacokinetics of levofloxacin in healthy subjects that have uncompromised renal function should be advantageous in treating disease population at large [47].

(e) The penetration of levofloxacin to body fluids (i.e., blister fluid), tissues of interest and respiratory cavities was shown to be adequate for levofloxacin to exhibit its promising pharmacodynamic activity [25, 34, 48, 49].

On the topic of drug-drug interaction liability of levofloxacin as either a perpetrator or a victim, a few studies have been published. Chien [46] showed that the pharmacokinetic profile of levofloxacin in HIV-infected patients was not altered by the concomitant administration of zidovudine; by the same token, the pharmacokinetics of zidovudine pharmacokinetics was unaffected by levofloxacin. Lee et al. [50] showed that co-administration of sucralfate, approximately 2 h post oral levofloxacin administration, had no bearing on the absorption and overall disposition of levofloxacin, and therefore, the non-inclusion of sucralfate was not considered essential in levofloxacin therapy. The co-administration of levofloxacin with other fluoroquinolones such as ciprofloxacin, norfloxacin and ofloxacin slightly increased the exposure by 10–17% [51]. The addition of levofloxacin to the steady-state regimen of theophylline marginally increased the serum concentration of theophylline [52]. Studies carried out with several oral antacid preparations such as aluminium or magnesium hydroxide preparations suggested that levofloxacin absorption and exposure was significantly affected by the simultaneous intake of levofloxacin with antacid preparations [53]. However, a 2-h window, either before or after levofloxacin dosing, was necessary, to permit the intake of antacids without any altered pharmacokinetics of levofloxacin [53]. Co-administration of cimetidine and probenecid resulted in the alteration in the pharmacokinetic profile of levofloxacin with respect to increased exposure and half-life by approximately 30–38% [54].

One important consideration is that how to put the various pharmacokinetic data of levofloxacin including drug-drug interaction potential in the context of burn injury patients. Although generally it appeared that pharmacokinetic parameters of levofloxacin were similar between burn injury patients and other studied population, there may be some situations of either a faster or slower clearance of levofloxacin that need to be anticipated in the therapy. One important caveat that

Table 1 Tabulated summary of clinical study design, objectives and pharmacokinetic data of levofloxacin in human studies

Study particulars	Pharmacokinetic data							
Subjects/design	Type	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg h/mL)	CL or CL/F (L/h)	V_d (L)	Remarks [Ref.]
Healthy								
$N = 18$ (M and F); randomized, double-blind, placebo-controlled	Clearance >80 mL/min ($N = 4$)			6.91		11.16	106.0	Levofloxacin was found to be well tolerated even after multiple dosing at 750 mg [15]
Single-centre, parallel group study. Subjects were classified in two groups based on creatinine clearance	Single dose Day 1	8.12	–		61.1			
	Steady state Day 10	8.71	–		67.4			
Levofloxacin 750 mg administered as IV infusion on day 1. Days 2 and 3, no treatment. Days 4–10, 7 once-daily IV infusions were administered	Clearance ≤80 mL/min ($N = 8$)			7.82		6.42	69.0	
	Single dose Day 1	12.9			121.0			
	Steady state Day 10	14.2	–		139.0			
$N = 10$ M multiple-dose, open-label, single-centre study	Plasma profile							No accumulation of levofloxacin was observed after multiple dosing for 7 days [16]
Levofloxacin (200 mg) was administered once on days 1 and 7 and twice from days 2 to 6 as IV infusion	Day 1	2.4	–	6.3	16.1	12.6	33.0	
	Day 7	2.9	–	6.2	23.0	9.3	39.0	
$N = 5$; 1 period, single centre Levofloxacin (200 mg) was given thrice daily after meal	Plasma profile	2.04	1.48	5.97	19.88	–	1.25	No accumulation or chiral conversion of levofloxacin was observed after multiple dosing and was found to be well tolerated [17]
$N = 15$, F; single-centre study	Follicular phase	128.4	1.0	9.93	1239.0	6.8	104.0	Systemic exposure of levofloxacin was higher during the follicular phase [18]
Subjects received levofloxacin 500 mg, on days 10 (follicular phase) and 21 (luteal phase) of menstrual cycle	Luteal phase	76.8	1.0	4.59	255.1	18.7	150	
$N = 20$ (11M, 9F); open-label, single-centre study	Men	5.66	–	7.69	42.13	11.67	120.35	Systemic exposure level of levofloxacin was higher in women as compared to men [19]
Dose was 500 mg single-dose intravenous	Women	8.10	–	6.47	54.27	9.05	79.27	
$N = 22$ paediatric subjects, single-centre study, HIV infected and 18 noninfected	Age group							HIV status and age did not have any effect on the pharmacokinetic profile of levofloxacin in paediatric subjects [20]
Single-oral dose of levofloxacin (15 mg/kg) dosed under fasted condition	0–2 years	7.0	1.33	1.79	29.89	–	–	
	2–6 years	6.86	1.56	3.22	31.69	–	–	
	≥6 years	4.98	1.50	3.37	27.49	–	–	
	HIV status							
	Infected	4.98	1.50	3.37	27.49	–	–	
	Noninfected	6.88	1.44	3.09	31.38	–	–	

N = 20 M, single-centre study	Serum	6.92	1.75	8.10	42.64	10.8	114.3	Levofloxacin was well tolerated and showed good tissue penetration [21]
Levofloxacin (500 mg) was administered as a single oral dose	Skin blister fluid	3.61	4.10	9.21	47.61	–	–	
N = 10 M; non-comparative, open-label, single-centre, randomized, phase I study Levofloxacin (750 mg) was dosed once daily for 3 days	Plasma	8.99	1.1	8.23	82.0	9.43	105.0	Tissue/plasma ratio was found to be 1.97 suggesting penetration of levofloxacin to deep tissues [22]
	Tissue[a]	11.77	3.3	–	161.0	–	–	
N = 6 M; open-label crossover study Subjects received 500 mg of levofloxacin orally every 12 h for five doses or 500 mg every 24 h for three doses, and then 6 weeks later, they received the other course	Plasma							Levofloxacin showed higher penetration to the inflammatory tissues [23]
	500 mg every 24 h	6.55	1.17	7.95	53.5	9.69	66.4	
	500 mg every 12 h	9.53	1.08	7.91	60.0	8.92	69.8	
	Skin blister fluid							
	500 mg every 24 h	4.33	3.67	7.95	54.1	20.9	35.2	
	500 mg every 12 h	6.79	2.33	7.91	55.9	29.7	27.7	
Respiratory infection								
N = 14; open-label study Levofloxacin (2 × 500 mg on day 1 and 1 × 500 mg from days 2 to 7) was administered as an IV infusion	Plasma profile	8.13	–	6.23	49.63	10.68	85.35	Levofloxacin was found to be effective in critically ill patients [24]
N = 24 patients with severe community-acquired pneumonia All subjects received 1-h intravenous infusions of 500 mg levofloxacin once or twice daily	Plasma							Levofloxacin exhibited excellent extracellular lung penetration of greater than 100% and a wide interindividual pharmacokinetic variability [25]
	500 mg once daily	12.6	0.2	11.5	151.0	45.3	41.0	
	500 mg twice daily	19.7	0.3	17.0	208.0	40.0	55.5	
	Epithelial lining fluid	11.9	–	–	–	–	–	
N = 16 subjects with chronic lung disease Levofloxacin 1000 mg was administered once daily for 3 days	Plasma	9.4	4.0	8.7	130.0	–	–	Levofloxacin showed deep tissue penetration at a single intravenous dose of 1000 mg once daily and well tolerated [48]
	Epithelial lining fluid	22.8	4.0	7.0	260.0	–	–	
	Alveolar cells	76.3	4.0	49.5	1492.0	–	–	
N = 17 (10M, 7F); patients with lower respiratory tract infection Levofloxacin 500 mg IV once daily administered as a 1 h intermittent infusion for 4–9 days followed by 500 mg oral once daily until the end of the therapy (total duration of therapy being 9–17 days)	IV	10.71	–	8.77	74.97	112.2	88.04	Levofloxacin exhibited more than 99% absolute oral bioavailability and clinical success rate of 94.1% [26]
	Oral	7.93	1.23	9.91	85.60	99.0	87.33	

(continued)

Table 1 (continued)

Study particulars	Pharmacokinetic data							
Subjects/design	Type	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg h/mL)	CL or CL/F (L/h)	V_d (L)	Remarks [Ref.]
N = 138 elderly and *N* = 45 young patients with CAP Levofloxacin was administered as 500, 750 and 1000 mg single IV dose	Plasma							Based on the AUC/MIC data, 750 mg provided optimum efficacy [28]
	Elderly patients			9.8		7.2	62.91	
	500 mg	–	–		49.9			
	750 mg	–	–		74.8			
	1000 mg	–	–		99.7			
	Young patients			7.4		10.4	68.22	
	500 mg	–	–		34.8			
	750 mg	–	–		52.3			
	1000 mg	–	–		69.7			
	Epithelial lining fluid							
	Elderly patients							
	500 mg	–	–		57.8			
	750 mg	–	–		86.7			
	1000 mg	–	–		115.7			
	Young patients							
	500 mg	–	–		40.4			
	750 mg	–	–		60.7			
	1000 mg	–	–		80.9			
N = 10 patients (8M, 2F) with early-onset ventilator-associated pneumonia Levofloxacin 500 mg was dosed twice daily for 8 days	Plasma	8.19	–	0.22	33.90	204.0	98.82	Intravenous levofloxacin 500 mg twice daily was found to be suitable in the treatment of early-onset VAP in ICU patients with normal renal function [29]
Renal impairment								
N = 11 patients and 2 controls, single-centre study Patients were classified as group 1, undergoing CVVH; group 2, NO CVVH, creatinine clearance ≥1.5 mg/dL; and group 3, NO CVVH, creatinine clearance ≤1.5 mg/dL. Dose of levofloxacin was 500 mg, intravenously administered	Group 1	4.9	–	28.7	34.9	15.9	–	Patients with normal renal function showed equivalent clearance as compared to healthy subjects. However, renal impaired patient showed longer half-life and delayed clearance [40]
	Group 2	6.8	–	4.2	46.5	7.61	–	
	Group 3	8.7	–	20.6	25.5	10.21	–	
	Control	7.9	–	7.7	40.5	8.23	–	
N = 5 critically ill patients undergoing CVVHDF and CVVH; single-centre study Patients received levofloxacin 500 mg/day. All patients received CVVHDF on day 1 and CVVH on day 2	Plasma profile	9.04	–	28.8	153.6	54.04	1.51	Dose adjustment is required in patients with renal impairment [45]

N = 8 female subjects with acute nonobstructive pyelonephritis Levofloxacin (750 mg) dosed intravenously once daily for 5 days	Plasma	12.5	1.0	6.7	85.4	–	–	Levofloxacin was well tolerated and exhibited optimum efficacy [43]
N = 8 patients with end-stage renal disease undergoing haemodialysis Levofloxacin (250 mg) was dosed over 1 h as infusion after a scheduled haemodialysis session	Serum	5.15	–	34.4	44.6	37.0	103.3	Optimum C_{max}-AUC ratio was obtained following 250 mg doses for 5 days [42]
N = 10 patients undergoing haemodialysis. Single-centre, 2-week washout period Phase 1: Single dose levofloxacin (500 mg), orally Phase 2: 500 mg for 3 consecutive days, orally	Single dose plasma profile	5.04	1.50	22.84	144.96	2.67	109.47	Levofloxacin showed accumulation following multiple dosing in patients with renal impairment, thus requiring dose titration [44]
	Multiple dose plasma profile	8.32	52.0	38.05	542.26	2.10	131.19	
Miscellaneous								
N = 6 patients with soft tissue injury Levofloxacin (500 mg) dosed once, intravenously	Plasma	8.73	0.52[b]	10.0	32.3	9.57	107.61	Levofloxacin exhibited deep tissue penetration. No significant difference was observed in levofloxacin concentration in healthy and inflamed tissue [30]
	Inflamed tissue	5.45	1.06[b]	–	25.5	–	–	
	Healthy tissue	4.42	1.39[b]	–	23.5	–	–	
N = 10 patients with cystic fibrosis Levofloxacin (240 mg) administered as inhaler once daily for 7 days	Plasma	1.71	0.34	7.49	14.77	–	–	Significant concentration of levofloxacin was attained in sputum samples following administration via nasal route [31]
	Sputum	4691	0.29	4.58	4507	–	–	
N = 10 HIV-infected patients; phase I, double-blind, randomized study Levofloxacin (350 mg) was administered orally once on day 1. From days 3 to 10, levofloxacin (350 mg) was dosed thrice daily	Single dose (day 1)	4.79	1.0[b]	5.66	29.94	12.30	98.76	Levofloxacin did not show any accumulation following multiple-dosing regimen [35]
	Multiple dose (day 10)	6.92	0.90[b]	6.50	31.24	11.18	104.10	
N = 6 in patients undergone cardiac surgery Levofloxacin 500 mg administered intravenously	Plasma[c]	15.9	0.33	–	32.6	–	–	Levofloxacin showed penetration to the lung tissue with tissue/plasma ratio of 0.6 [37]
	Tissue[c]	6.8	0.67	–	18.6	–	–	
N = 22 (12 patients with cystic fibrosis and 10 non-cystic fibrotic subjects) Levofloxacin (500 mg) dosed orally q.d. for 14 days	Cystic fibrotic patient	7.06	2.20	6.44	71.32	130.2	70.6	Systemic exposure of levofloxacin was 1.5 times higher in cystic fibrotic patients as compared to their normal counterparts [32]
	Non-cystic fibrotic subjects	5.72	1.10	6.81	47.5	174.5	102.0	
N = 9 Caucasian ICU patients Levofloxacin (500 mg) administered as infusion Assessment	Plasma	8.43	–	13.01	110.29	6.57	101.38	Pharmacokinetic profile of levofloxacin ICU patients was similar to that of healthy subjects [55]

(continued)

Table 1 (continued)

Study particulars	Pharmacokinetic data							
Subjects/design	Type	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC (μg h/mL)	CL or CL/F (L/h)	V_d (L)	Remarks [Ref.]
N = 12 (6 healthy and 6 typhoid patients) Levofloxacin (500 mg) dosed once orally	Healthy	7.57	1.87	11.15	106.23	4.76	76.36	No significant difference was observed in the pharmacokinetic profile of levofloxacin in healthy subjects and typhoid patients [38]
	Typhoid patients	7.59	1.82	10.02	102.38	4.89	70.43	
N = 28 critically ill patients (18 with normal hepatic function and 10 with hepatic dysfunction); open-label study Levofloxacin (500 mg) administered intravenously for 5 days	Normal hepatic function	7.5	–	7.6	61.0	135	93.5	Hepatic dysfunction had no effect on the disposition of levofloxacin [33]
	Hepatic dysfunction	7.4	–	8.7	67.9	132	98.3	
Burn injury								
N = 10 severely burned ICU patients Levofloxacin (750 mg) dosed as infusion	Plasma profile	11.3	–	7.8	93.0	9.0	101.2	Pharmacokinetic profile of levofloxacin in burn injury patients was similar to that of critically ill patients. Higher inter-patient variability was observed in burn injury subjects [6]
Drug-drug interaction								
N = 6 HIV-infected patients Levofloxacin (350 mg, orally) was dosed once on days 1 and 10 and thrice a day from days 3 to 9. Zidovudine (100 mg, orally) dosed once on days 1 and 10	Day 1	3.82	1.0	6.2	17.2	11.4	98.0	No pharmacokinetic interaction was observed [46]
	Day 10	7.06	1.1	7.2	37.4	9.4	109.0	
N = 24 (12M, 2F), single-dose, open-label, randomized crossover study Levofloxacin (500 mg) was administered alone and in combination with 1000 mg sucralfate under fasted condition	Levofloxacin alone	5.9	1.0	6.2	50.5	10.1	–	Sucralfate did not affect the pharmacokinetics of levofloxacin [50]
	Levofloxacin + sucralfate	6.7	1.0	6.1	47.9	10.7	–	

Data expressed as mean for all parameters except for t_{max} which are expressed as median

HV human volunteers, *M* male, *F* female

[a]Values expressed as median

[b]Tissue concentration expressed as μg/g

[c]T_{max} expressed as mean

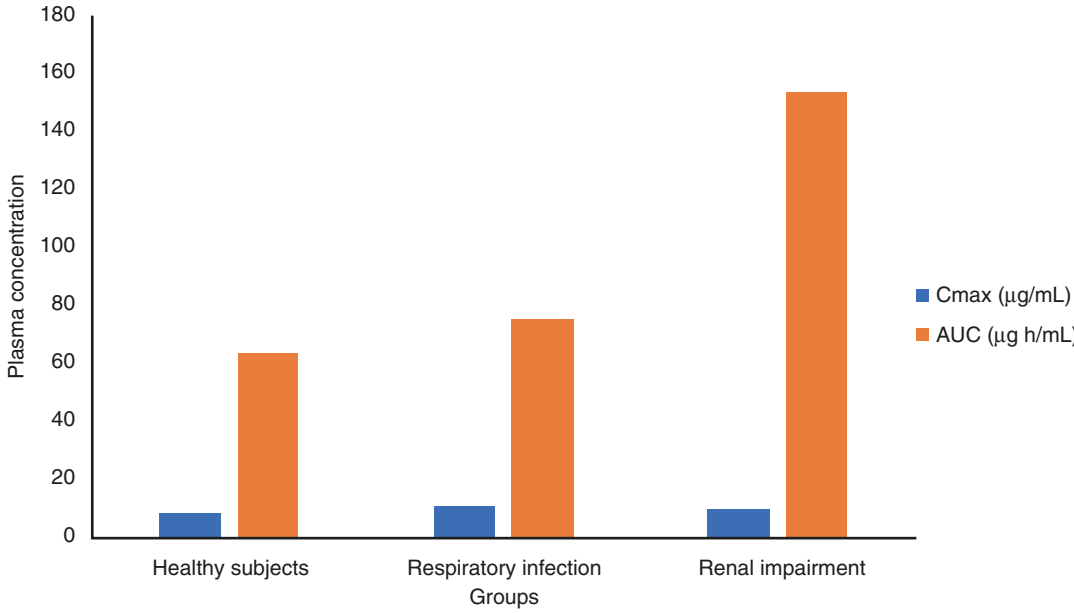

Fig. 4 Dose-normalized C_{max} and AUC values of levofloxacin in healthy subjects and renal impaired patients with respect to the dose administered in respiratory infection patients (500 mg). Data represented for healthy subjects, respiratory infection and renal impaired patients corresponds to Refs. [6, 19, 26], respectively

needs to be considered is that if burn injury patients have an underlying renal impairment issue, it is quite possible that levofloxacin pharmacokinetics will likely be altered and consideration of dose adjustment in such patients would become critical. Another observation worthy of discussion is the high degree of variability in the inter-patient pharmacokinetics of levofloxacin in the burn injury patients. As pointed out earlier, burn injury has the potential to alter several physiological process key for the drug disposition, which in turn contributes for the observed variability. Such high degree of variability in burn injury patients was not unique for levofloxacin but has been also reported for other antibiotics such as vancomycin, ciprofloxacin, etc. [11, 12]. From the drug-drug interaction perspective, only thing of relevance was with respect to the observed pharmacokinetic interaction between levofloxacin and cimetidine/probenecid. Because both probenecid and cimetidine interfere in the renal excretory process of levofloxacin, they tended to decrease the excretion of levofloxacin and increase its half-life and exposure. Hence, as a precautionary measure for treating burn injury patients with levofloxacin, other co-medications that influence the urinary excretory processes should be replaced with other agents that will not interfere in the urinary excretory process of levofloxacin (Fig. 4).

Despite the high degree of variability in the pharmacokinetic parameters of levofloxacin in burn injury patients, a simple linear regression model was proposed to predict the pharmacokinetics of levofloxacin with a limited sampling strategy [5]. Furthermore, the developed linear regression model using pharmacokinetic data in burn injury patients was also shown to be applicable for the prediction of levofloxacin pharmacokinetics in healthy subjects who were dosed either orally or intravenously [5].

Conclusions

The focus of the review was to provide a comprehensive report on the pharmacokinetics of levofloxacin in healthy subjects, critical care patients and burn injury patients. In addition to describing the general clinical pharmacokinetics of levofloxacin across the population spread, other important factors that may play a

role in the pharmacokinetics of levofloxacin such as age, sex, renal impairment status, etc. have been summarized. Based on the review, levofloxacin showed comparable pharmacokinetics across the varied population including burn injury patients. The burn injury patients tended to exhibit higher degree of pharmacokinetic variability. Regardless of the population, renal function status was shown to alter the pharmacokinetics of levofloxacin. Therefore, dose adjustment decision of levofloxacin in burn injury patients and/or critical care patients should consider the renal function. Another important consideration in clinical therapy with levofloxacin was to examine the probable role of co-medication on the renal functionality in burn injury or critical care patients who were stabilized with an appropriate dose of levofloxacin.

References

1. Hooper DC (2000) New uses for new and old quinolones and the challenge of resistance. Clin Infect Dis 30(2):243–254
2. Zhanel GG, Ennis K, Vercaigne L, Walkty A, Gin AS, Embil J, Smith H, Hobon DJ (2002) A critical review of the fluoroquinolones: focus on respiratory infections. Drugs 62(1):13–59
3. Zhanel GG, Walkty A, Vercaigne L, Karlowsky JA, Embil J, Gin AS, Hoban DJ (1999) The new fluoroquinolones: a critical review. Can J Infect Dis 10:207–238
4. O'Donnell JA, Gelone SP (2000) Fluoroquinolones. Infect Dis Clin North Am 14:489–513
5. Srinivas NR (2016) Predictions of the pharmacokinetics in burn injury patients using regression models - case study with levofloxacin. Drug Res (Stuttg) 66(10):555–560
6. Kiser TH, Hoody DW, Obritsch MD, Wegzyn CO, Bauling PC, Fish DN (2006) Levofloxacin pharmacokinetics and pharmacodynamics in patients with severe burn injury. Antimicrob Agents Chemother 50(6):1937–1945
7. Jaehde U, Sorge F (1995) Clinical pharmacokinetics in patients with burns. Clin Pharmacokinet 29(1):15–28
8. Epstein MD, Tchervenkov JI, Alexander JW, Johnson JR, Vester JW (1991) Increased gut permeability following bum trauma. Arch Surg 126:198–200
9. Zini R, Riant P, Barre J, Tillement JP (1990) Disease-induced variations in plasma protein levels. Implications for drug dosage regimens (part 1). Clin Pharmacokinet 19(2):147–159
10. Rowland M, Tozer TN (1989) Clinical pharmacokinetics: concepts and applications. Lea &Febiger, Philadelphia, pp 145–146. 187–8
11. Garrelts JC, Jost G, Kowalsky SF, Krol GJ, Lettieri JT (1996) Ciprofloxacin pharmacokinetics in burn patients. Antimicrob Agents Chemother 40:1153–1156
12. Garrelts JC, Peterie JD (1988) Altered vancomycin dose vs. serum concentration relationship in burn patients. Clin Pharmacol Ther 44:9–13
13. Zaske DE, Sawchuk RJ, Gerding DN, Strate RG (1976) Increased dosage requirements of gentamicin in burn patients. J Trauma 16:824–828
14. Zaske DE, Sawchuk RJ, Strate RG (1978) The necessity of increased doses of amikacin in burn patients. Surgery 84:603–608
15. Chow AT, Fowler C, Williams RR, Morgan N, Kaminski S, Natarajan J (2001) Safety and pharmacokinetics of multiple 750-milligram doses of intravenous levofloxacin in healthy volunteers. Antimicrob Agents Chemother 45(7):2122–2125
16. Zhang L, Li JT, Lu Y, Li MN, Zhang YL, Liu Y, Li TY, Zhang JW (2002) Pharmacokinetics of multiple intravenous instillation of levofloxacin in Chinese healthy subjects. Acta Pharmacol Sin 23(4):381–384
17. Nakashima M, Uematsu T, Kanamaru M, Okazaki O, Hakusui H (1992) Phase I study of levofloxacin, (S)-(-)-ofloxacin. Jpn J Clin Pharmacol Ther 23(2):515–520
18. Madhavi M, Priyanka UM (2014) Influence of follicular and luteal phase on levofloxacin pharmacokinetics in female volunteers. Int J Pharm Bio Sci 5(1):305–312
19. Abuelkheir MM (2009) Effect of pharmacokinetic differences between men and women on pharmacodynamic target attainment of levofloxacin against Streptococcus pneumoniae. Saudi Pharm J 17(1):29–39
20. Thee S, Garcia-Prats AJ, McIlleron HM, Wiesner L, Castel S, Norman J, Draper HR, van der Merwe PL, Hesseling AC, Schaaf HS (2014) Pharmacokinetics of ofloxacin and levofloxacin for prevention and treatment of multidrug-resistant tuberculosis in children. Antimicrob Agents Chemother 58(5):2948–2951
21. Trampuz A, Wenk M, Rajacic Z, Zimmerli W (2000) Pharmacokinetics and pharmacodynamics of levofloxacin against *Streptococcus pneumoniae* and *Staphylococcus aureus* in human skin blister fluid. Antimicrob Agents Chemother 44(5):1352–1355
22. Chow AT, Chen A, Lattime H, Morgan N, Wong F, Fowler C, Williams RR (2002) Penetration of levofloxacin into skin tissue after oral administration of multiple 750 mg once-daily doses. J Clin Pharm Ther 27(2):143–150
23. Child J, Mortiboy D, Andrews JM, Chow AT, Wise R (1995) Open-label crossover study to determine pharmacokinetics and penetration of two dose regimens of levofloxacin into inflammatory fluid. Antimicrob Agents Chemother 39(12):2749–2751
24. Benko R, Matuz M, Doro P, Peto Z, Molnar A, Hajdu E, Nagy E, Gardi J, Soos G (2007) Pharmacokinetics and pharmacodynamics of levofloxacin in critically

ill patients with ventilator-associated pneumonia. Int J Antimicrob Agents 30(2):162–168
25. Boselli E, Breilh D, Rimmelé T, Djabarouti S, Saux MC, Chassard D, Allaouchiche B (2005) Pharmacokinetics and intrapulmonary diffusion of levofloxacin in critically ill patients with severe community-acquired pneumonia. Crit Care Med 33(1):104–109
26. Furlanut M, Brollo L, Lugatti E, Di Qual E, Dolcet F, Talmassons G, Pea F (2003) Pharmacokinetic aspects of levofloxacin 500 mg once daily during sequential intravenous/oral therapy in patients with lower respiratory tract infections. J Antimicrob Chemother 51(1):101–106
27. Lubasch A, Keller I, Borner K, Koeppe P, Lode H (2000) Comparative pharmacokinetics of ciprofloxacin, gatifloxacin, grepafloxacin, levofloxacin, trovafloxacin, and moxifloxacin after single oral administration in healthy volunteers. Antimicrob Agents Chemother 44(10):2600–2603
28. Noreddin AM, Marras TK, Sanders K, Chan CK, Hoban DJ, Zhanel GG (2004) Pharmacodynamic target attainment analysis against *Streptococcus pneumoniae* using levofloxacin 500 mg, 750 mg and 1000 mg once daily in plasma (P) and epithelial lining fluid (ELF) of hospitalized patients with community acquired pneumonia (CAP). Int J Antimicrob Agents 24(5):479–484
29. Pea F, Di Qual E, Cusenza A, Brollo L, Baldassarre M, Furlanut M (2003) Pharmacokinetics and pharmacodynamics of intravenous levofloxacin in patients with early-onset ventilator-associated pneumonia. Clin Pharmacokinet 42(6):589–598
30. Bellmann R, Kuchling G, Dehghanyar P, Zeitlinger M, Minar E, Mayer BX, Müller M, Joukhadar C (2004) Tissue pharmacokinetics of levofloxacin in human soft tissue infections. Br J Clin Pharmacol 57(5):563–568
31. Geller DE, Flume PA, Griffith DC, Morgan E, White D, Loutit JS, Dudley MN (2011) Pharmacokinetics and safety of MP-376 (levofloxacin inhalation solution) in cystic fibrosis subjects. Antimicrob Agents Chemother 55(6):2636–2640
32. Lee CK, Boyle MP, Diener-West M, Brass-Ernst L, Noschese M, Zeitlin PL (2007) Levofloxacin pharmacokinetics in adult cystic fibrosis. Chest 131(3):796–802
33. Rebuck JA, Fish DN, Abraham E (2002) Pharmacokinetics of intravenous and oral levofloxacin in critically ill adults in a medical intensive care unit. Pharmacotherapy 22(10):1216–1225
34. Drusano GL, Preston SL, Van Guilder M, North D, Gombert M, Oefelein M, Boccumini L, Weisinger B, Corrado M, Kahn J (2000) A population pharmacokinetic analysis of the penetration of the prostate by levofloxacin. Antimicrob Agents Chemother 44(8):2046–2051
35. Goodwin SD, Gallis HA, Chow AT, Wong FA, Flor SC, Bartlett JA (1994) Pharmacokinetics and safety of levofloxacin in patients with human immunodeficiency virus infection. Antimicrob Agents Chemother 38(4):799–804
36. Piscitelli SC, Spooner K, Baird B, Chow AT, Fowler CL, Williams RR, Natarajan J, Masur H, Walker RE (1999) Pharmacokinetics and safety of high-dose and extended-interval regimens of levofloxacin in human immunodeficiency virus-infected patients. Antimicrob Agents Chemother 43(9):2323–2327
37. Hutschala D, Skhirtladze K, Zuckermann A, Wisser W, Jaksch P, Mayer-Helm BX, Burgmann H, Wolner E, Müller M, Tschernko EM (2005) In vivo measurement of levofloxacin penetration into lung tissue after cardiac surgery. Antimicrob Agents Chemother 49(12):5107–5111
38. Usman M, Ashraf M, Khokhar MI, Ashiq B, Masood MI, Afzal S, Omer O, Ali M, Qadir MI (2013) Comparative pharmacokinetics of levofloxacin in healthy volunteers and in patients suffering from typhoid Fever. Iran J Pharm Res 12(1):147–154
39. Weinrich M, Scheingraber S, Stremovskaia T, Schilling MK, Kees F, Pistorius GA (2006) Liver tissue concentrations of levofloxacin after single intravenous administration of 500 mg for antibiotic prophylaxis in liver surgery. Int J Antimicrob Agents 28(3):221–225
40. Bellmann R, Egger P, Gritsch W, Bellmann-Weiler R, Joannidis M, Dunzendorfer S, Wiedermann CJ (2002) Elimination of levofloxacin in critically ill patients with renal failure: influence of continuous venovenous hemofiltration. Int J Clin Pharmacol Ther 40(4):142–149
41. Malone RS, Fish DN, Abraham E, Teitelbaum I (2001) Pharmacokinetics of levofloxacin and ciprofloxacin during continuous renal replacement therapy in critically ill patients. Antimicrob Agents Chemother 45(10):2949–2954
42. Sowinski KM, Lucksiri A, Kays MB, Scott MK, Mueller BA, Hamburger RJ (2003) Levofloxacin pharmacokinetics in ESRD and removal by the cellulose acetate high performance-210 hemodialyzer. Am J Kidney Dis 42(2):342–349
43. Nicolle L, Duckworth H, Sitar D, Bryski L, Harding G, Zhanel G (2008) Pharmacokinetics/pharmacodynamics of levofloxacin 750 mg once daily in young women with acute uncomplicated pyelonephritis. Int J Antimicrob Agents 31(3):287–289
44. Tsaganos T, Kouki P, Digenis P, Giamarellou H, Giamarellos-Bourboulis EJ, Kanellakopoulou K (2008) Pharmacokinetics of levofloxacin after single and multiple oral doses in patients undergoing intermittent haemodialysis. Int J Antimicrob Agents 32(1):46–49
45. Guenter SG, Iven H, Boos C, Bruch HP, Muhl E (2002) Pharmacokinetics of levofloxacin during continuous venovenous hemodiafiltration and continuous venovenous hemofiltration in critically ill patients. Pharmacotherapy 22(2):175–183
46. Chien SC, Chow AT, Rogge MC, Williams RR, Hendrix CW (1997) Pharmacokinetics and safety of oral levofloxacin in human immunodeficiency virus-infected individuals receiving concomitant zidovudine. Antimicrob Agents Chemother 41(8): 1765–1769

47. Fish DN, Chow AT (1997) The clinical pharmacokinetics of levofloxacin. Clin Pharmacokinet 32(2):101–119
48. Conte JE Jr, Golden JA, McIver M, Little E, Zurlinden E (2007) Intrapulmonary pharmacodynamics of high-dose levofloxacin in subjects with chronic bronchitis or chronic obstructive pulmonary disease. Int J Antimicrob Agents 30(5):422–427
49. Kempker RR, Barth AB, Vashakidze S, Nikolaishvili K, Sabulua I, Tukvadze N, Bablishvili N, Gogishvili S, Singh RS, Guarner J, Derendorf H, Peloquin CA, Blumberg HM (2015) Cavitary penetration of levofloxacin among patients with multidrug-resistant tuberculosis. Antimicrob Agents Chemother 59(6):3149–3155
50. Lee LJ, Hafkin B, Lee ID, Hoh J, Dix R (1997) Effects of food and sucralfate on a single oral dose of 500 milligrams of levofloxacin in healthy subjects. Antimicrob Agents Chemother 41(10):2196–2200
51. Radandt JM, Marchbanks CR, Dudley MN (1992) Interactions of fluoroquinolones with other drugs: mechanisms, variability, clinical significance, and management. Clin Infect Dis 14:272–284
52. Okimoto N, Niki Y, Soejima R (1992) Effect of levofloxacin on serum concentration of theophylline. Chemotherapy 40(Suppl 3):68–74
53. Shiba K, Sakai O, Shimada J, Okazaki O, Aoki H, Hakusui H (1992) Effect of antacids, ferrous sulfate, and ranitidine on absorption of DR-3355 in humans. Antimicrob Agents Chemother 36:2270–2274
54. Gaitonde MD, Mendes P, House ESA, Lehr KH (1995) The effects of cimetidine and probenecid on the pharmacokinetics of levofloxacin [abstract no. A41]. American Society for Microbiology: Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, 35; 8
55. Sánchez Navarro A, Colino Gandarillas CI, Alvarez Lerma F, Menacho YA, Domínguez-Gil A (2005) Pharmacokinetics and pharmacodynamics of levofloxacin in intensive care patients. Clin Pharmacokinet 44(6):627–635

Bacteriology of *Naja atra* Snakebite Wound and Its Implications for Antibiotic Therapy

Yan-Chiao Mao, Po-Yu Liu, Liao-Chun Chiang, and Chen-Chang Yang

1 Introduction

Six major venomous snake species are distributed throughout Taiwan, including *Trimeresurus stejnegeri*, *Protobothrops mucrosquamatus*, *Deinagkistrodon acutus*, and *Daboia siamensis* in the Viperidae family and *Naja atra* and *Bungarus multicinctus* in the Elapidae family. *N. atra*, the only cobra species, is also distributed throughout Southeastern Asia, including Vietnam, Laos, and Southern China (Fig. 1) [1]. In Taiwan, *N. atra* infrequently bites humans and causes 6% (range, 0%–36%) of all snakebite cases [2]. However, once envenomated, the majority of patients develop wound infections, including cellulitis, tissues necrosis, finger or toe gangrene, and/or extensive necrotizing fasciitis (Figs. 2 and 3); therefore, empirical antibiotic therapy is frequently advocated [3]. In Taiwan, bacteriology studies of *N. atra* bite wounds remain scarce and fragmented [4–6]. Although studies of the oral bacteriology of *N. atra* have been conducted in Hong Kong [7, 8], little is known about snakebite wound bacteriology and the effects of geographic differences in the same species [9–11]. To better understand the bacteriology of *N. atra* bite wounds, we retrospectively analyzed 112 cases from two referring medical centers: Taichung Veterans General Hospital (VGH-TC) in central Taiwan and Taipei Veterans General Hospital (VGH-TP) in Northern Taiwan.

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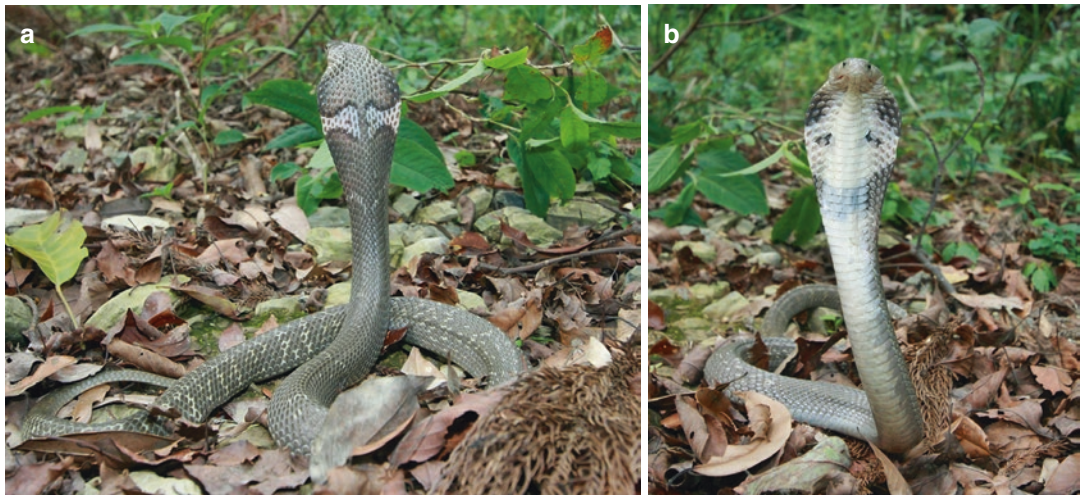

Fig. 1 Features of *Naja atra* (pictures were provided and used with the permission from Chih-Ming Lai). (**a**) *N. atra* (dorsal side). The hood mark shape is variable from spectacle, mask to horseshoe, or O-shape and is often linked to light throat area on at least one side. (**b**) *N. atra* (ventral side). The throat area is clearly defined light which is usually with a pair of clearly defined lateral spots

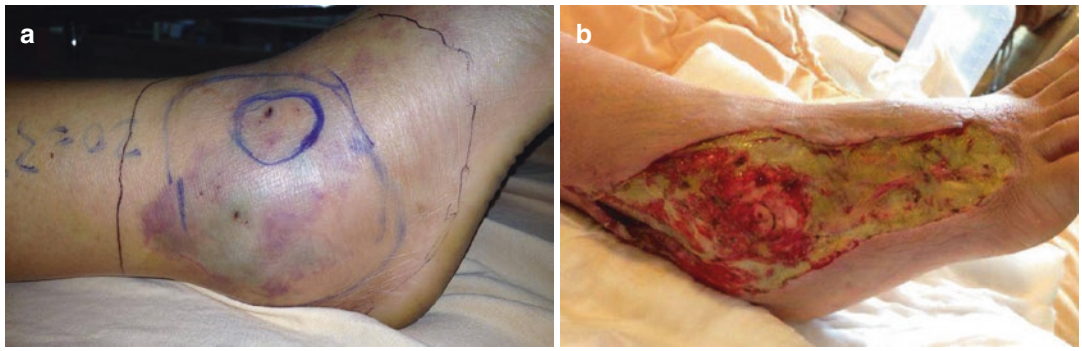

Fig. 2 (**a**) *N. atra* bite over right foot, manifesting local tissue necrosis and abscess formation which occurred 33 h later. (**b**) Second debridement was performed 7 days after the bite. *M. morganii* and *E. faecalis* were identified in the deep tissue biopsy culture

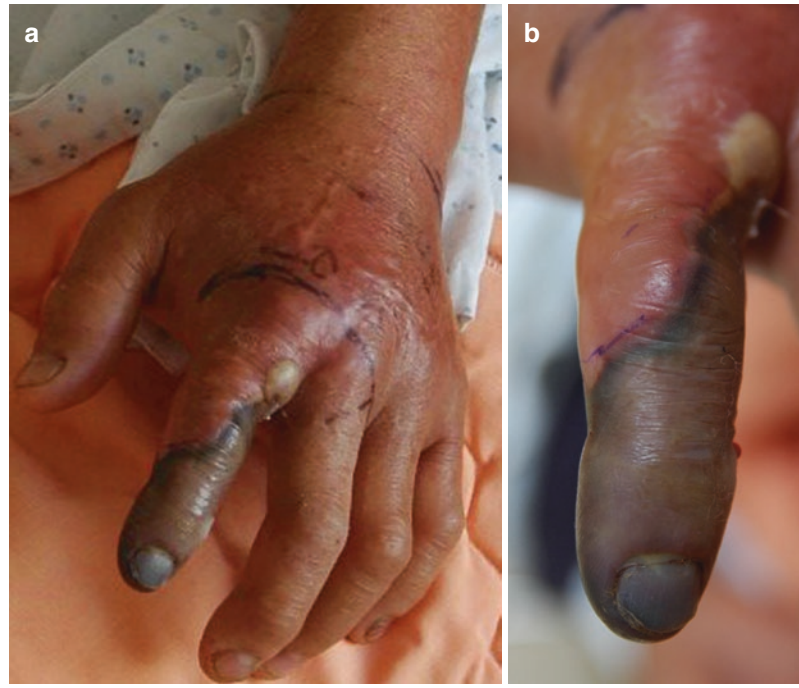

Fig. 3 (**a**) *N. atra* bite over left index finger. Swelling extended to the ipsilateral shoulder, and gangrenous change in the finger developed 3 days later. (**b**) A close-up picture. Patient underwent finger amputation 5 days post-bite. *A. hydrophila* was identified in the wound discharge culture

2 Study Population

This was a retrospective cohort study. The study protocol followed the principles of the Declaration of Helsinki. All cases of *N. atra* envenomation were admitted to VGH-TC between April 2005 and July 2009 (4 years) and to VGH-TP between October 1995 and September 2009 (14 years). Cases were identified by searching the computerized databases at both VGH-TC and VGH-TP, using the keywords "snake," "cobra," "*N. atra*," and "*N. n. atra*" both in English and Chinese. Two authors independently reviewed the medical records of all subjects with possible cobra envenomations. A definite diagnosis was made by the identification of the culprit snake, which included the examination of the snake, identification of the snake by the patient through a picture, or laboratory testing of the venom by the treating physician [12–14]. Patients with typical manifestations, as determined through physical examination, serial wound inspection, a relevant history, and clinical improvement after receiving specific antivenom for *N. atra*, were included in the "clinical case" group (Table 1) [2, 3, 15]. After a careful review of the medical records, patients with snakebites other than those of *N. atra* [e.g., patients with snakebites of the other five medi-

Table 1 Bacteria isolated from *Naja atra* bite cases and diagnostic methods of its envenomation

	Diagnostic methods							
	VGH-TC	VGH-TP	Overall	*P* value	Definitive case[a]	Clinical case[b]	Overall	*P* value
Case numbers	*N* = 78	*N* = 34	*N* = 112		79 (54/25)[c]	33 (24/9)[c]	112	0.646[d]
First aid								
Topical herbs	2	6	8		–	–	–	
Rope binding[e]	4	0	4		–	–	–	
Incision and suction	2	1	3		–	–	–	
Wound infection	52	34	86		61	25	86	
Any surgery	36	25	61		42	19	61	
Any form of bacterial culture	32	27	59		41	18	59	
Positive bacterial cultures	29	21	50		34	16	50	
Aerobic gram-positive bacteria	13	11	24		16	8	24	
Bacillus cereus	1	0	1	1	1	0	1	1
Enterococcus spp.	10	11	21	0.015	13	8	21	0.336
Coagulase -negative	2	2	4	0.584	3	1	4	1
Staphylococcus species								
Staphylococcus aureus	1	1	2	0.517	2	0	2	1
Aerobic gram-negative bacteria	27	22	49		33	16	49	
Acinetobacter baumannii	1	0	1	1	1	0	1	1
Aeromonas hydrophila	6	1	7	0.673	6	1	7	0.672
Citrobacter freundii	1	1	2	0.517	1	1	2	0.504
Escherichia coli	2	3	5	0.163	3	2	5	0.63
Klebsiella pneumoniae	0	1	1	0.304	0	1	1	0.295
Morganella morganii	17	15	32	0.016	17	15	32	0.011
Proteus spp.	4	4	8	0.242	6	2	8	1
Proteus mirabilis	1	2	3	0.218	3	0	3	0.554
Proteus penneri	1	1	2	0.517	2	0	2	1
Proteus vulgaris	3	2	5	0.638	3	2	5	0.63
Pseudomonas aeruginosa	2	4	6	0.068	4	2	6	1
Providencia spp.	4	2	6	1	3	3	6	0.358
Providencia alcalifaciens	1	1	2	0.517	1	1	2	0.504
Providencia rettgeri	3	1	4	1	2	2	4	0.58
Serratia spp.	1	2	3	0.218	3	0	3	0.554
Serratia liquefaciens	0	1	1	0.304	1	0	1	1
Serratia marcescens	1	1	2	0.517	2	0	2	1
Shewanella spp.	3	2	5	0.638	4	1	5	1
Yokenella regensburgei	1	0	1	1	1	0	1	1
Anaerobic bacteria								
Bacteroides spp.	5	2	7		5	2	7	
Bacteroides fragilis	5	2	7	1	5	2	7	1
Bacteroides uniformis	0	1	1	0.304	1	0	1	1
Fungus								
Candida parapsilosis	1	0	1	1	1	0	1	1
Polymicrobial (≥2 pathogens)	14	18	32	< 0.001	21	11	32	0.471

[a]Diagnosed by examining the snake, having the patients to identify snake through a picture, or laboratory testing of the venom

[b]Diagnosed by physical examination, serial wound inspection, a relevant history, and clinical improvement after receiving specific antivenom for *N. atra* bite

[c]The bracket number represents the cases in Taichung Veterans General Hospital/Taipei Veterans General Hospital, respectively

[d]No significant variation in the diagnostic methods between the two hospitals

[e]Included any form of rope, rubber band, or towel/clothes bindings

cally important snakes (*T. stejnegeri*, *P. mucrosquamatus*, *D. acutus*, *D. siamensis*, and *B. multicinctus*) and less toxic or nonvenomous snakes] and patients with equivocal manifestations and a negative identification of culprit snake were excluded.

3 Definition of Wound Infection

Besides purulence/abscess and organisms isolated from the fluid/tissue/blood, the appearance of certain symptoms or signs such as pain, erythema, local warmth, swelling, lymphangitis, delayed healing, malodor, crepitus in soft tissues, discolored or friable granulation tissue, or wound breakdown or dehiscence was also indicative of wound infections [16–18]. Since this was a retrospective study, we employed stricter criteria for infected wound following snakebites, which are defined as the presence of two of the following three criteria: onset of new or increasing pain, localized erythema or swelling at the bite site, or purulence at the bite site. The presence of fever and one of the above criteria also satisfied the definition of wound infection [19]. Fever is arbitrarily defined as a body temperature above 38 °C as measured with a tympanic thermometer, a device commonly used in both hospitals. If no abnormalities were mentioned in the case notes, it was assumed that no complication was present.

4 Bacteriology and Statistics

An aerobic and/or anaerobic bacterial culture was performed when infection was suspected in a snakebite wound. A deep tissue or biopsy culture was performed during surgical debridement, and blood culture was performed during febrile episodes. The culture sampling technique has been described in the literature [20]. Polymicrobial infection was defined as the growth of two or more microbes on the same infected or purulent wound [21]. Bacterial identification was performed using traditional biochemical methods with the VITEK 2 system (BioMérieux, Inc., Durham, NC, USA). Susceptibility to antimicrobial agents was determined by the disk diffusion method. Inhibition zone diameters were interpreted according to the zone diameter breakpoints recommended by the Clinical and Laboratory Standards Institute. All positive cultures were subjected to antibiotic susceptibility test analysis to maximize the test precision. The distribution of bacterial species between VGH-TC and VGH-TP and different diagnostic methods were compared using chi-square or Fisher's exact test. All data were analyzed with Statistical Package for the Social Sciences, version 22.0 (2013 release, IBM Corp. Armonk, NY, USA). A two-tailed p value <0.05 was considered statistically significant.

5 Results

Fifteen patients received first aid, including topical herbs in eight, rope binding in four, and incision and suction in three. According to the two diagnostic methods, 79 patients were diagnosed as "definitive case" (positive snake identification), including 54 at VGH-TC and 25 at VGH-TP and 33 as "clinical case" (typical manifestations), including 24 at VGH-TC and 9 at VGH-TP (Table 1). No patients received antibiotics prior to reaching the study hospitals. Clinically suspected wound infection, including cellulitis, tissue necrosis, finger or toe gangrene, or necrotizing fasciitis, developed in 86 out of 112 (77%) envenoming cases. Sixty-one (54%) patients eventually underwent various types of surgery, including local debridement, incision and drainage, fasciotomy or fasciectomy, finger or toe amputation, and skin grafting, which were all performed in the study hospitals. Bacterial cultures from any type of biological sample, including wound discharge, deep tissue or biopsy, or blood, were obtained from 59 of the 86 cases. Fifty patients (50/59, 85%) had positive bacterial cultures, and more than two organisms were isolated from 32 (32/50, 64%) patients. A total of 23 organisms were identified (Table 1). Gram-negative rod bacteria, such as members of the *Enterobacteriaceae* family, were more frequently identified than gram-positive cocci. The following pathogens were detected (in descending order): *Morganella morganii*, 32 cases; *Enterococcus* spp., 21; *Proteus* spp., 8; *Aeromonas hydrophila*, 7; and anaerobic *Bacteroides* spp., 7. *Bacteroides* spp. were the

only anaerobes implicated in these cases. Statistically, a higher incidence of *Morganella*, *Enterococcus* spp., and polymicrobial infection (≥2 pathogens) was observed at VGH-TP.

In this study, all 59 patients produced more than one set of bacterial cultures during hospitalization. Overall, 155 wound discharge, 23 deep tissue or biopsy, and 44 blood samples were obtained. Anaerobic culture was not always concomitantly performed with aerobic culture; therefore, only 47 and 2 anaerobic cultures were obtained from wound discharge and deep tissue or biopsy, respectively. The positive proportions of bacterial culture were 62.6% (97/155) in wound discharge, 78.3% (18/23) in deep tissue or biopsy, and 6.8% (3/44) in blood samples. Only members of the *Bacteroides fragilis* and *Shewanella* species were isolated from blood samples. The results of antibiotic susceptibility tests of *Enterococcus* and *Bacteroides* spp. and the most frequently occurring gram-negative pathogens are listed in Tables 2 and 3, respectively.

6 Discussion

The oral flora of snakes comprises a wide range of aerobic and anaerobic microorganisms, particularly fecal gram-negative rods, because their prey (e.g., rodents or reptiles) usually defecate while being ingested [10, 22, 23]. The bacterial compositions vary among snake species and may be influenced by venom properties [9, 19, 24] and the fecal flora of the prey in different geographic regions [23]. Laboratory bacteriological investigations of aerobic isolates from the venom and oral cavities of the North American pit viper *Crotalus atrox* demonstrated a preponderance of enteric and coliform organisms, particularly *Aerobacter*, *Proteus*, and *Pseudomonas*, with *Clostridium* as the most common anaerobic genus [25]. In the venom of *C. viridis helleri* and *C. scutulatus scutulatus*, *Proteus* spp., *P. aeruginosa*, and coagulase-negative *Staphylococcus* spp. are the most common aerobic species, and *Clostridium* is the most common anaerobic species [23]. In Thailand, *Enterobacter*, *Pseudomonas*, and *Staphylococcus* spp. are the most common aerobic species, and *Clostridium* is the most common anaerobic species in the venom or mouth of the Malayan pit viper (*Calloselasma rhodostoma*). In a recent study conducted by Shek et al. in Hong Kong, *M. morganii*, *Aeromonas hydrophila*, *Proteus* spp., *Enterococcus faecalis*, coagulase-negative *Staphylococcus*, and anaerobic *Clostridium* were the most commonly isolated pathogens in the oropharynx of *N. atra* [7].

The mouth of *N. atra* harbors larger numbers of bacterial species associated with snakebite wound infections than crotaline or colubrid snake species [7, 8]. In our study, *M. morganii* was the most predominant bacteria isolated from bite wounds, followed by *Enterococcus* spp., *Proteus* spp., *A. hydrophila*, *P. aeruginosa*, and *Providencia* spp., in descending order. Our human case study is largely consistent with the experimental findings of Shek et al. [7] with the exception of anaerobic pathogen species. *Bacteroides* spp. were the only anaerobe isolated from the *N. atra* bite wounds in our study. Notably, a previously healthy 31-year-old man developed *Bacteroides fragilis* bacteremia after a *N. atra* bite over his hand. This patient recovered after antibiotic therapy and serial wound debridement and grafting. Another previously healthy 35-year-old man developed *Shewanella* bacteremia after a *N. atra* bite over his finger. He also recovered after the administration of antibiotics, finger amputation, and grafting surgery. In both cases, polymicrobial wound infections were also present: *M. morganii*, *P. rettgeri*, *P. aeruginosa*, *Shewanella* sp., and *Enterococcus* spp. in the first case and *Enterococcus* spp., *P. mirabilis*, *P. penneri*, *Shewanella* sp., and *B. fragilis* in the second case. Although *Bacteroides* and *Shewanella* bacteremia are usually associated with an underlying immunocompromised status (e.g., malignancy), hepatobiliary disease, and high mortality rates [26, 27], the pathogenic effects of these types of bacteremia in immunocompetent patients and in the context of polymicrobial infection remain poorly understood. Snakebite may be a benign cause of *Bacteroides* or *Shewanella* bacteremia with a favorable outcome.

Table 2 Antibiotic susceptibility test of *Enterococcus* and *Bacteroides* spp. isolated from *Naja atra* bite wounds

	Ampicillin	Clindamycin	Gentamicin	Chloramphenicol	Penicillin	Sulfamethoxazole-trimethoprim	Vancomycin	Teicoplanin	Erythromycin	Metronidazole
Enterococcus spp.										
VGH-TC	17/17	–	18/18	–	18/18	–	18/18	–	–	–
(*N* = 18)[a]										
VGH-TP	13/15	3/12	9/15	12/15	14/15	10/12	15/15	15/15	7/15 (*I* = 2)	–
(*N* = 15)										
Bacteroides spp.										
VGH-TC	–	10/10	–	12/10	–	–	–	–	–	10/10
(*N* = 10)										
VGH-TP	–	6/7 (*I* = 1)	–	7/7	–	–	–	–	–	6/7 (*I* = 1)
(*N* = 7)										

VGH-TC Taichung Veterans General Hospital, *VGH-TP* Taipei Veterans General Hospital
[a]The bracket number represents the total numbers of positive culture

Table 3 Antibiotic susceptibility of the most common gram-negative pathogens isolated from *Naja atra* bite wounds[a]

Susceptibility strains	Ampicillin	Ampicillin–sulbactam	Piperacillin–tazobactam	Cefazolin	Cefotaxime	Ceftazidime	Cefmetazole	Flomoxcf	Gentamicin	Amikacin	Ciprofloxacin	Levofloxacin	Imipenem	Sulfamethoxazole-trimethoprim	Chloramphenicol
VGH-TC															
Aerobic gram-negative bacteria															
Aeromonas	0/11	0/5	11/11	0/11	11/11	5/5	5/5	4/11	11/11	11/11	11/11	5/5	6/6	10/10	5/5
hydrophila (12)[b]								(*I* = 7)[c]							
Escherichia	0/2	1/1	2/2	1/2	2/2	1/1	1/1	2/2	2/2	2/2	1/2	1/1		1/2	1/1
coli (2)															
Morganella	3/26	7/7	25/25	0/25	26/26	7/7	7/7	25/26	26/26	26/26	26/26	7/7	11/11	26/26	7/7
morganii (26)	(*I* = 4)							(*I* = 1)							
Proteus spp. (6)	1/6	3/3	6/6	0/6	6/6	3/3	3/3	6/6	6/6	6/6	6/6	3/3	1/1	6/6	3/3
Pseudomonas			8/8			8/8			7/8	7/8	6/8	6/8	8/8		
aeruginosa (8)										(*I* = 1)	(*I* = 2)	(*I* = 1)			
Providencia	4/10	0/5	11/11	0/11	11/11	5/5	5/5	11/11	11/11	11/11	11/11	5/5	6/6	11/11	2/5
spp.(11)	(*I* = 5)			(*I* = 1)											
Shewanella	1/1	3/3	3/3	0/1	1/1	3/3	1/1	1/1	3/3	3/3	3/3	3/3	2/2	3/3	1/1
spp. (3)															
VGH-TP															
Aerobic gram-negative bacteria															
A. hydrophila (1)	0/1			0/1	1/1	1/1		0/1	1/1	1/1				1/1	1/1
E. coli (3)	1/2	0/1	1/1	1/2	2/2	2/2	1/1	2/2	2/2	0/1	1/1	1/1	1/1	2/2	2/2
M. morganii (16)	3/14	10/10	13/13	3/14	14/14	14/14	10/10	14/14	14/14	9/9	13/13	5/5	13/13	14/14	14/14
	(*I* = 1)														
Proteus spp. (6)	0/6	4/4	4/4		6/6	6/6	4/4	6/6	6/6	5/5	4/4	3/3	4/4	6/6	2/6
															(*I* = 1)
P. aeruginosa (5)			5/5			5/5			5/5	4/4	5/5	2/2	5/5	0/4	1/4
Providencia	1/2	1/1	1/1	1/2	2/2	2/2	1/1	1/1	2/2	2/2	1/1		1/1	2/2	2/2
spp.(2)															
Shewanella	1/4	2/2	2/2	0/4	3/4	4/4	2/2	4/4	4/4	4/4	2/2		1/2	4/4	4/4
													(*I* = 1)		
spp.(4)															
Overall															
Aerobic gram-negative bacteria															
A. hydrophila (13)	0/12	0/5	11/11	0/12	12/12	6/6	5/5	4/12	12/12	12/12	11/11	5/5	6/6	11/11	6/6
								(*I* = 7)							
E. coli (5)	0/4	1/2	3/3	2/4	4/4	3/3	2/2	4/4	4/4	2/3	2/3	2/2	1/1	3/4	3/3
M. morganii (42)	6/40	17/17	38/38	3/39	40/40	21/21	17/17	39/40	40/40	35/35	39/39	12/12	14/14	40/40	21/21
	(*I* = 5)							(*I* = 1)							
Proteus spp. (12)	1/12	7/7	10/10	0/6	12/12	9/9	7/7	12/12	12/12	11/11	10/10	6/6	5/5	12/12	5/9

															(*I* = 1)
P. *aeruginosa* (13)			13/13			13/13			12/13	11/12	11/13	8/10	13/13	0/4	1/4
										(*I* = 1)	(*I* = 2)	(*I* = 1)			
Providencia	5/12	1/6	12/12	1/13	13/13	7/7	6/6	12/12	13/13	13/13	12/12	5/5	7/7	13/13	4/7
spp. (13)	(*I* = 5)			(*I* = 1)											
Shewanella	2/5	5/5	5/5	0/5	4/5	7/7	3/3	5/5	7/7	7/7	5/5	3/3	3/4	7/7	5/5
													(*I* = 1)		
spp. (7)															

VGH-TC Taichung Veterans General Hospital, *VGH-TP* Taipei Veterans General Hospital

[a]To maximize the precision of the antibiotic susceptibility test, all positive cultures were included in the analysis

[b]The bracket number represents the total numbers of positive culture

[c]Intermediate susceptibility

In-hospital snakebite management comprises the administration of antivenom, antibiotics for wound infections, or surgery to ameliorate infectious complications. In Brazil, Jorge et al. suggested chloramphenicol as the antibiotic of choice for the management of *Bothrops* envenomation because the most frequently isolated pathogens from these wounds include *M. morganii*, *P. rettgeri*, *Enterobacter* spp., *Escherichia coli*, *Enterococcus* spp., and *Bacteroides* spp. [28]. In Northern Thailand, Threaten et al. recommended benzylpenicillin with gentamicin as a prophylactic antibiotic regimen after Malayan pit viper (*Calloselasma rhodostoma*) envenomation because *Enterobacter* spp., *Pseudomonas* spp., and occasionally *Staphylococcus* and *Clostridia* have been cultured from the venom and mouth of this snake species [9]. However, a positive bacterial culture obtained from the mouth or venom of a snake does not necessarily correspond to a high risk of snakebite wound infection. Hence, the use of prophylactic antibiotics during snakebite management remains controversial [22]. For example, a low incidence of wound infection was documented in snakebites from certain crotaline species, despite the isolation of several pathogens from the snake venom [19, 23, 29]. Furthermore, the antibacterial effect of crotaline snake venom was previously described [7–9, 24], and prophylactic antibiotics have not been found to reduce the incidence of wound infection in prospective evaluations [30, 31].

In Taiwan, the crotaline snakes *T. stejnegeri* and *P. mucrosquamatus* cause more than 70% of all snakebite incidents [2]; however, these species rarely induce wound infections after envenomation. Chen et al. previously investigated snakebites from *T. stejnegeri* and *P. mucrosquamatus* and found that 6% and 26% cases, respectively, developed clinically suspected wound infections and 0% and 9% cases, respectively, underwent surgery, including dermatomy/fasciotomy, skin graft, and digit amputation, after envenomation [32]. In our study, 77% (86/112) of the cases developed clinically suspected wound infections, and 54% (61/112) required surgery secondary to tissue necrosis, finger or toe gangrene, and/or necrotizing fasciitis. *N. atra* venom comprises cardiotoxins, neurotoxins, hemotoxins, and phospholipase A_2, among others. Cardiotoxins and neurotoxins represent the major components and account for 55% and 10% of the dry weight of crude venom, respectively [3]. Although neurotoxins are the most lethal fraction in small mammals, they causes only mild neurotoxicity in humans; instead, the major concern in humans is cardiotoxins, which work synergistically with phospholipase A_2 to induce local tissue necrosis after snakebites, predispose the wound to bacterial infection from the indigenous oral flora of the snake, and necessitate limb amputation or cause mortality in rare circumstances [3, 33].

In our study, we identified "clinical cases" of *N. atra* bites by the typical presentations of *N. atra* envenomation. *N. atra* bites induce distinct effects, including wound necrosis (63%–100%), fever, necrotizing fasciitis, gastrointestinal effects, and systemic neurotoxicity, which are rarely or not found in crotaline (*T. stejnegeri* and *P. mucrosquamatus*) bites [3, 32, 34]. Most *N. atra* bite cases can be accurately diagnosed and treated using the diagnostic algorithm established by the Taiwan Poison Control Center, which includes physical examination, serial wound inspection, a relevant history, and clinical improvement after receiving a specific antivenom [2, 3, 15]. Only a few cases with equivocal manifestations necessitated laboratory testing of the venom to establish a definitive diagnosis [12–14]. Moreover, we did not find significant variations in the distribution of bacteriology between definitive and clinical cases, which might favor the misclassification of infected crotaline snakebite wounds into *N. atra* bites among clinical cases [7, 8].

The diagnosis of wound infection following snake envenomation remains problematic not only because the venom causes toxicological effects similar to those caused by pathogenic flora (e.g., local swelling, heat, tenderness, regional lymphadenopathy, fever, and increased white blood cell counts) [25, 35] but also because no validated physical criteria are available for the diagnosis of this particular type of wound infection [17, 19]. Nevertheless, we have tried our best to employ stricter criteria in the diagnosis of wound infection (i.e., clinical symptoms/signs

supporting the diagnosis of wound infection and organisms isolated from the wound discharge, deep tissue or biopsy, or blood). Although the incidence of wound infection might still have been overestimated in this study, we believed the overestimate was likely to be of limited magnitude given that a very high proportion of positive bacterial cultures was obtained in cases with clinically suspected infection and more than half of the patients with a diagnosis of wound infection underwent surgery because of infectious complications. Furthermore, a high incidence of wound necrosis (63%–100%), which has been recognized as a factor significantly associated with certain types of wound infection [16], was frequently observed with *N. atra* envenomation in contrast to crotaline envenomation in Taiwan [3, 32, 34]. The importance of wound infection following *N. atra* envenomation should not be overlooked. We suggested that snakebite wound infection should be considered a special wound infection entity. More objective measurements such as sonographic, laboratory, and/or validated physical criteria for snakebite wound infections should be established in the future [36–39].

The judicious use of antibiotics based on local bacteriology patterns should be considered to improve the management of *N. atra* bite wound infections. Chen et al. inspected 21 snakebite cases with wound infections and isolated at least 17 bacterial species from these wounds, including 17 caused by *N. atra*, 1 by *T. stejnegeri*, 1 by *P. mucrosquamatus*, and 2 by unknown snake species [4]. *M. morganii*, *Enterococcus* spp., and *P. aeruginosa* were the most common aerobic species and *Bacteroides* spp. the most common anaerobe species isolated from snakebites. Huang et al. analyzed 17 cases of snakebite with wound infections, including 16 caused by *N. atra* snakebite and 1 by *T. stejnegeri*, and isolated 13 bacterial species [5]. *M. morganii*, *Enterococcus* spp., and *A. hydrophila* were the most common aerobic species, and *Bacteroides* spp. were the only anaerobic species isolated in that study. Although those two studies did not specify the bacteria with respect to snake species, our findings suggest that these pathogens more likely arose from *N. atra* snakebite wounds. Accordingly, we do not recommend the routine use of antibiotics in the management of crotaline snakebites [7, 8]. In our study, no significant differences in bacterial distribution or antibiotic resistance were observed between the two hospitals, except for an increased incidence of *M. morganii*, *Enterococcus* spp., and polymicrobial infections among cases from VGH-TP, which may have been related to variations in the fecal flora of prey and oral flora of individual snakes in different geographic areas in Taiwan [40]. As *M. morganii* is naturally resistant to benzylpenicillin, aminopenicillins, oxacillin, first- and second-generation cephalosporins, and sulfamethoxazole, and given the safety profile of chloramphenicol, monotherapy with ureidopenicillin or combination therapy with aminopenicillin and a third-generation cephalosporin or fluoroquinolone may be the initial drugs of choice for the management of *N. atra* snakebite wound infection [41]. However, as increased antibiotic resistance of gram-negative bacteria to fluoroquinolone and of *Enterococcus* spp. to penicillins has been observed, we recommend the continuous surveillance of antibiotic resistance among these pathogens [42, 43].

7 Limitations

This study has several limitations. First, there is always a time delay in bacterial culture collection from snakebite wounds because of the natural course of *N. atra* envenoming [3]. Patients may have received several forms of treatment (e.g., wound cleansing, application of topical medicines, surgical debridement, or antimicrobial therapy) in a prehospital setting or during transportation or hospitalization that may have altered the bacterial composition before bacterial culture collection; therefore, the management timing cannot always be addressed in detail.

Second, in our study, anaerobic cultures were not always concomitantly performed with aerobic cultures; therefore, the incidence and numbers of cases affected by anaerobic infection may have been underestimated [22, 44].

Third, both VGH-TC and VGH-TP are referral centers; therefore, the incidence of wound infection

and the bacteriological pattern in this study may not be generalizable to all primary care facilities because of possible referral bias. Furthermore, this is a retrospective study, which suffers certain inherent limitations of the study design; hence, the results should be interpreted cautiously. Nevertheless, this is the first study to investigate a single snake species that most frequently causes snakebite wound infections in Taiwan, and the findings may have important clinical implications in the better management of *N. atra* bite.

Conclusions

A high incidence of clinically suspected wound infection was observed in cases of *N. atra* envenomation. No significant differences were observed in the distribution of bacteriology between the study hospitals, except for an increase in the incidence of *M. morganii*, *Enterococcus* spp., and polymicrobial infections at VGH-TP, which may have been related to variations in the fecal flora of prey and oral flora of individual snakes in different geographic areas in Taiwan. With the exception of anaerobic pathogens, our human case study findings support the experimental findings obtained in Hong Kong [7]. Based on the bacteriological findings, we suggest that either monotherapy with ureidopenicillin or combination therapy with aminopenicillin and a third-generation cephalosporin or fluoroquinolone is the preferred drug of choice in the initial management of *N. atra* snakebite wound infections.

References

1. Uetz P, Freed P, Hošek J (eds) (2016) The Reptile Database Retrieved from http://www.reptile-database.org/. Accessed 22 Mar 2017
2. Mao YC, Hung DZ (2015) Epidemiology of snake envenomation in Taiwan. In: Gopalakrishnakone P, Faiz MA, Fernando R, Gnanathasan CA, Habib AG, Yang C-C (eds) Clinical toxinology in Asia Pacific and Africa. Springer, Singapore, pp 3–22
3. Mao YC, Hung DZ (2015) Management of snake envenomation in Taiwan. In: Gopalakrishnakone P, Faiz MA, Fernando R, Gnanathasan CA, Habib AG, Yang C-C (eds) Clinical toxinology in Asia Pacific and Africa. Springer, Singapore, pp 23–52
4. Chen CM, Wu KG, Chen CJ, Wang CM (2011) Bacterial infection in association with snakebite: a 10-year experience in a northern Taiwan medical center. J Microbiol Immunol Infect 44(6):456–460
5. Huang LW, Wang JD, Huang JA, Hu SY, Wang LM, Tsan YT (2012) Wound infections secondary to snakebites in central Taiwan. J Venom Anim Toxins incl Trop Dis 18(3):272–276
6. Liao WB, Lee CW, Tsai YS, Liu BM, Chung KJ (2000) Influential factors affecting prognosis of snakebite patients management: Kaohsiung Chang Gung Memorial Hospital experience. Chang Gung Med J 23(10):577–583
7. Shek KC, Tsui KL, Lam KK, Crow P, Ng KH, Ades G, Yip KT, Grioni A, Tan KS, Lung DC, Lam TS, Fung HT, Que TL, Kam CW (2009) Oral bacterial flora of the Chinese cobra (Naja atra) and bamboo pit viper (Trimeresurus albolabris) in Hong Kong SAR. China Hong Kong Med J 15(3):183–190
8. Lam KK, Crow P, Ng KH, Shek KC, Fung HT, Ades G, Grioni A, Tan KS, Yip KT, Lung DC, Que TL, Lam TS, Simpson ID, Tsui KL, Kam CW (2011) A cross-sectional survey of snake oral bacterial flora from Hong Kong, SAR, China. Emerg Med J 28(2):107–114
9. Theakston RD, Phillips RE, Looareesuwan S, Echeverria P, Makin T, Warrell DA (1990) Bacteriological studies of the venom and mouth cavities of wild Malayan pit vipers (Calloselasma rhodostoma) in southern Thailand. Trans R Soc Trop Med Hyg 84(6):875–879
10. Garg A, Sujatha S, Garg J, Acharya NS, Chandra Parija S (2009) Wound infections secondary to snakebite. J Infect Dev Ctries 3(3):221–223
11. Wong OF, Lam TS, Fung HT, Choy CH (2010) Five-year experience with Chinese cobra (Naja atra)—related injuries in two acute hospitals in Hong Kong. Hong Kong Med J 16(1):36–43
12. Huang YP, Yu YJ, Hung DZ (2002) Sandwich enzyme-linked immunosorbent assay for Taiwan cobra venom. Vet Hum Toxicol 44(4):200–204
13. Hung DZ, Liau MY, Lin-Shiau SY (2003) The clinical significance of venom detection in patients of cobra snakebite. Toxicon 41(4):409–415
14. Hung DZ, Lin JH, Mo JF, Huang CF, Liau MY (2014) Rapid diagnosis of Naja atra snakebites. Clin Toxicol (Phila) 52(3):187–191
15. Warrell DA (2010) Guidelines for the clinical management of snake-bites, 2nd edn. WHO Regional Office for South-East Asia, New Delhi, pp 1–151
16. Gardner SE, Frantz RA, Doebbeling BN (2001) The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair Regen 9(3):178–186
17. Cutting KF, White R (2004) Defined and refined: criteria for identifying wound infection revisited. Br J Community Nurs 9(3):S6–S15
18. World Union of Wound Healing Societies (2008) Wound infection in clinical practice. An interna-

tional consensus. Medical Education Partnership Ltd, London
19. Clark RF, Selden BS, Furbee B (1993) The incidence of wound infection following crotalid envenomation. J Emerg Med 11(5):583–586
20. Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14(2):244–269
21. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, Deery HG, Embil JM, Joseph WS, Karchmer AW, Pinzur MS, Senneville E (2012) 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis 54(12):e132–e173
22. Kerrigan KR (1992) Bacteriology of snakebite abscess. Trop Dr 22(4):158–160
23. Goldstein EJ, Citron DM, Gonzalez H, Russell FE, Finegold SM (1979) Bacteriology of rattlesnake venom and implications for therapy. J Infect Dis 140(5):818–821
24. Chen LW, Kao PH, Fu YS, Lin SR, Chang LS (2011) Membrane-damaging activity of Taiwan cobra cardiotoxin 3 is responsible for its bactericidal activity. Toxicon 58(1):46–53
25. Ledbetter EO, Kutscher AE (1969) The aerobic and anaerobic flora of rattlesnake fangs and venom: therapeutic implications. Arch Environ Health 19(6):770–778
26. Cheng CW, Lin HS, Ye JJ, Yang CC, Chiang PC, Wu TS, Lee MH (2009) Clinical significance of and outcomes for Bacteroides fragilis bacteremia. J Microbiol Immunol Infect 42(3):243–250
27. Liu PY, Lin CF, Tung KC, Shyu CL, Wu MJ, Liu JW, Chang CS, Chan KW, Huang JA, Shi ZY (2013) Clinical and microbiological features of shewanella bacteremia in patients with hepatobiliary disease. Intern Med 52(4):431–438
28. Jorge MT, Ribeiro LA, da Silva ML, Kusano EJ, de Mendonca JS (1994) Microbiological studies of abscesses complicating Bothrops snakebite in humans: a prospective study. Toxicon 32(6):743–748
29. LoVecchio F, Klemens J, Welch S, Rodriguez R (2002) Antibiotics after rattlesnake envenomation. J Emerg Med 23(4):327–328
30. Jorge MT, Malaque C, Ribeiro LA, Fan HW, Cardoso JL, Nishioka SA, Sano-Martins IS, Franca FO, Kamiguti AS, Theakston RD, Warrell DA (2004) Failure of chloramphenicol prophylaxis to reduce the frequency of abscess formation as a complication of envenoming by Bothrops snakes in Brazil: a double-blind randomized controlled trial. Trans R Soc Trop Med Hyg 98(9):529–534
31. Kerrigan KR, Mertz BL, Nelson SJ, Dye JD (1997) Antibiotic prophylaxis for pit viper envenomation: prospective, controlled trial. World J Surg 21(4):369–372. discussion 72-3
32. Chen YW, Chen MH, Chen YC, Hung DZ, Chen CK, Yen DH, Huang CI, Lee CH, Wang LM, Yang CC (2009) Differences in clinical profiles of patients with Protobothrops mucrosquamatus and Viridovipera stejnegeri envenoming in Taiwan. Am J Trop Med Hyg 80(1):28–32
33. Wu PL, Chiu CR, Huang WN, Wu WG (2012) The role of sulfatide lipid domains in the membrane pore-forming activity of cobra cardiotoxin. Biochim Biophys Acta 1818(5):1378–1385
34. Wang W, Chen QF, Yin RX, Zhu JJ, Li QB, Chang HH, Wu YB, Michelson E (2014) Clinical features and treatment experience: a review of 292 Chinese cobra snakebites. Environ Toxicol Pharmacol 37(2):648–655
35. Blaylock R (1999) Antibiotic use and infection in snakebite victims. S Afr Med J 89(8):874–876
36. Blokhuis-Arkes MH, Haalboom M, van der Palen J, Heinzle A, Sigl E, Guebitz G, Beuk R (2015) Rapid enzyme analysis as a diagnostic tool for wound infection: comparison between clinical judgment, microbiological analysis and enzyme analysis. Wound Repair Regen 23:345–352
37. Huang MN, Chang YC, Wu CH, Hsieh SC, Yu CL (2009) The prognostic values of soft tissue sonography for adult cellulitis without pus or abscess formation. Intern Med J 39(12):841–844
38. Adhikari S, Blaivas M (2012) Sonography first for subcutaneous abscess and cellulitis evaluation. J Ultrasound Med 31(10):1509–1512
39. Vohra R, Rangan C, Bengiamin R (2014) Sonographic signs of snakebite. Clin Toxicol (Phila) 52(9):948–951
40. Abrahamian FM, Goldstein EJ (2011) Microbiology of animal bite wound infections. Clin Microbiol Rev 24(2):231–246
41. Stock I, Wiedemann B (1998) Identification and natural antibiotic susceptibility of Morganella morganii. Diagn Microbiol Infect Dis 30(3):153–165
42. Sheng WH, Chen YC, Wang JT, Chang SC, Luh KT, Hsieh WC (2002) Emerging fluoroquinolone-resistance for common clinically important gram-negative bacteria in Taiwan. Diagn Microbiol Infect Dis 43(2):141–147
43. Wang JT, Chang SC, Wang HY, Chen PC, Shiau YR, Lauderdale TL, Hospitals T (2013) High rates of multidrug resistance in Enterococcus faecalis and E. faecium isolated from inpatients and outpatients in Taiwan. Diagn Microbiol Infect Dis 75(4): 406–411
44. Nishioka Sde A, Silveira PV (1992) Bacteriology of abscesses complicating bites of lance-headed vipers. Ann Trop Med Parasitol 86(1):89–91

Part V

Wound Assessment, Care, Measurement

Managing Patients with Fistulas

Kersten Reider

1 Introduction

Fistulas are abnormal communications between two epithelialized surfaces. Most occur after surgical intervention. Enteric fistulas represent abnormal connections between the gastrointestinal tract and the skin or open surgical wound. Common etiologies include surgical intervention, Crohn's disease, trauma, foreign bodies, infectious disease, and tumors. An enterocutaneous fistula is a devastating complication for both surgeon, clinical provider, and patient. Prior to the advent of sophisticated treatment plans, enterocutaneous fistulas held a large mortality rate. In current era, the mortality rate has been reduced from 5 to 20% [1]. However, the development and management of an ECF remain a chronic, debilitating condition associated with prolonged intensive care stay, increased length of hospital stay, and hospital costs of over $500,000 [1].

The primary goals of a patient with an enterocutaneous fistula include skin protection, control, containment, quantification of fistula effluent, and patient comfort. Control of effluent is critical to protect the wound bed from the corrosive effects of the enteric contents and to allow the surrounding wound bed to develop granulation tissue before skin grafting or closure. Failure to control effluent has been associated with poor wound healing.

2 Goals of Treatment

Protection and prevention of skin breakdown surrounding the ECF are essential components in the wound care aspects of treatment. There are several causes of impaired skin integrity at the site of the ECF. The four most common causes are mechanical trauma, allergic responses, infections, and chemical irritants [2]. Frequent dressing changes with pouches that contain abrasive adhesives along with poor pouching techniques can cause repetitive mechanical trauma to the peri-wound skin. Weeping skin, edema, and erythema can be seen in an allergic response to the materials that construct the pouches. When the pouches begin to lift along the wound edge, effluent gets trapped against the skin causing moisture-related skin damage, fungal rashes, and skin infections. The most common chemical irritant is bowel contents. The enzymatic contents of the effluent can be very caustic to skin integrity. Healing the surrounding skin, preventing further skin breakdown, and minimizing wound contamination are key components in wound management [2].

Skin irritation and discomfort can seriously compromise the healing potential of the patient. The proper application of an appropriate intervention can prevent unnecessary patient discomfort and promote adequate wound healing.

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Mobility should not be restricted in ambulatory patients as this may hinder their recovery. Treatment plans should be individualized to meet the needs desired by each patient.

Effluent containment is key to improving skin integrity. Enteric contents can spill onto the surrounding skin leading to persistent tissue inflammation and infection and, if left untreated, can develop into sepsis [2]. Containment of effluent can be accomplished with pouching devices, specialized dressings, or a combination of both techniques. Patient status will ultimately dictate the best treatment option.

Odor from the ECF can be a cause of great anxiety for these patients. Containment of effluent is obviously the best way to control odor. Most pouches have odor-proof linings, and external deodorizers are also available. Deodorizers are available in tablet, liquid, and powder form from a multitude of distributors.

Measurement of the fluid and electrolyte balances in these patients is another important goal in the management of the ECF patient. Certain dressings can give an inaccurate reading of actual fluid loss while others can be more precise. These factors must be considered while managing these patients. In patients with short bowel and proximal ECFs, fluid losses can be up to multiple liters daily [2]. Electrolyte abnormalities can lead to secondary conditions that may be life-threatening. Nutritional needs of these patients are often overlooked. Accurate measurement of the effluent will help guide supplementation and caloric intake.

3 Techniques

There are several methods to managing the patient with a complex abdomen and fistula. The goals of treatment are to contain effluent, to maintain periwound skin integrity, to promote granulation tissue, and most importantly to support the patient's emotional well-being. Initial treatment consists of an overall assessment of the patient, nature of the fistula, and assessment of the wound. The patient also needs to be evaluated for sepsis and electrolyte abnormalities, and imaging of the fistula needs to be completed. Radiologic fistulograms and abdominal CT scans generally assist in identifying the source and anatomy of the fistulas and indicate whether there is a distal bowel obstruction [3]. Once radiographic studies are completed, patients are typically made NPO, and nutritional support with total parenteral nutrition is initiated. Nasogastric tubes may or may not be placed depending on the location of the fistula. Nonoperative medical management continues to be the main objective when tackling fistulas in the hopes for spontaneous closure. When spontaneous closure does not occur, containment of fistula content and protection of the periwound skin become an imperative part of the treatment plan [3].

Containment of fistula effluent can be achieved in several ways. Over the years, there have been several enhancements in the products and clinical techniques in achieving containment. One of the techniques used include the use of roll gauze, an occlusive dressing, and wall suction. Roll gauze is placed into the wound ensuring that it comes into contact with the entire wound base. Once the base is covered, a red rubber catheter is placed on top of the gauze. This procedure is accomplished by cutting extra holes into the catheter to facilitate the removal of drainage. The catheter is then covered with the remaining roll gauze ensuring that the entire wound space is filled. The roll gauze is then held in place with an occlusive dressing that is applied over the entire wound. Barrier rings may be used to encompass the catheter or applied in skin folds to achieve an airtight seal. The catheter is then connected to low continuous bedside suction. This technique has proven to contain drainage and quantify output, but it does not allow the patient to be ambulatory, dressing changes tend to cause the patient discomfort, granulation tissue promotion is minimal, and it also becomes a barrier to discharge from an acute care facility (Fig. 1).

Another method to contain effluent from a functioning fistula is to apply a fistula management pouch. Fistula management pouches are available in many shapes and sizes. These pouches can be applied by a clinician, or the patient can be taught to change the pouch independently. Depending on the size of the wound and location

Fig. 1 The use of roll gauze, a red rubber catheter with extra holes placed into it, and occlusive dressing connected to bedside suction

of the fistula, the fistula management pouch is cut to encompass the entire area. Barrier rings may be utilized to fill creases and gaps where leaking may occur. Pouches are typically changed on a regular basis to eliminate the risk of leaking and periwound skin breakdown. Just like the prior technique, this method also helps to contain drainage and quantify output. This technique allows the patient to be ambulatory, pouch application can be taught to the patient, and barriers to discharge can be minimal but are dependent on each individual case. Despite more positive outcomes with this technique, it also has some less attractive clinical outcomes which include the following. The caustic effluent from the fistula may wash away the barrier of the fistula management pouch and barrier rings quickly causing frequent leaking episodes and periwound skin breakdown. Application of the pouches can also be labor-intensive. Pouches may be costly depending on the patient's need and insurance coverage (Fig. 2).

A newer method of treatment includes the use of fistula isolation devices along with negative-pressure wound therapy (NPWT). This method has proven to contain effluent while promoting wound healing. By isolating the fistula with an isolation device, it provides a conduit to allow the effluent to easily pass into a pouching system [4]. There are three types of fistula isolation devices that can be used to isolate a fistula. These products include a wound crown, fistula funnel, and isolator strip (Table 1). Location of the fistula and depth of the wound will be the deciding factors as to what product will be best suited to contain fistula output while promoting wound healing.

The wound is cleansed with a wound cleanser. The fistula isolation device is then cut to contour the wound bed while also encompassing the fistula. The base of the wound crown and fistula funnel is cut open with a scissors and trimmed to fit around the fistula before placement over the fistula. The base of these isolation devices are intended to be cut to best fit the contours of the wound bed and should be tailored for each individual patient to mirror the wound bed and create the best possible seal. Depending on the

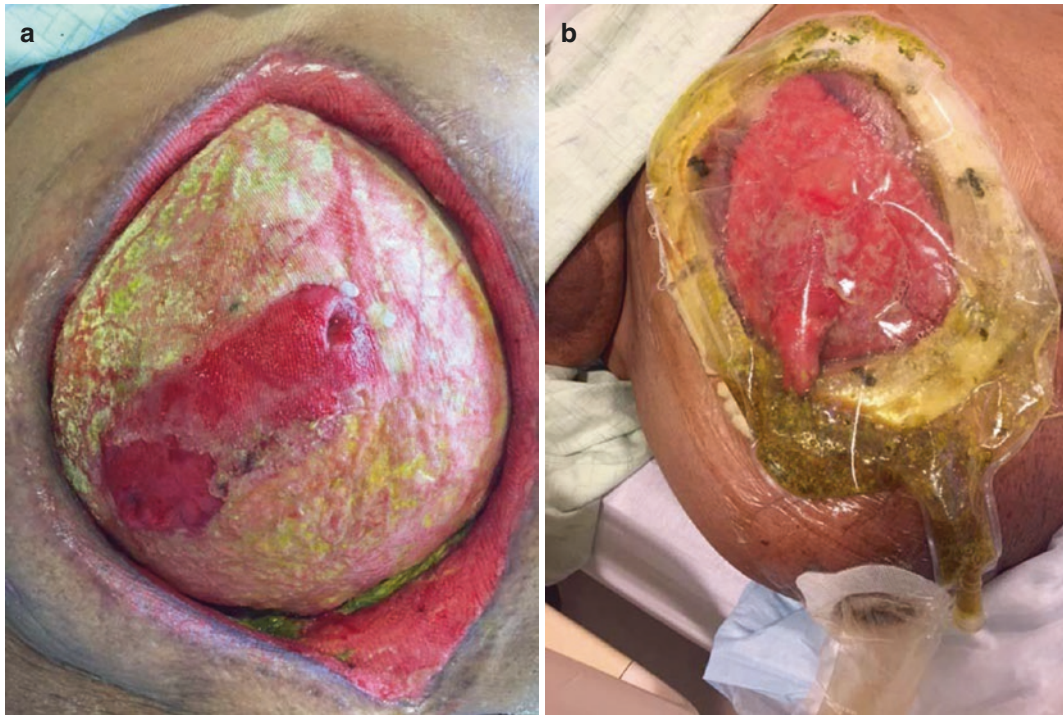

Fig. 2 The use of a fistula management pouch to contain output

Table 1 KCI fistula devices

KCI
An Acelity Company

FISTULA SOLUTION® Devices

One–piece, compressible isolation devices to contain and control effluent

You Need to Isolate	Solution	
• Small intestinal fistula • Ileostomy	**Wound Crown®** • General applications • Isolates and controls the effluent of enteric fistulas and ostomy stomas	 Item #00860013000301
• Small sized fistulas • Sidewell fistulas • Deep crevice wound bed areas	**Fistula Funnel®** • Tapered design flexes to isolate sidewall fistulas • Sizeable to 1, 2, or 3 centimeter isolation area diameter	 Item #00860013000325
• Large fistulas • Group of fistulas • Large or uniquely shaped wound bed areas	**Isolator Strip®** • Flexible strip designed to be shaped as needed for specific isolation applications	 Item #00860013000318

contour of the wound bed, a barrier ring may be used between the fistula isolation device and the wound bed to assist with the seal. Table 2 describes the procedure for a dressing application with negative-pressure wound therapy and a fistula isolation device. Figure 3 demonstrates the use of a fistula isolation device and negative-pressure wound therapy on an actual patient.

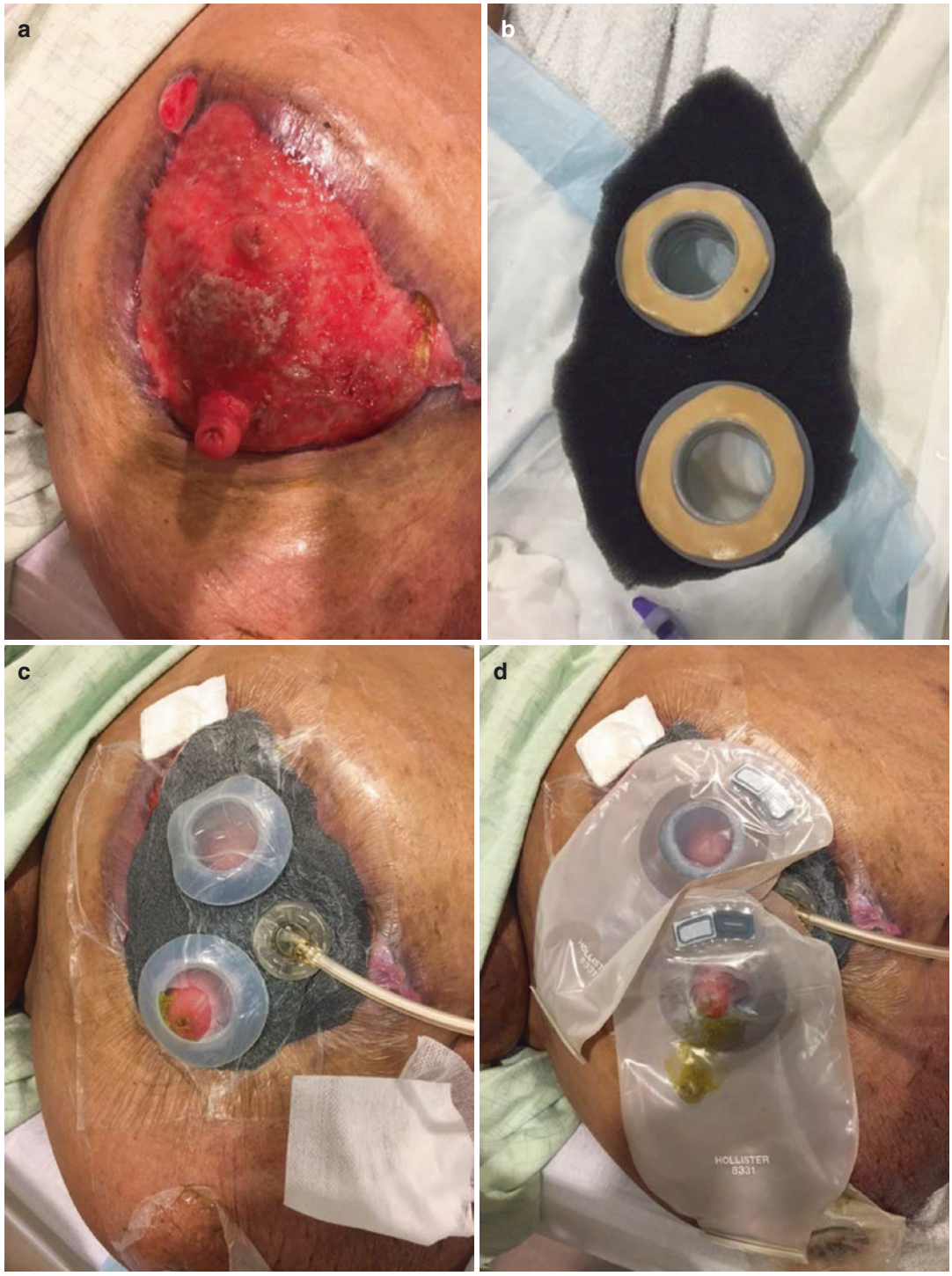

Fig. 3 (**a**) Presentation of the wound with two stomatized fistulas. (**b**) Fistula isolation device is fit into holes cut into NPWT foam dressing that is cut to fit the dimensions of the wound. (**c**) Fistula isolation devices and NPWT in place. (**d**) Pouching system applied over the fistula isolation device to contain effluent

4 Discussion

Enteric fistulas are a relatively common complications of bowel surgery where the bowel has been exposed. The primary goal when a fistula develops is to ensure the patient is stabilized systemically [5]. Fluid and electrolyte imbalance, sepsis, nutrition, and skin care are the focus areas of clinical concern. Control of the effluent is critical not only to protect the skin from the corrosiveness of the enteric contents but also to facilitate adequate nursing care of the patient until definitive closure can be undertaken [6]. Application of a dressing to help manage effluent and promote healing of the abdominal wound is imperative. Dressings and techniques used will be dependent upon the output and depth of the fistula along with the surgeons specific recommendations [6]. Closure of the fistula using acellular dermal matrix and fibrin glue has been described in the literature and sounds attractive but neither tends to be successful in clinical practice. The same can be said for local, extraperitoneal repair of the hole in the bowel followed by split-thickness skin graft [7]. Intubating the fistula with a tube draining system may result in the creation of a larger lesion that is more difficult to control. The tube may enlarge the hole causing erosion into the adjacent bowel [8]. Until recently, fistulas were commonly managed with large bags such as Eakin fistula bags, which could be placed over the wound to collect and contain effluent. While this is satisfactorily efficient at containing effluent, there was no active treatment applied to the wound bed to promote granulation tissue and wound contracture [5]. In addition depending on the wound, contour of the patient abdomen, and amount of effluent, the bags tend to leak causing periwound skin breakdown and patient dissatisfaction.

The most promising techniques combine NPWT with fistula isolation devices and ostomy appliances. This technique enables isolation of the fistula necessary for effective containment of effluent while protecting the surrounding wound bed and promoting sufficient granulation tissue to accept a split-thickness skin graft or reconstruction and closure [4].

Table 2 Procedure for applying negative-pressure wound therapy and fistula isolation device

1. Foam is measured and cut to fit the wound bed
2. After tailoring the foam, a hole is cut into the foam where the fistula would be centered
3. The collapsible fistula isolation device is then inserted into the hole created into the polyurethane foam until the top and bottom flanges lie flush against the foam
4. The flanges anchor the collapsible fistula isolation device within the polyurethane foam and create a channel to capture the effluent draining from the fistula
5. The assembled dressing is then placed into the wound bed so that the collapsible fistula isolation device base is centered over the fistula
6. The device and foam is then covered by clear drape and negative-pressure wound therapy at 125 mmHg continuous
7. After a seal is achieved, an opening is cut in the clear drape at the top of the fistula isolation device
8. An ostomy appliance is then applied to the top flange of the fistula isolation device to contain effluent

Conclusions

Enterocutaneous fistulas are uniquely challenging for care providers and the patient. Goals for treatment of an ECF include effective containment of effluent from the fistula, along with topical and systemic therapies designed to promote granulation of the surrounding wound bed essential to primary healing, or secondary surgical closure. Utilizing a collapsible fistula isolation device along with NPWT has proven to be the most effective form of treatment.

References

1. Bleier J, Hedrick T (2010) Metabolic support of the enterocutaneous fistula patient. Clin Colon Rectal Surg 23(3):142–148
2. Hoedema R, Suryadevara S (2010) Enterostomal therapy and wound care of the enterocutaneous fistula patient. Clin Colon Rectal Surg 23(3):161–168
3. Draus J, Huss S, Niall H, Cheadle W, Larson G (2006) Enterocutaneous fistula: are treatments improving? Surgery 140:570–578
4. Heineman J, Garcia L, Obst M, Chong HS, Langin JG, Humpal R, Pezzella PA, Dries DJ (2015) Collapsible enteroatmospheric fistula isolation device: a novel, simple solution to a complex problem. J Am Coll Surg 221(2):e7–e14

5. Timmons J, Russell F (2014) The use of negative-pressure wound therapy to manage enteroatmospheric fistula in two patients with large abdominal wounds. Int Wound J 11:723–729
6. Schecter W, Hershberg A, Chang D, Harris H, Napolitano LM, Wexner SD, Dudrick SJ (2009) Enteric fistulas: principles of management. J Am Coll Surg 209(4):484–491
7. Majercik S, Kinikini M, White T (2012) Enteroatmospheric fistula: from soup to nuts. Nutr Clin Pract 27(4):507–512
8. Terzi C, Egeli T, Canda A, Arslan N (2014) Management of enteroatmospheric fistulae. Int Wound J 11(Suppl 1):17–21

Wound Measurement, Score

Katherine M. Marsh and Ersilia L. Anghel

1 Introduction

Wound assessment is an important aspect of monitoring wound progression to healing and the efficacy of treatment. There are many aspects that contribute to wound assessment including wound size, wound edge, site, wound bed, the presence of necrotic tissue, wound depth, surrounding skin, the presence of infection, and pain [1]. Of these variables, wound measurement is a helpful quantitative finding that provides a practical approach to track wound healing. In fact, specific measurements such as wound measurement were the most frequently used outcome measure across research studies involving chronic wounds [2]. Changes in wound measurement can also be used as a predictive tool for wound resolution, particularly if used early in the course [3, 4].

Though there is currently no gold standard technique to quantitatively evaluate wound healing, manual metric measurement has historically been most often utilized. More recently, software-based and advance device-based methods were developed to provide more accurate and precise measurements. Digital alternatives including digital planimetry, stereophotogrammetry, and other digital imaging methods have now become preferred measurement techniques over traditional manual metric measurement.

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2 Techniques

In general, there are six main approaches for measuring wound area (Table 1). These include manual metric measurement, mathematical models, manual planimetry, digital planimetry, stereophotogrammetry, and digital imaging methods [5]. Other less common methods include the volume-based method [6], bipolar bioimpedance measurement [7], histogram planimetry [8], or high-frequency ultrasound [9, 10].

2.1 Manual Metric Measurement

Wound measurement has traditionally been completed using a ruler-based technique. This method typically involves using a ruler to measure the longest length and widest width of a wound and then multiplying these two numbers to estimate wound area. It is quick, convenient, simple, and inexpensive. However, manual metric measurement not only has shown poor inter-rater reliability [11], but it is also inaccurate and tends to overestimate wound size [12, 13]. Furthermore, the measurements tend to become even less reliable as wounds became larger and more irregularly shaped [14].

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Table 1 Summary of the advantages and disadvantages of the six most common wound measurement techniques

Technique	Advantages	Disadvantages
Manual		
Manual metric measurement	Cost efficient Time efficient	Unreliable Overestimates size
Mathematical models	Cost efficient Time efficient	Unreliable Underestimates size
Manual planimetry	Accurate and reliable	Requires wound contact Time-consuming
Digital		
Digital planimetry	Accurate and reliable	Time-consuming
Stereophotogrammetry	Accurate and reliable	High cost Time-consuming
Digital imaging	Accurate and reliable Time efficient	Poor depth measurement

Regardless of the evidence against manual metric measurement, there is no current gold standard for wound measurement. Therefore, most studies are compared to this technique, and it is still widely in use today.

2.2 Mathematical Models

Manual metric measurement typically involves multiplying the measured length and width of the wound. This formula assumes the wound is a rectangular or square shape. Mathematical models such as the elliptical method apply basic geometric principles to calculate the area of an elliptical instead, as most wounds are closer to an elliptical shape. This method involves measuring the shortest and longest radii of the wound and using the following formula: $\text{Area}\,(\text{mm}^2) = \text{Length}\,(\text{mm}) \times \text{Width}\,(\text{mm}) \times 0.25 \times \pi$ [15]. While manual metric measurement generally overestimates size, the elliptical method often underestimates size in small wounds [16].

2.3 Manual Planimetry

Another manual measurement technique method is acetate tracing/contact planimetry. Manual planimetry involves placing a transparency with a metric grid above a wound and counting the number of square centimeters within the wound perimeter. Inter- and intrarater reliability are higher than the manual metric measurement, though still inferior to computerized or digitalized methods overall [17]. Since this method involves direct contact with the wound, several disadvantages exist including contamination of the wound bed and discomfort to the patient [18].

2.4 Digital Planimetry

Digital planimetry is similar to manual planimetry, though it involves using a computer to perform calculations instead of manually counting squares on a metric grid [19]. Overall, digital planimetry is more accurate and precise than manual planimetry, though both can be more time-consuming than other measurement methods [5, 20]. Digital planimetry devices such as Visitrak™ require contact with the wound and come with the same disadvantages of doing so [5]. This process involves tracing the wound onto a transparent sheet and then retracing the outline onto a digital device that calculates the surface area. However, some digital planimetry techniques require minimal or no wound contact [21, 22]. Noncontact digital planimetry is discussed further in the digital imaging section below.

2.5 Stereophotogrammetry

Unlike some planimetry methods, stereophotogrammetry using structured light devices does

not require contact with the wound. In this method, a stereographical camera is used to take an image of the wound. The camera is linked to a computer, where the clinician then traces the wound perimeter using a cursor. The wound area, length, and width are calculated via the computer software, and wound size can be measured in two or three dimensions. Stereophotogrammetry with the 3D LifeViz™ camera was found to be as accurate as digital planimetry, and the wound measurements were taken significantly quicker [23]. However, overall stereophotogrammetry is still a time-consuming method, especially when compared to newer measurement methods.

2.6 Digital Imaging Methods

Digital imaging methods are similar to stereophotogrammetry and digital planimetry, where an image of a wound is captured and transferred to a computer. If the computer software uses a scale placed near the wound in the photo to estimate the area of the wound and then calculate the wound area, this is sometimes referred to as noncontact digital planimetry. This and other noncontact photographic methods have been found to be as accurate as traditional digital planimetry [24].

In addition to noncontact digital planimetry, there are multiple other types of digital imaging methods including optical imaging, hyperspectral imaging, thermal imaging, laser Doppler imaging, confocal microscopy, optical coherence tomography, and NIR spectroscopy imaging [25]. Other innovative wound measurement techniques involve a structured light or laser approach. Laser-assisted wound measurement devices do not require wound contact and involve the use of a digital camera and projected laser beams. The main limitation of this method is an artificially low measurement of wound depth, likely attributed to the decreasing resolution of imaging shallow wounds [26].

One laser-assisted device in particular has recently shown encouraging results [27]. The 3D wound measurement device, inSight (eKare Inc., Fairfax, VA), demonstrated high inter-rater and intra-rater values for both wound area and volume. It functions by retrofitting a standard iPad with an infrared laser and utilizing associated software to measure the wound. Similar to other laser-assisted wound measurement devices, the major limitation of the device is an accurate measurement of wound depth.

Besides the inSight (eKare Inc., Fairfax, VA) 3D wound measurement device, multiple other devices are also now in use. Other devices include Silhouette Mobile® system (ARANZ Medical, Christchurch, New Zealand) [28, 29], a smartphone wound measurement device (WMD) [30], SilhouetteStar™ (System E; ARANZ Medical, Christchurch, New Zealand) [26], VeV MD Vista Medical (Winnipeg, Manitoba, Canada) [31], and the TeleDiaFoS® (Nalecz Institute of Biocybernetics and Biomedical Engineering, Warsaw, Poland) [32, 33], to name a few. Overall, digital imaging devices have been superior to most other wound measurement methods by reducing clinician measurement variability and improving accuracy and reliability. Additionally, many of the devices are inexpensive and have the potential to integrate into patients' electronic medical records.

3 Discussion

Wound measurement is of particular value in the setting of diabetic foot ulcers, venous ulcers, pressure ulcers, burns, ostomy sites, and other postoperative sites such as amputations. Ideally, measurement techniques should maximize inter-rater and intra-rater reliability, account for anatomical variations, and allow for sequential wound assessment and documentation. Tracking wound area over time allows clinicians to assess responses to treatment and tailor intervention accordingly. Proper wound assessment is vital, particularly within the first 1–4 weeks of treatment. The total reduction in wound area during this time is a strong predictor of healing [3, 4, 34]. When assessing healing rate, the wound size measurements do not necessarily need to be accurate as long as they are reliable and the percent change can be followed [35]. Early identification of wounds with less percentage change and there-

fore less healing potential with standard therapy could ultimately direct clinicians to provide earlier or more aggressive interventions. Identifying these at-risk patients would likely lead to improved outcomes and lower cost, though these particular questions have not yet been studied.

The number of risk factors for poor wound healing is increasing as the population ages and lives with more comorbidities. These risk factors include diabetes, smoking, alcohol use, older age, male sex, heart failure, the inability to stand or walk without help, end-stage renal disease, larger wound size, history of poor wound healing, peripheral neuropathy, and peripheral artery disease [36–38]. Patients with the potential for poor healing can be identified, perhaps more aggressive treatments initiated, and wound progress tracked. Ideally, both treatment and wound monitoring would be individualized, conceivably using more involved wound measurement methods for at-risk patients.

Conclusions

Wound measurement is an important aspect of wound assessment, tracking progression to healing, and identification of at-risk patients. Multiple wound measurement techniques are available, with digital methods preferred due to higher accuracy and reliability. Newer devices significantly reduce clinician measurement variability and show potential for replacing commonly used manual metric measurement. With the emergence of new techniques and technology, there is a possibility of measuring more wound dimensions and is the topic of current study in the field.

References

1. Grey JE, Enoch S, Harding KG (2006) Wound assessment. Br Med J 332(7536):285–288
2. Liu Z, Saldanha IJ, Margolis D, Dumville JC, Cullum NA (2017) Outcomes in Cochrane systematic reviews related to wound care: an investigation into prespecification. Wound Repair Regen 25(2):292–308
3. Sheehan P, Jones P, Caselli A, Giurini JM, Veves A (2003) Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. Diabetes Care 26(6):1879–1882
4. Lavery LA, Barnes SA, Keith MS, Seaman JW Jr, Armstrong DG (2008) Prediction of healing for postoperative diabetic foot wounds based on early wound area progression. Diabetes Care 31(1):26–29
5. Jorgensen LB, Sorensen JA, Jemec GB, Yderstraede KB (2016) Methods to assess area and volume of wounds - a systematic review. Int Wound J 13(4): 540–553
6. Schubert V, Zander M (1996) Analysis of the measurement of four wound variables in elderly patients with pressure ulcers. Adv Wound Care 9(4):29–36
7. Kekonen A, Bergelin M, Eriksson JE, Vaalasti A, Ylanen H, Viik J (2017) Bioimpedance measurement based evaluation of wound healing. Physiol Meas 38(7):1373–1383
8. Yesiloglu N, Yildiz K, Cem Akpinar A, Gorgulu T, Sirinoglu H, Ozcan A (2016) Histogram planimetry method for the measurement of irregular wounds. Wounds 28(9):328–333
9. Zang CY, Cao YQ, Xue WJ, Zhao R, Zhang M, Zhang YH, Feng Z, Wang YB (2017) Application of high-frequency ultrasound in dermabrasion of patients with deep partial-thickness burns. Zhonghua Shao Shang Za Zhi 33(2):97–102
10. Agabalyan NA, Su S, Sinha S, Gabriel V (2017) Comparison between high-frequency ultrasonography and histological assessment reveals weak correlation for measurements of scar tissue thickness. Burns 43(3):531–538
11. Bryant JL, Brooks TL, Schmidt B, Mostow EN (2001) Reliability of wound measuring techniques in an outpatient wound center. Ostomy Wound Manage 47(4):44–51
12. Rogers LC, Bevilacqua NJ, Armstrong DG, Andros G (2010) Digital planimetry results in more accurate wound measurements: a comparison to standard ruler measurements. J Diabetes Sci Technol 4(4):799–802
13. Langemo D, Anderson J, Hanson D, Hunter S, Thompson P (2008) Measuring wound length, width, and area: which technique? Adv Skin Wound Care 21(1):42–45
14. Bilgin M, Gunes UY (2013) A comparison of 3 wound measurement techniques: effects of pressure ulcer size and shape. J Wound Ostomy Continence Nurs 40(6):590–593
15. Bowling FL, King L, Fadavi H, Paterson JA, Preece K, Daniel RW, Matthews DJ, Boulton AJ (2009) An assessment of the accuracy and usability of a novel optical wound measurement system. Diabet Med 26(1):93–96
16. Shaw J, Hughes CM, Lagan KM, Bell PM, Stevenson MR (2007) An evaluation of three wound measurement techniques in diabetic foot wounds. Diabetes Care 30(10):2641–2642
17. Thawer HA, Houghton PE, Woodbury MG, Keast D, Campbell K (2002) A comparison of computer-assisted and manual wound size measurement. Ostomy Wound Manage 48(10):46–53

18. Gethin G, Cowman S (2006) Wound measurement comparing the use of acetate tracings and Visitrak digital planimetry. J Clin Nurs 15(4):422–427
19. Wunderlich RP, Peters EJ, Armstrong DG, Lavery LA (2000) Reliability of digital videometry and acetate tracing in measuring the surface area of cutaneous wounds. Diabetes Res Clin Pract 49(2–3):87–92
20. Sugama J, Matsui Y, Sanada H, Konya C, Okuwa M, Kitagawa A (2007) A study of the efficiency and convenience of an advanced portable wound measurement system (VISITRAK). J Clin Nurs 16(7):1265–1269
21. Wendelken ME, Berg WT, Lichtenstein P, Markowitz L, Comfort C, Alvarez OM (2011) Wounds measured from digital photographs using photodigital planimetry software: validation and rater reliability. Wounds 23(9):267–275
22. Bien P, De Anda C, Prokocimer P (2014) Comparison of digital planimetry and ruler technique to measure ABSSSI lesion sizes in the ESTABLISH–1 study. Surg Infect (Larchmt) 15(2):105–110
23. Stockton KA, McMillan CM, Storey KJ, David MC, Kimble RM (2015) 3D photography is as accurate as digital planimetry tracing in determining burn wound area. Burns 41(1):80–84
24. Chang AC, Dearman B, Greenwood JE (2011) A comparison of wound area measurement techniques: Visitrak versus photography. Eplasty 11:e18
25. Mukherjee R, Tewary S, Routray A (2017) Diagnostic and prognostic utility of non-invasive multimodal imaging in chronic wound monitoring: a systematic review. J Med Syst 41(3):46
26. Davis KE, Constantine FC, Macaslan EC, Bills JD, Noble DL, Lavery LA (2013) Validation of a laser-assisted wound measurement device for measuring wound volume. J Diabetes Sci Technol 7(5):1161–1166
27. Anghel EL, Kumar A, Bigham TE, Maselli KM, Steinberg JS, Evans KK, Kim PJ, Attinger CE (2016) The reliability of a novel mobile 3-dimensional wound measurement device. Wounds 28(11):379–386
28. Hammond CE, Nixon MA (2011) The reliability of a handheld wound measurement and documentation device in clinical practice. J Wound Ostomy Continence Nurs 38(3):260–264
29. Miller C, Karimi L, Donohue L, Kapp S (2012) Interrater and intrarater reliability of silhouette wound imaging device. Adv Skin Wound Care 25(11):513–518
30. Sprigle S, Nemeth M, Gajjala A (2012) Iterative design and testing of a hand-held, non-contact wound measurement device. J Tissue Viability 21(1):17–26
31. Haghpanah S, Bogie K, Wang X, Banks PG, Ho CH (2006) Reliability of electronic versus manual wound measurement techniques. Arch Phys Med Rehabil 87(10):1396–1402
32. Foltynski P, Ladyzynski P, Sabalinska S, Wojcicki JM (2013) Accuracy and precision of selected wound area measurement methods in diabetic foot ulceration. Diabetes Technol Ther 15(8):712–721
33. Ladyzynski P, Foltynski P, Molik M, Tarwacka J, Migalska-Musial K, Mlynarczuk M, Wojcicki JM, Krzymien J, Karnafel W (2011) Area of the diabetic ulcers estimated applying a foot scanner-based home telecare system and three reference methods. Diabetes Technol Ther 13(11):1101–1107
34. Khoo R, Jansen S (2016) The evolving field of wound measurement techniques: a literature review. Wounds 28(6):175–181
35. Santamaria N, Ogce F, Gorelik A (2012) Healing rate calculation in the diabetic foot ulcer: comparing different methods. Wound Repair Regen 20(5): 786–789
36. Prompers L, Schaper N, Apelqvist J, Edmonds M, Jude E, Mauricio D, Uccioli L, Urbancic V, Bakker K, Holstein P, Jirkovska A, Piaggesi A, Ragnarson-Tennvall G, Reike H et al (2008) Prediction of outcome in individuals with diabetic foot ulcers: focus on the differences between individuals with and without peripheral arterial disease. The EURODIALE Study. Diabetologia 51(5):747–755
37. Parker CN, Finlayson KJ, Shuter P, Edwards HE (2015) Risk factors for delayed healing in venous leg ulcers: a review of the literature. Int J Clin Pract 69(9):967–977
38. Harris LS, Luck JE, Atherton RR (2017) Suboptimal identification of patient-specific risk factors for poor wound healing can be improved by simple interventions. Int Wound J 14(1):138–141

Influence of Sensory Innervation on Epithelial Renewal and Wound Healing

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1 Introduction

The main function classically attributed to peripheral somatosensory system is to receive, transduce, and channel external or internal information toward central regions of the nervous system. However, there are numerous examples throughout the body of mammals which indicate that some neurons from sensory ganglia are not restricted to generate afferent impulses. This population of neurons is characterized by its capacity to release neuropeptides from their peripheral terminals. It is postulated that through this neurosecretory character, peptidergic neurons of dorsal root ganglia influence diverse processes in their targets (efferent function). This notion is also supported by the presence of receptor sites and degrading enzymes for neuropeptides in all tissues innervated by peptidergic neurons. Nevertheless, it is often assumed that efferent functions of sensory ganglia are only relevant in clearly pathological events (e.g., neurogenic inflammation). Indeed, it is the fact that sensory nerves participate in pathological events that explains a resurgence of the study and an effort to characterize the effects and mechanisms that govern the interaction between sensory nerves and their peripheral targets such as the skin.

The synthesis and transport of neuropeptides to the peripheral terminals of dorsal root ganglion (DRG) neurons have been documented in various species [1–4]. Thus, efferent functions of DRG neurons may represent a conserved mechanism for tissue renewal and functional maintenance during normal physiological conditions. There are systematic observations about the deleterious effects related to sensory denervation which provokes major changes of gene regulation on its targets [5, 6]. Moreover, the generation of antibodies to label fine terminals at the periphery has revealed that peptidergic terminals are in almost every part of the mammalian body, including the skin, muscle, bone, immune organs, teeth, blood vessels, and viscera. In these regions it has been observed both the existence of synaptic-like contacts between peptidergic endings and some target cells and the expression of neuropeptide receptors by different cell types [7–10]. Overall the anatomical and functional studies suggest that peptidergic innervation plays an active and continuous role on epithelial renewal, wound repair, glandular secretion, and mineralized tissue formation that is just beginning to be understood.

In this chapter, we will discuss several aspects of sensory innervation and the proposed mechanisms by which sensory terminals influence epithelial homeostasis. A brief survey of the main anatomical and neurochemical characteristics of

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the nerve terminals that innervate the skin will be made. All of this will be discussed under the context of the noceffector concept proposed by Kruger [11] which states that peptidergic neurons of DRG devote most of its biological existence to have an effector or trophic influence on its target.

2 Cytology and Neurochemistry of Dorsal Root Ganglion Neurons: An Overview

Broadly, two main classes of neurons have been described in sensory ganglia based on cell body size, cytoplasmic appearance, axonal diameter, and axonal myelin content. Due to light or dark appearance of its cytoplasm in electron and light microscopy studies, DRG neurons are subdivided into large light (also named A cells) and small dark neurons (also named B cells) [12, 13]. Furthermore, it has been determined by immunohistochemical studies that light appearance is given by a rich content of the 150 and 200 kDa neurofilament subunits [14]. Likewise, dark neurons have a cytoskeleton primarily constituted by the intermediate filament protein called peripherin [15, 16]. Besides these cytological features, it is known that electrical properties such as conduction velocity correlate with soma size and fiber diameter [17]. Thus, large light neurons (soma diameter > 35 mm) correspond to neurons with myelinated fibers [14, 18, 19]. These large-caliber and myelinated axons are the well-known A-fibers which are divided into three subgroups, namely, A, B, and C, from fastest to slowest. In addition, the small dark neurons (<20 mm) give rise to C-fibers which are unmyelinated fibers and, consequently, the thinnest and slowest fibers in sensory nerves [17, 20]. This relationship between anatomical parameters and functional properties does not necessarily apply to medium-sized neurons (20–35 mm). For instance, some A cells skewed toward the large population are neurofilament-negative, and, conversely, neurons skewed toward the small population are neurofilament-positive [18, 21]. Rather than a clear subdivision of neuronal populations, there is a perplexing scenario of subpopulations with overlapping phenotypic and functional properties.

Besides its afferent (i.e., sensory) role, C- and Aδ-fiber neurons are mainly implicated in tissue management [22–24]. Although neuropeptide content is associated with pain modulation, it has been recently documented that a fraction of peptidergic neurons does not process exclusively nociceptive stimuli [2, 23, 25–27]. Moreover, efforts to define a biochemical profile to predict receptive modalities have not been successful at all. Some DRG neurons have the intrinsic genetic program to express neuropeptides, and others acquire a peptidergic phenotype only after they have contacted a target in late embrionary stages [28–30]. Apparently, peptidergic phenotype is related to localization of peripheral terminals in the target tissue rather than to a sensory modality [2, 23, 25]. Indeed, it has been postulated that peptidergic neurons constitute a nocifensor system that probably lacks a sensory function [31]. In fact, there is still much debate about the existence of two separate populations for afferent and efferent role in dorsal root ganglion. For the sake of convenience, we will refer for those cranial and DRG neurons having an efferent role only as peptidergic neurons or noceffectors, assuming that if a neuron presents vesicles with peptides in the peripheral terminals, it conveys a specific message that helps maintain tissue homeostasis, regardless if this neuron transmits a sensory stimuli or whether it is a noxious/non-noxious stimuli [11].

In elegant studies using genetic axonal tracers, the peptidergic and non-peptidergic populations in mice are shown to be topographically segregated. For instance, in mouse epidermis, non-peptidergic fibers terminate in the stratum granulosum, while most of the peptidergic fibers terminate in the stratum spinosum [32]. Similarly, this segregation continues in the spinal cord and in ascending pathways. Peptidergic neurons project to spinal lamina I and the outer region of lamina II (II_o), and these spinal neurons project heavily to the brain stem (parabrachial nuclei) and thalamus, while non-peptidergic neurons

connect with second-order interneurons in the internal region of lamina II (II_{inner}). These interneurons project to lamina V which then project to several limbic and striatal regions [32, 33]. The spatially segregated pathways suggest that these groups of neurons have at least different sensory processing capacities. If this anatomical separation could also be relevant for efferent functions of sensory neurons remains to be determined.

Neuropeptide content in DRG neurons has been reported in various vertebrates as rodents, primates, felines, birds, and reptiles [1–4]. The proportion of peptidergic neurons varies depending on the species, and inside a species varies according to the spinal cord level [34]. Regardless of the animal species, the peptidergic population is consistently composed by a subpopulation of C-fibers neurons and in smaller fraction by a subpopulation of A-fiber neurons [2, 3, 25, 35]. The major peptides synthesized by DRG neurons are substance P (SP) and calcitonin gene-related peptide (CGRP). In addition, DRG neurons also synthesize other peptides such as somatostatin, neuropeptide Y, galanin, vasoactive intestinal polypeptide, pituitary adenylate cyclase-activating polypeptide-38, and opioids.

3 CGRP and Substance P in Dorsal Root Ganglia: Synthesis, Release, and Receptors

3.1 CGRP

The calcitonin gene peptide superfamily consists of four members with potent vasoactive properties that include calcitonin, CGRP, adrenomedullin, and amylin [36, 37]. CGRP exists in two isoforms encoded by different genes, α and β in rat and I and II in human. While the rat isoforms differ in one amino acid residue, in humans they differ in three [38, 39]. The most noticeable site of synthesis of α-CGRP in the peripheral nervous system is the DRG, whereas β-CGRP is preferentially expressed by enteric neurons. The translation of I-CGRP mRNA generates a 121 and 128 amino acid precursor in rats and humans, respectively. The first 25 amino acids of this precursor correspond to the signal peptide, a sequence that assists the targeting of the messenger to the endoplasmic reticulum. The next 103 residues correspond to the proCGRP [40]. The final 37 amino acid peptide is created by proteolytic cleavage of flanking peptides in proCGRP [41].

CGRP receptor belongs to the G-protein-coupled receptor superfamily. A molecular, biological approach has revealed that CGRP receptor is a heterodimer composed of the calcitonin receptor-like receptor protein (CRLR or CLR) and receptor activity-modifying protein 1 (RAMP-1) [37]. The latter is required to transport CRLR to the plasma membrane and to control a specific pattern of glycosylation that determines the affinity for CGRP [42]. The CGRP receptor is associated with the formation of cAMP through the activation of adenylyl cyclase. The biological effects of CGRP end with a proteolytic cleavage by proteases as neutral endopeptidase, insulin-degrading enzyme, and endothelin-converting enzyme-1 [43, 44].

3.2 Substance P

SP is a member of the tachykinin family that includes peptides with a conserved FXGLM-NH2 C-terminal sequence. The mRNAs that encode SP, neurokinin A, neuropeptide K, and neuropeptide G are derived from the preprotachykinin 1 gene. In DRG neurons, alternative RNA splicing of the primary transcript results in the generation of four mRNAs called α-, β-, γ-, and δ-TAC1 [45, 46]. SP precursor sequences are encoded by all four TAC1 mRNAs, but what directs the alternative splicing in the range of tissues where tachykinins are expressed is still unknown [47–49]. Putatively, the posttranslational processing of all these precursors gives rise to the active form of substance P that consists of 11 amino acid residues [50, 51].

The effects of tachykinins are mediated through a group of three G-protein-coupled metabotropic receptors: neurokinin-1 (NK1), neurokinin-2 (NK2), and neurokinin-3 (NK3). Substance P binds preferentially to NK1 receptor

[52, 53]. The activation of tachykinin receptor leads to inositol phosphate accumulation [54]. NK1 receptor stimulation in tracheal smooth muscle causes Ca^{2+} release from intracellular stores through the activation of both inositol triphosphate and ryanodine receptors. In muscle cells, the Ca^{2+} release from the sarcoplasmic reticulum in response to NK1 activation is coupled to Ca^{2+} influx through channels located in the plasma membrane [55]. Once released, SP is inactivated by the action of the neutral endopeptidase and the angiotensin-converting enzyme [56].

4 Release of SP and CGRP from Somatosensory Nerves

CGRP and SP are strongly expressed in normal DRG neurons, which suggests that they are ready to use whenever it is needed. A great portion of the neurons with capacity of peptide release are recognized for being capsaicin sensitive. Capsaicin is the pungent ingredient in hot chili peppers of the *Capsicum* genus, and it has been a valuable pharmacological and clinical tool, because it has allowed studying both afferent and efferent functions of DRG neurons [57]. The notion that C- and Aδ-fibers have a neurosecretory function dates from the early years of the twentieth century. Experiments by Bayliss [58] assigned an efferent role for the nerve fibers that emerge from posterior roots. They noticed that, when central ends of these fibers were excited at lumbar level, the impulse generated (i.e., antidromic process) provoked vascular dilatation at their peripheral ends in the hind limbs of various species. Nowadays it is known that antidromic stimulation of C and Aδ produces vasodilatation and increases plasma extravasation [59, 60]. Immunohistochemical and pharmacological experiments had revealed that CGRP induces arterial vasodilatation, whereas SP provokes an increase in vascular permeability [61, 62]. Overall, these vascular changes and concomitant activation of mast cells, lymphocytes, and neutrophils lead to what is called neurogenic inflammation. Thus, the main efforts to understand peptide release from peripheral terminals of peptidergic DRG neurons have been centered on factors involved in inflammation. In this regard, capsaicin is widely known for its capacity to induce neurogenic inflammation by releasing SP and CGRP from peripheral terminals. It is believed that capsaicin releases neuropeptides exclusively via activation of the vanilloid receptor 1 (TRPV1), but other members of TRPV family might be involved [63]. TRPV1 is a nonselective cation channel that allows entry of calcium and, besides capsaicin, is also gated by nociceptive stimuli such as low pH and heat [64, 65].Classical exocytosis occurs when Ca^{2+} influx into the terminals and initiates exocytotic mechanisms that release neuropeptides and/or other neurotransmitters [66]. The addition of capsaicin to nerve, skin, and mucosal explants induces peptide release, but it is prevented if explants are incubated in Ca-free medium containing EGTA [67–69]. The notion that this effect is partially mediated by TRPV1 is supported by the fact that a competitive antagonist of TRPV1, namely, capsazepine, diminished CGRP concentrations in eluates quantified by immunoassay or radioimmunoassays [68, 69]. Ruthenium red, a noncompetitive channel blocker of TRPV1, attenuates neuropeptide release in response to capsaicin [67]. In addition, acidic stimulation promotes CGRP release in the nerves and skin through TRPV1-dependent mechanism [70]. Noxious heat (40–50 °C) evokes CGRP release in a calcium-dependent manner, as shown that both incubating in calcium-free medium and skin loaded with (BAPTA) diminished CGRP release [71, 72]. However, it has been shown that neither capsazepine nor Ruthenium Red abolished completely peptide release from nerve and skin explants [71, 73]. It is proposed that other heat-activated channels of TRPV subfamily (V1–V4) might be involved in neuropeptide release from peripheral terminals [71, 73]. This is supported by the fact that neonatal capsaicin denervation does not eliminate all peptidergic fibers in different targets. Likewise, TRPV1 is not expressed by all peptidergic neurons, and its presence in fibers varies with the type of

target [74]. It is noteworthy that TRPV members are coexpressed in DRG neurons and potentially different members may heteromultimerize, contributing to functional heterogeneity and a more complex pharmacology [75–77]. In considering TRPV channels as key elements for regulating peptide release from peripheral terminals, it must be taken into account that these channels are sensitized by vanilloids, temperature, and proinflammatory mediators, which results in distinct biophysical and regulatory properties [78]. TRP participation in peptide release on both patho- and physiological conditions awaits further investigation to define its precise contribution.

Regarding factors coming from a target, there are some inflammatory mediators capable to evoke or sensitize SP and CGRP release in certain tissues and conditions. For instance, bradykinin alone can induce neuropeptide release in the rat trachea and skin and in the heart of guinea pig [72, 79, 80]. Bradykinin evokes a significantly CGRP release only in the trachea, whereas in the skin, it only stimulates release of SP [72, 80]. The effects of bradykinin seem to be mediated through the activation of B_2 receptor which activates phospholipase C, resulting in formation of diacylglycerol and activation of protein kinase C [72, 80, 81]. The sole action of histamine, serotonin, prostaglandin E2, or proinflammatory cytokines seems not to be sufficient to promote exocytosis in peripheral terminals [69, 79, 80, 82, 83]. The action of these mediators is favored by conditions such as acid pH or noxious heator in combination with other inflammatory mediators. The interaction of serotonin and histamine sensitizes bradykinin effect on CGRP and SP release [72, 80]. Near inflammation zones and tumors, leukocytes and thrombocytes produced proinflammatory cytokines. In this regard, stimulation of rat skin from hind paw with IL-1b and TNF-a augmented heat-induced release of CGRP in a dose-dependent manner [82]. As in the case of bradykinin, cytokines activate receptors coupled with kinases which may sensitize heat-activated ion channels by phosphorylation and lead to a major release of peptides [84]. It has also been observed that noceffector activity is also exerted to inhibitory modulation. Plasma extravasation in rat skin, bronchoconstriction of guinea pig and human, and contraction of the left atrium of guinea pig heart are blocked by the presence of nociceptin, an opioid-related peptide [85–88]. These processes require neuropeptides release from noceffector terminals. Indeed, release of substance P and CGRP from rat isolated trachea in response to electrical field stimulation was diminished by nociception [89]. It has been proposed that nociceptin stimulates the G-protein-coupled orphan receptor ORL_1 to activate an inward-rectifier K^+ channel. The latter reduces neuropeptide release from noceffector endings via a membrane hyperpolarization which probably counteracts TRPV1 gating [86]. Likewise, μ-/κ-/δ-opioid receptor agonist inhibited electrical-induced release from noceffector endings in several preparations, although not all agonists are effective in all sites tested [83, 90–93]. The actual effect of endogenous opioids and its physiological relevance for efferent functions remains to be elucidated. Apart from these factors that can be found in most tissues, apparently there are some tissue-specific signals capable to evoke peptide release. That is the case of the conversion of trans-urocanic acid to cis-urocanic acid by ultraviolet radiation in the stratum corneum of the skin. In rodents cis-urocanic acid may increment microvascular blood flow of hind paw and diminished contact hypersensitivity by means of releasing SP and CGRP [94].

As could be inferred for the depleting effects of capsaicin in neuropeptide contents in different preparations in vitro, long-term synthesis of neuropeptides is intimately related with the amount of these neuropeptides that are available for release from the noceffector endings. Several reports indicate that neuropeptide exocytosis can be achieved by two means: local factors that stimulate direct or indirectly TRP channels and antidromically stimulations of peripheral endings which rely in axonal conduction by activation of voltage-dependent calcium channels. Since much of the research has dealt with inflammatory conditions, little is known if the same factors could modulate synthesis and release of

neuropeptides in noninjury conditions. Although capsaicin has helped to elucidate the pharmacology of noceffector terminals, it remains unclear which are the endogenous ligands that have similar effects as capsaicin and the dynamics of production and sources of such TRPV1 agonist in normal and pathophysiological conditions. Only a few molecules such as anandamide, arachidonate, and diacylglycerol have been shown to activate TRPV1 in a capsaicin-like manner [95, 96]. An intriguing issue that deserves further study is the role of antidromic process in vivo. It is known that a suprathreshold stimulus depolarizes primary afferents in the spinal cord (i.e., dorsal root reflex), which could trigger efferent action of noceffector [97]. Furthermore, dorsal rhizotomy, periaqueductal gray matter stimulation, and blockage in the spinal cord of GABAA, non-NMDA, or 5-HT3 receptors interfere with development of neurogenic cutaneous inflammation [98, 99]. This data implies that local mechanisms and/or central nervous mechanisms could modulate exocytotic release at periphery. The understanding of these mechanisms may clarify how noceffectors coordinate normal processes, such as hair growth, bone metabolism, gland secretion, and vascular tone.

5 Efferent Effects of Peptides Released by Somatosensory Nerves on Skin Physiology

Noceffectors establish synaptic-like contacts with Langerhans cells, melanocytes, and mast cells in the skin [7–9]. In other targets like the smooth muscle, epithelium, viscera, lymphoid organs, blood vessels, teeth, and bone, where noceffectors lack specialized contacts, they appear to establish a paracrine way of communication [10, 11, 100]. Cellular elements located in the aforementioned targets not only possess receptors for the peptides released by noceffectors from their C- and Aδ-fiber terminals but also express peptidases that terminate with the biological effects of such peptides [28, 29]. The anatomical and physiological evidence so far summarized suggests that, besides its ability to send information to the spinal cord (afferent role), the anatomical and functional organizations of ganglion sensory neurons render them capable of releasing the content of its vesicles and transmit a specific message to their peripheral targets (efferent role).

The skin receives innervation that originates from DRG and trigeminal ganglion. Nerve plexus of large caliber arrive at the deepest part of the dermis. As nerves ascend through the skin, they ramify in thinner plexuses. At the border between the dermis and the epidermis, individual fibers cross the basal membrane and terminate as free nerve endings in either stratum spinosum or stratum granulosum (Fig. 1). Free nerve endings also innervate structures immersed in the dermis like hair follicles, blood vessels, and sebaceous glands [101–103]. Many of these free nerve endings present immunoreactivity for SP and CGRP, and its distribution within the skin is conserved between individuals of the same species. The presence of SP and CGRP receptors in keratinocytes, fibroblasts, melanocytes, endothelial cells, and immune cells has been elucidated by immunohistochemical studies and functional assays [8, 104, 105].

A long-standing issue in the field of dermatology is related to the observation that cutaneous denervation is followed by trophic changes which are manifested as alterations in skin, nails, and subcutaneous tissues [106, 107]. Not until recent investigation, anecdotal observations have been replaced for a careful quantification of efferent activity of peptidergic DRG neurons. Recently, it has been established that skin noceffector is involved in modulating expression of genes of cytoskeleton, extracellular matrix, transcription factors, proteases, receptors, intracellular transducers, and adhesion molecules [5]. Taken altogether, these findings indicate that noceffector activity influences several kinds of cellular elements in its targets. Therefore, it is conceivable that malfunction of noceffectors may be a causal factor in some dermatological diseases.

In rodents and humans, epidermis becomes thinner after nerve injury [108, 109]. Sciatic nerve transection in rodents diminishes keratinocyte incorporation of analogs of

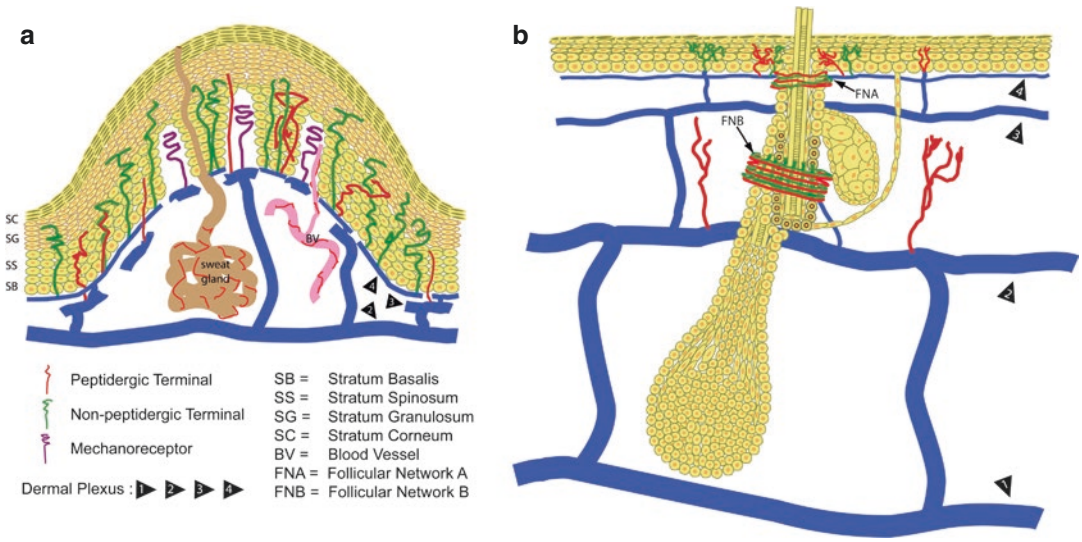

Fig. 1 General arrangement of sensory innervation in mammalian skin. In (**a**) glabrous skin and (**b**) hairy skin axons from dorsal root ganglia are grouped in the dermis as large plexuses. As axons reach the superficial layers of the skin, they travel in smaller plexuses. Sebaceous glands, blood vessels, and epidermis are innervated by ramified terminal fibers

thymidine up to 40% which suggest a reduction of keratinocyte proliferation [109–111]. Both epidermal thickness and proliferation are restored if reinnervation is permitted [108, 110, 111]. Due to alterations in motor innervation also affect keratinocyte proliferation, it is argued that lack of movement rather than neuropeptide secretion from noceffectors is the cause of skin atrophy. Dorsal rhizotomy or ganglionectomy, procedures that conserve normal gait, also produces epidermal thinning. Sensory denervation of dorsal skin, which does not support body weight, produces epidermal thinning [112, 113]. An insight into the mechanism of this phenomenon comes from in vitro and in vivo studies. In cultures of keratinocytes, fibroblasts, and endothelial cells, substance P promotes cell proliferation [114, 115], while CGRP promotes the proliferation of melanocytes and endothelial cells [8].

The hair follicles receive peptidergic innervation which shows immunoreactivity for substance P and CGRP (Fig. 1). Normal hair cycle is accompanied by substantial morphological, cellular, and biochemical changes in many skin compartments, such as changes in the thickness of the epidermis and dermis, reorganization of the skin vasculature and the extracellular matrix, as well as variations in the number and functional activity of major skin cell populations [116]. This tissue remodeling is associated with tightly regulated sprouting and regression of peptidergic fibers. The number of CGRP and SP fibers increases from telogen to anagen in the dermis and subcutis [117, 118]. Peptidergic nerve fibers are concentrated around and above the bulge region where one major population of epithelial hair follicle stem cells resides. Thus, it is conceivable that noceffectors participate actively in hair cycle modulation and concomitant tissue remodeling. SP-releasing microcapsules implanted at resting growth phase of hair (telogen) stimulate growth phase (anagen) in mice skin [119], while treatment with substance P in anagen induces a premature regression of hair follicles (catagen) [120]. CGRP subcutaneous implants failed to promote transition of telogen to anagen [118]. Further investigations are required to define the precise role of the combination of nerve-derived signals in hair cycle.

Overall, the evidence indicates that noceffectors interact with almost all cell populations in the skin. By means of this interaction, the

optimum functioning of major physiological processes that maintain the skin in a healthy state is preserved. Neuropeptide release is involved in modulating epidermal renewal, hair growth, blood flow, and immunological priming. Accordingly, it is not surprising that alteration in the synthesis and release of neuropeptides may result in disturbance of skin homeostasis. That could be the case of some variants of dermatological diseases like atopic dermatitis, psoriasis, urticaria, or vitiligo whose etiology is unknown and sometimes attributed to a neurological origin. A common denominator in these diseases is an elevated number of nerve fibers in the dermis and epidermis containing SP and CGRP compared with healthy skin [121–123]. In addition, more frequent contacts of nerve fibers with mast cells and blood vessels are observed [124, 125]. Until now little is known if peripheral nerve fiber sprouting responds to a diminishing in peptide release which in turn evokes secretion of neurotrophic signals from a target organ. For instance, keratinocytes in psoriatic lesions have reduced expression of the transcription factor Jun B with concomitant augmented levels of mRNA of two chemotactic proteins, S100A8 and S100A9, which are involved in the onset of psoriasis [126]. Remarkably, sensory denervation leads to an upregulation of S100A8 and S1009 genes [5]. Likewise, psoriatic lesions contain an increased number of keratinocytes expressing NGF, whose synthesis is promoted by neuropeptide release [105, 127].

6 Role of Peptidergic Nerves on Epithelial Renewal and Wound Repair

To get a better understanding on how sensory nerves influence epithelium physiology, we performed a series studies using neonatal capsaicin treatment. This chemical denervation model allowed us to reduce the amount of peptidergic terminals in the skin and to determine whether the reduction of peptidergic terminals affects epithelial homeostasis both in noninjury conditions and during wound repair [128, 129]. We employed design-based stereological methods to assure an unbiased quantification of biological structures (Fig. 2). Most of the dermatological research has relied on qualitative or 2D sampling which may overestimate or underestimate the magnitude of a certain cellular responses. For example, the data of cell number is usually expressed as a ratio quantity (i.e., cell/unit area) which can be misinterpreted if the reference space is not the same between experimental conditions. In contrast, stereological estimations of the number, length volume, or area of biological objects are performed by a systematic random sampling without any assumption of spatial distribution, size, shape, and object orientation. Stereological probes facilitate the comparison of experimental conditions by expressing the data of measured parameters as an absolute quantity (i.e., millions of cells). Rather than to offer a guide on how to design a stereological study, the main intention of this section is to show how this methodology was used to study the role of innervation during wound healing [130–132].

Although it is well-known that neonatal capsaicin treatment eliminates a great number of DRG neurons with C- and Aδ-axons, there was no quantitative data about the repercussion of capsaicin treatment on the development of epidermal innervation. For this purpose, we quantified the amount of intraepidermal nerve fibers (IENF) immunoreactive for protein gene product 9.5 (PGP^+) and calcitonin gene-related peptide ($CGRP^+$) in the glabrous skin of the rat [129]. In control animals, the total estimated length of PGP^+ and $CGRP^+$ fibers remained relatively constant at 1, 3, and 6 months. These findings suggest that nerve supply generated during development is only redistributed as animal ages. Moreover, we also observed changes on IENF morphology which indicate that nerve fibers undergo continual remodeling over time (Fig. 3). Accordingly, the arborization and location of sensory endings in the mouse cornea showed substantial changes over

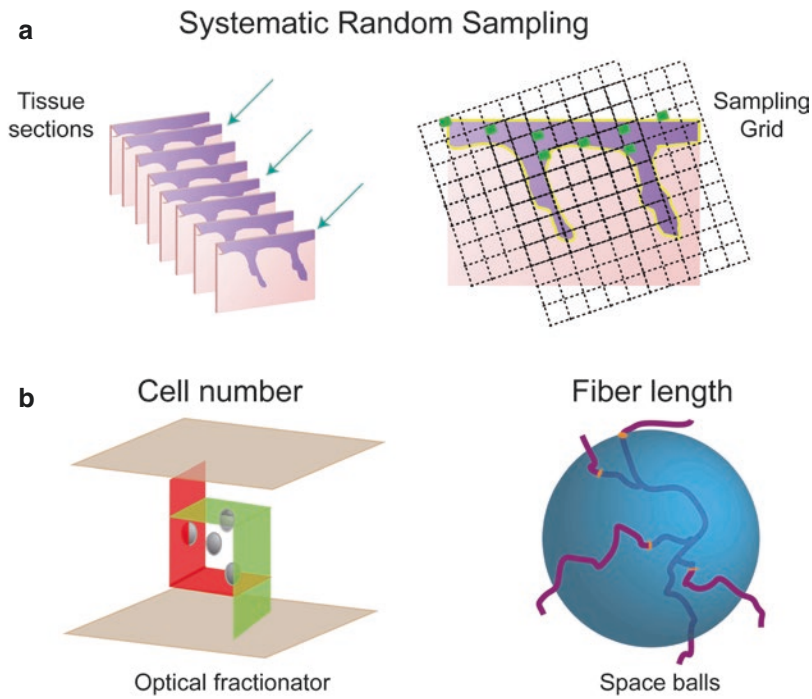

Fig. 2 Stereological quantification of cell number and fiber length. Reliable and unbiased estimates of volume, number, area, and length of biological objects are obtained by design-based stereology methods. (**a**) Sections and counting sites are determined by a systematic random sampling which assures a representative sampling through the analyzed area. (**b**) The estimation of the total number of cells is performed by counting the cells inside a virtual box or optical dissector. The fiber length is obtained by counting the intersections of the nerve fibers with a stereological probe called space balls. Both procedures require thick tissue sections (>20 mm)

a 1-month period [133]. Capsaicin treatment reduced the total length of PGP^+ fibers on average by 80%, and that of $CGRP^+$ fibers was reduced by 55%. While IENF showed an intricate morphology in control rats, the nerve endings in the epidermis of treated animals had a straight thick morphology and were poorly ramified. Despite the reduction of the nerve supply to the epidermis, the keratinocyte proliferation was not altered in capsaicin-treated rats. Interestingly, the quantitative analysis of IENF on capsaicin-treated rats revealed that peptidergic fibers were the predominant type of fibers in the epidermis as was also confirmed by a double-immunofluorescence staining for CGRP and beta III tubulin (Fig. 3). Thus, we hypothesized that the remaining peptidergic innervation is sufficient to maintain adequate epithelial renewal in noninjury conditions, but in conditions of high cell demand, denervated epithelia are not able to generate the number of cells required for epithelial expansion.

Until recently, all efforts to show a beneficial action of sensory nerves during skin wound repair have been limited to document the impact of denervation upon the time of wound closure [134–138]. Since the discovery of adult stem cells in different parts of the body, it became clear that the innervation is an essential part of the stem cell niche. Little is known about the exact interaction between neurons and progenitor cells. By using the neonatal capsaicin denervation, we explored whether sensory innervation was involved in the modulation of the epithelial progenitors that participate in reepithelialization of the hairy skin [128]. The hair follicle is an

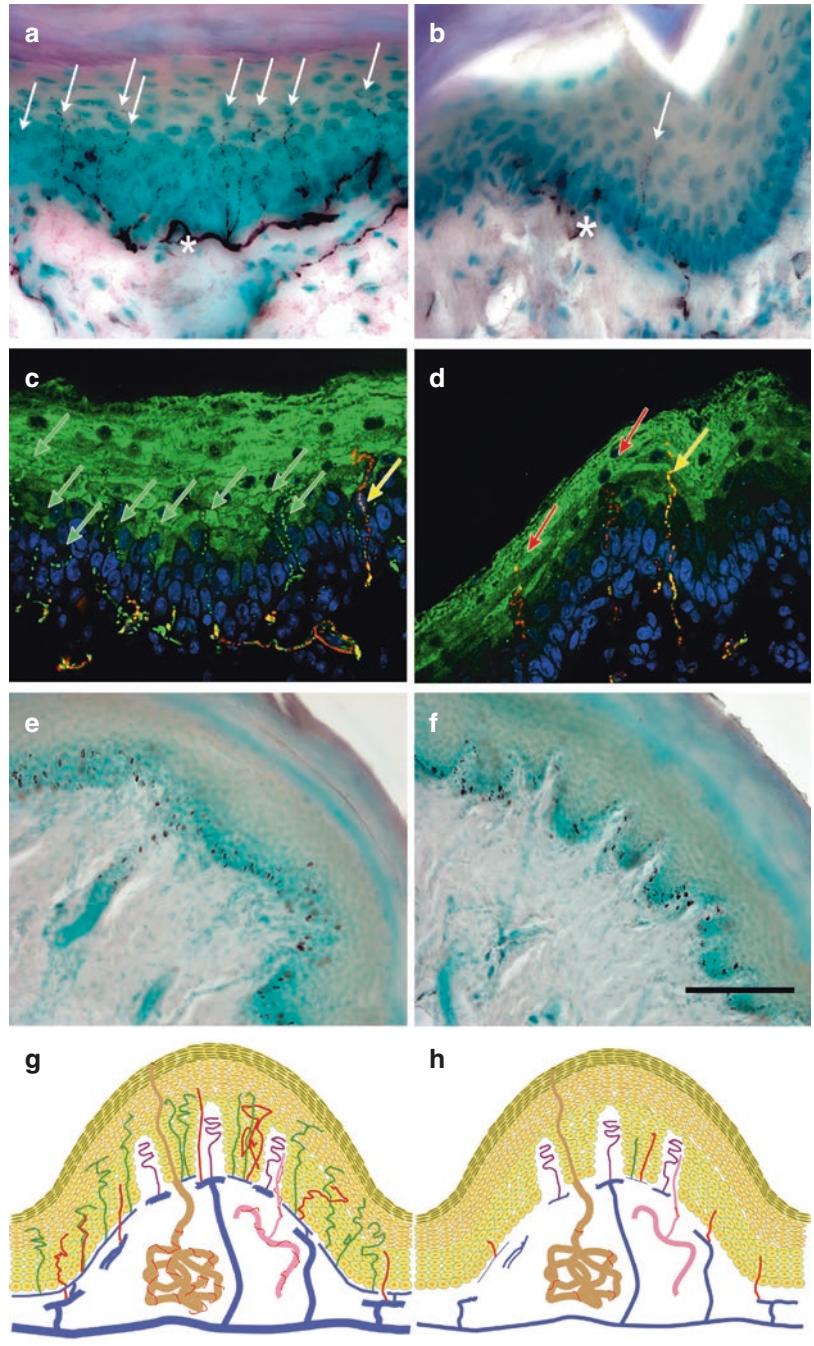

Fig. 3 Effects of neonatal capsaicin treatment on skin innervation. (**a**) Glabrous skin sections were immunostained for protein gene product 9.5. (**b**) Capsaicin treatment decreased the number of intraepidermal nerve fibers and fiber complexity. While in (**c**) control rats the most abundant type of nerve fibers was of non-peptidergic type (green arrows: immunoreactivity for beta III tubulin), (**d**) the epidermis of treated rats showed almost exclusively peptidergic fibers (red and yellow arrows: immunoreactivity for CGRP). Despite the reduction of intraepidermal nerve fibers in capsaicin-treated rats (F), the keratinocyte proliferation was similar in (**e**) control and (**f**) treated rats. (**g**) Sensory innervation in the epidermis of glabrous skin of control rats. (**h**) Summary of the changes induced by neonatal capsaicin treatment. Scale bar = 150 mm

excellent model to study the signals and mechanisms that may govern the neural modulation of stem cells. Based on the anatomical location of sensory fibers in the bulge region of the hair follicle, we evaluated the possibility that nerve-derived signals may influence the activation or migration of epithelial progenitors (Fig. 4). During the first 47 h post-wound, the epidermal proliferation was reduced in the capsaicin-treated rats, while the proliferation in the hair follicles was the same as in control rats. To determine if the low number of

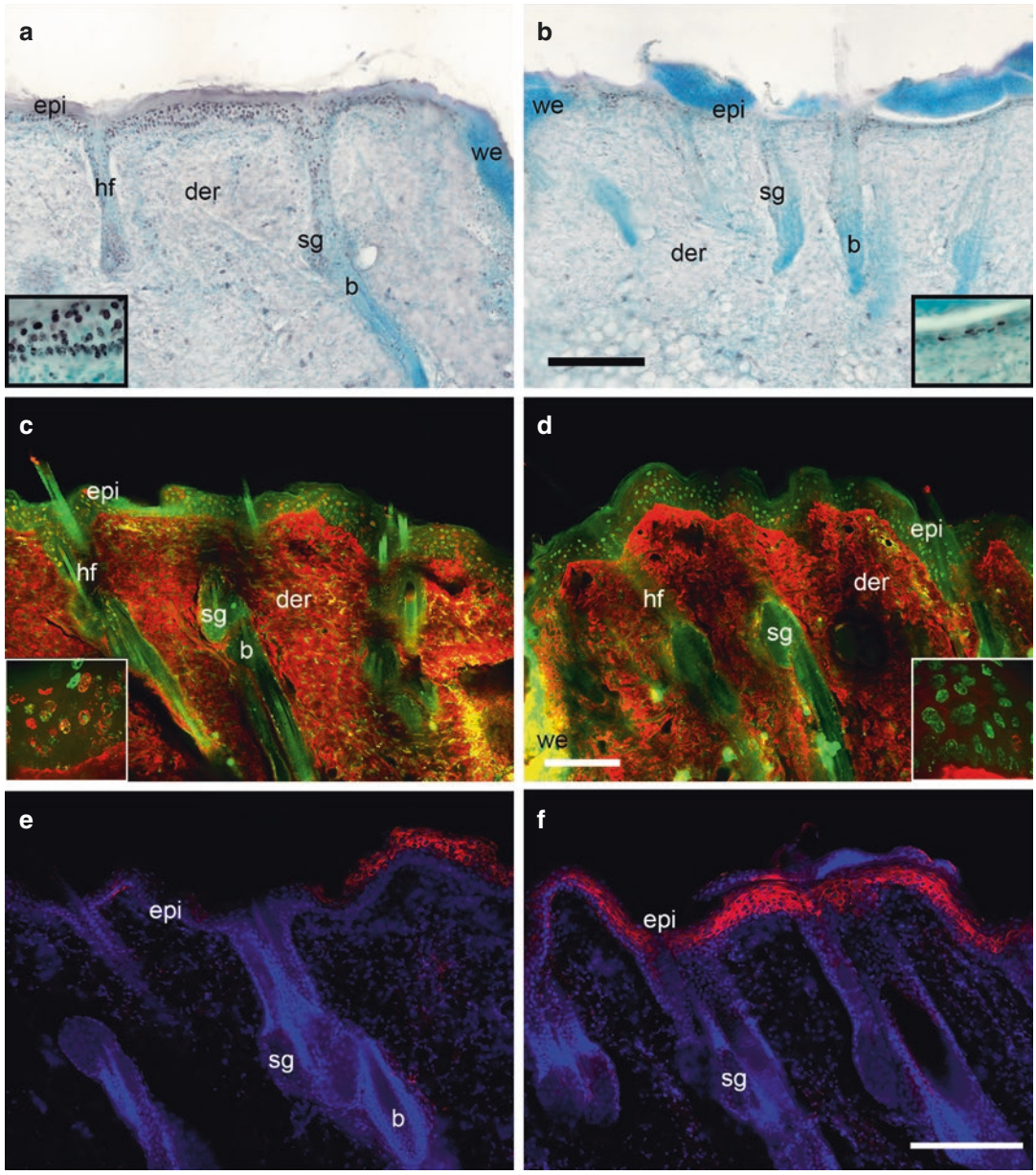

Fig. 4 Effects of capsaicin treatment on wound healing. After 47h after wounding, the epidermis of (**a**) control rats was thicker and showed more $BrdU^+$ nuclei than (**b**) capsaicin-treated rats. At 61h after wounding, the epidermis of (**c**) control rats presented more IdU/CldU labeled nuclei than (**d**) treated rats, which suggest that denervation is related to less migration of stem cell progeny from the hair follicle. Noteworthy, at 61 h after wounding, we observed an increased area of epidermis expressing keratin 6 in (**f**) capsaicin-treated rats than in (**e**) controls. *epi* epidermis, *der* dermis, *sg* sebaceous gland, *b* bulge, *we* wound edge, *hf* hair follicle. Scale bar = 200 mm. Modified from [128]

bromodeoxyuridine-positive cells ($BrdU^+$) in the epidermis of treated rats resulted from a reduced mobilization of transit amplifying cells from the hair follicle, we performed pulse and chase experiments with halogenated thymidine analogs (iododeoxyuridine, IdU; chlorodeoxyuridine,

CldU). This procedure is based on the principle that cells in the hair follicle proliferate faster than the cells in the epidermis allowing to track the fate of the double-labeled cells in different skin compartments. Remarkably, the proportion of $IdU^+/CldU^+$ cells in the epidermis increased over time only in the control group. This finding suggests that the deficiency of sensory nerves hampers the traffic of cells from the follicle toward the epidermis. Although it has been shown that cells from the hair follicle are dispensable for reepithelialization, the migration of these cells accelerates the reestablishment of the epidermis [139, 140]. In capsaicin-treated animals, the efflux of hair follicle cells is diminished which correlates with an extended time for wound closure. Moreover, treated rats showed an extended recruitment of epithelial precursors as indicated by the broader area of epidermis expressing keratin 6, a marker of epidermal activation. Our results revealed that epithelial precursors must migrate more distance to reach the border of the wound in denervated rats. Taken together, our findings may explain the delay in reepithelialization observed in several models of denervation. From a clinical perspective, it would be desirable to understand the mechanism and signals behind the activation of distinct regions of the epithelium to better contend with chronic wounds. In this regard, it is noteworthy that the stem cell niche of the bulge showed the presence of receptors for substance P and CGRP (Fig. 5).

Conclusions

This chapter summarizes the evidence that sensory neurons of dorsal root ganglia are not restricted to transmit information toward the central nervous system. These neurons are thought to be crucial participants in the maintenance of tissue integrity and functionality. Nevertheless, we are just glimpsing the potential of neurosecretory function of the so-called sensory neurons for body health. Perhaps the notion that these neurons are merely transducers of noxious information has delayed advancement toward the understanding of efferent functions. Moreover, it is frequently assumed that peripheral release of neuropeptides is restricted to a noxious condition just because at spinal cord level, neuropeptides serves as cotransmitters of painful transmission. This view, however, responds in great extent to technical limitations for recording peripheral activity. Therefore, this field awaits for future improvement in procedures to investigate peripheral release in more physiological terms. This issue is extremely important because the available preparations only permit to study local factors that regulate noceffector activity and overlook systemic factors which may be more important during normal conditions. Although tissue homeostasis does not rely entirely on noceffectors, they seem to be an essential component because different cell populations express receptors and degradatory enzymes for neuropeptides. Accordingly, alterations in the communication between noceffectors and peripheral targets could lead to a variety of functional modifications in the innervated target. Although, at first glance, it could be considered that neuropeptide effects on different organs are non-related with each other, we think that such effects must be the manifestation whereby the brain interacts with the body regulating central issues for its homeostasis both in health and disease.

Regarding wound healing, dorsal root ganglion neurons are involved in processes such as reepithelialization, angiogenesis, and inflammation. Here we described a mechanism based on neural regulation of epithelial SC physiology. Peptidergic neurons seem to promote the mobilization of stem cell progeny from the hair follicle and to modulate the activation of epidermal progenitors (Fig. 6). The myriad of nerve-derived signals is not limited to neuropeptides. Both sympathetic and sensory neurons could act in concert to regulate diverse aspects of adult stem cells [141, 142]. Next research efforts should reveal the molecular pathways that the nervous system modulate to understand how neurons regulate the activation and differentiation of SC in different niches of the body and its possible implications for tumor formation.

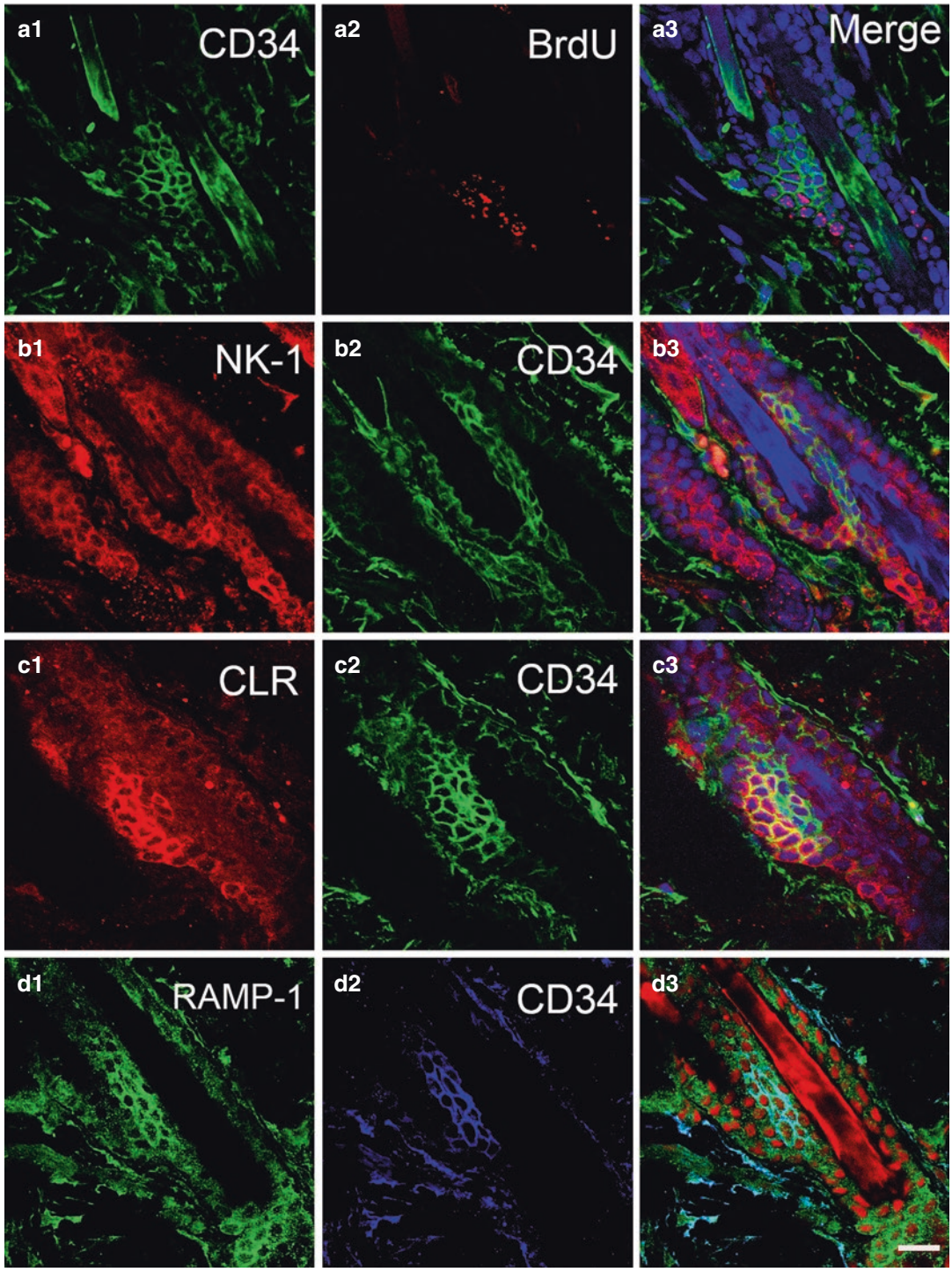

Fig. 5 Neuropeptide receptors in the bulge region of the hair follicles. (**a**) Label-retaining cells were found in the bulge region of the rat hair follicles after 8 weeks of BrdU pulses in a region displaying expression of CD34. (**b**) By confocal microscopy, we found that (**b**) substance P receptor (NK-1) and (**c**, **d**) CGRP receptor (CLR and RAMP-1) were expressed by stem cells from the hair follicle. Scale bar = 20 mm. Modified from [128]

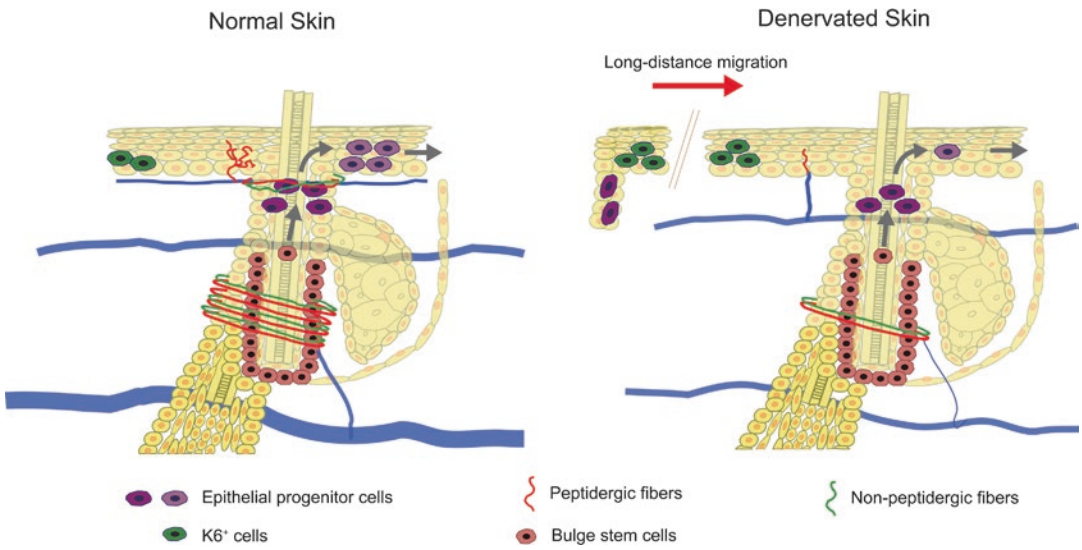

Fig. 6 Wound model explaining the effector function of sensory nerves. (Left) In normal skin, epithelial progenitor cells are activated both in the epidermis and the hair follicles which migrate toward the wound edge to promote reepithelialization. (Right) In partially denervated skin, there is less migration from stem cell progeny from the hair follicles toward the epidermis. Late on time, there is a recruitment of epidermal progenitors far from the wound edge. The lateness of this event and the longer distance of migration by epithelial progenitors to reach the wound edge may explain the delay in wound closure commonly observed in different denervation models

References

1. Holford LC, Case P, Lawson SN (1994) Substance P, neurofilament, peripherin and SSEA4 immunocytochemistry of human dorsal root ganglion neurons obtained from post-mortem tissue: a quantitative morphometric analysis. J Neurocytol 23(9):577–589
2. Lawson SN, Crepps BA, Perl ER (1997) Relationship of substance P to afferent characteristics of dorsal root ganglion neurons in guinea-pig. J Physiol Lond 505(1):177–191
3. McCarthy PW, Lawson SN (1989) Cell type and conduction velocity of rat primary sensory neurons with substance P-like immunoreactivity. Neuroscience 28(3):745–753
4. Gibson SJ, Polak JM, Bloom SR, Sabate IM, Mulderry PM, Ghatei MA, McGregor GP, Morrison JF, Kelly JS, Evans RM (1984) Calcitonin gene-related peptide immunoreactivity in the spinal cord of man and of eight other species. J Neurosci 4(12):3101–3111
5. Fundin BT, Rice FL, Ernfors P (2002) Patterned gene programs and target remodeling following axotomy at a major site for sensory innervation. J Neurobiol 53(3):370–380
6. Meyer MH, Etienne W, Meyer RA Jr (2004) Altered mRNA expression of genes related to nerve cell activity in the fracture callus of older rats: a randomized, controlled, microarray study. BMC Musculoskelet Disord 5(1):24
7. Hosoi J, Murphy GF, Egan CL, Lerner EA, Grabbe S, Asahina A, Granstein RD (1993) Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. Nature 363(6425):159–163
8. Hara M, Toyoda M, Yaar M, Bhawan J, Avila EM, Penner IR, Gilchrest BA (1996) Innervation of melanocytes in human skin. J Exp Med 184(4):1385–1395
9. Egan CL, Viglione-Schneck MJ, Walsh LJ, Green B, Trojanowski JQ, Whitaker-Menezes D, Murphy GF (1998) Characterization of unmyelinated axons uniting epidermal and dermal immune cells in primate and murine skin. J Cutan Pathol 25(1):20–29
10. Hukkanen M, Konttinen YT, Rees RG, Gibson SJ, Santavirta S, Polak JM (1992) Innervation of bone from healthy and arthritic rats by substance P and calcitonin gene related peptide containing sensory fibers. J Rheumatol 19(8):1252–1259
11. Kruger L (1996) The functional morphology of thin sensory axons: some principles and problems. Prog Brain Res 113:255–272
12. Lawson SN (1979) The postnatal development of large light and small dark neurons in mouse dorsal root ganglia: a statistical analysis of cell numbers and size. J Neurocytol 8(3):275–294
13. Lawson SN, Caddy KW, Biscoe TJ (1974) Development of rat dorsal root ganglion neurons. Studies of cell birthdays and changes in mean cell diameter. Cell Tissue Res 153(3):399–413
14. Lawson SN, Harper AA, Harper EI, Garson JA, Anderton BH (1984) A monoclonal antibody against

neurofilament protein specifically labels a subpopulation of rat sensory neurons. J Comp Neurol 228(2):263–272
15. Lawson SN (1992) Morphological and biochemical cell types of sensory neurons. In: Scott SA (ed) Sensory neurons: diversity, development, and plasticity. Oxford University Press, New York, pp 27–59
16. Goldstein ME, House SB, Gainer H (1991) NF-L and peripherin immunoreactivities define distinct classes of rat sensory ganglion cells. J Neurosci Res 30(1):92–104
17. Harper AA, Lawson SN (1985) Conduction velocity is related to morphological cell type in rat dorsal root ganglion neurons. J Physiol 359:31–46
18. Lawson SN, Waddell PJ (1991) Soma neurofilament immunoreactivity is related to cell size and fibre conduction velocity in rat primary sensory neurons. J Physiol 435:41–63
19. Fang X, Djouhri L, McMullan S, Berry C, Okuse K, Waxman SG, Lawson SN (2005) trkA is expressed in nociceptive neurons and influences electrophysiological properties via Nav1.8 expression in rapidly conducting nociceptors. J Neurosci 25(19):4868–4878
20. Perl ER (1992) Function of dorsal root ganglion neurons: an overview. In: Scott SA (ed) Sensory neurons: diversity, development, and plasticity. Oxford University Press, New York, pp 3–23
21. Lee KH, Chung K, Chung JM, Coggeshall RE (1986) Correlation of cell body size, axon size, and signal conduction velocity for individually labelled dorsal root ganglion cells in the cat. J Comp Neurol 243(3):335–346
22. Albrecht PJ, Hines S, Eisenberg E, Pud D, Finlay DR, Connolly MK, Pare M, Davar G, Rice FL (2006) Pathologic alterations of cutaneous innervation and vasculature in affected limbs from patients with complex regional pain syndrome. Pain 120(3):244–266
23. Dux M, Sann H, Schemann M, Jancsó G (1999) Changes in fibre populations of the rat hairy skin following selective chemodenervation by capsaicin. Cell Tissue Res 296(3):471–477
24. Boilly B, Faulkner S, Jobling P, Hondermarck H (2017) Nerve dependence: from regeneration to cancer. Cancer Cell 31(3):342–354
25. Lawson SN, Crepps B, Perl ER (2002) Calcitonin gene-related peptide immunoreactivity and afferent receptive properties of dorsal root ganglion neurons in guinea-pigs. J Physiol Lond 540(3):989–1002
26. Fang X, McMullan S, Lawson SN, Djouhri L (2005) Electrophysiological differences between nociceptive and non-nociceptive dorsal root ganglion neurons in the rat in vivo. J Physiol Lond 565(3):927–943
27. Djouhri L, Bleazard L, Lawson SN (1998) Association of somatic action potential shape with sensory receptive properties in guinea-pig dorsal root ganglion neurons. J Physiol Lond 513(3):857–872
28. Goldstein ME, Grant P, House SB, Henken DB, Gainer H (1996) Developmental regulation of two distinct neuronal phenotypes in rat dorsal root ganglia. Neuroscience 71(1):243–258
29. Hall AK, Ai X, Hickman GE, MacPhedran SE, Nduaguba CO, Robertson CP (1997) The generation of neuronal heterogeneity in a rat sensory ganglion. J Neurosci 17(8):2775–2784
30. O'Brien C, Woolf CJ, Fitzgerald M, Lindsay RM, Molander C (1989) Differences in the chemical expression of rat primary afferent neurons which innervate skin, muscle or joint. Neuroscience 32(2):493–502
31. Holzer P, Maggi CA (1998) Dissociation of dorsal root ganglion neurons into afferent and efferent-like neurons. Neuroscience 86(2):389–398
32. Zylka MJ, Rice FL, Anderson DJ (2005) Topographically distinct epidermal nociceptive circuits revealed by axonal tracers targeted to Mrgprd. Neuron 45(1):17–25
33. Braz JM, Nassar MA, Wood JN, Basbaum AI (2005) Parallel "pain" pathways arise from subpopulations of primary afferent nociceptor. Neuron 47(6):787–793
34. Yang Y, Ozawa H, Lu H, Yuri K, Hayashi S, Nihonyanagi K, Kawata M (1998) Immunocytochemical analysis of sex differences in calcitonin gene-related peptide in the rat dorsal root ganglion, with special reference to estrogen and its receptor. Brain Res 791(1–2):35–42
35. McCarthy PW, Lawson SN (1990) Cell type and conduction velocity of rat primary sensory neurons with calcitonin gene-related peptide-like immunoreactivity. Neuroscience 34(3):623–632
36. Wimalawansa SJ (1997) Amylin, calcitonin gene-related peptide, calcitonin, and adrenomedullin: a peptide superfamily. Crit Rev Neurobiol 11(2–3):167–239
37. Russell FA, King R, Smillie SJ, Kodji X, Brain SD (2014) Calcitonin gene-related peptide: physiology and pathophysiology. Physiol Rev 94(4):1099–1142
38. Amara SG, Arriza JL, Leff SE, Swanson LW, Evans RM, Rosenfeld MG (1985) Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide. Science 229(4718):1094–1097
39. Steenbergh PH, Hoppener JW, Zandberg J, Lips CJ, Jansz HS (1985) A second human calcitonin/CGRP gene. FEBS Lett 183(2):403–407
40. Gkonos PJ, Born W, Jones BN, Petermann JB, Keutmann HT, Birnbaum RS, Fischer JA, Roos BA (1986) Biosynthesis of calcitonin gene-related peptide and calcitonin by a human medullary thyroid carcinoma cell line. J Biol Chem 261(31):14386–14391
41. Wimalawansa SJ (1996) Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials. Endocr Rev 17(5):533–585
42. McLatchie LM, Fraser NJ, Main MJ, Wise A, Brown J, Thompson N, Solari R, Lee MG, Foord SM (1998) RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature 393(6683):333–339

43. Kim YG, Lone AM, Nolte WM, Saghatelian A (2012) Peptidomics approach to elucidate the proteolytic regulation of bioactive peptides. Proc Natl Acad Sci U S A 109(22):8523–8527
44. Hartopo AB, Emoto N, Vignon-Zellweger N, Suzuki Y, Yagi K, Nakayama K, Hirata K (2013) Endothelin-converting enzyme-1 gene ablation attenuates pulmonary fibrosis via CGRP-cAMP/EPAC1 pathway. Am J Respir Cell Mol Biol 48(4):465–476
45. Marchand JE, Zaccheo TS, Connelly CS, Kream RM (1993) Selective in situ hybridization histochemical analyses of alternatively spliced mRNAs encoding beta- and gamma-preprotachykinins in rat central nervous system. Brain Res Mol Brain Res 17(1–2):83–94
46. Harmar AJ, Hyde V, Chapman K (1990) Identification and cDNA sequence of delta-preprotachykinin, a fourth splicing variant of the rat substance P precursor. FEBS Lett 275(1–2):22–24
47. Carter MS, Krause JE (1990) Structure, expression, and some regulatory mechanisms of the rat preprotachykinin gene encoding substance P, neurokinin A, neuropeptide K, and neuropeptide gamma. J Neurosci 10(7):2203–2214
48. Krause JE, Chirgwin JM, Carter MS, Xu ZS, Hershey AD (1987) Three rat preprotachykinin mRNAs encode the neuropeptides substance P and neurokinin A. Proc Natl Acad Sci 84(3):881–885
49. Page NM (2005) New challenges in the study of the mammalian tachykinins. Peptides 26(8):1356–1368
50. Carraway R, Leeman SE (1979) The amino acid sequence of bovine hypothalamic substance P. Identity to substance P from colliculi and small intestine. J Biol Chem 254(8):2944–2945
51. Chang MM, Leeman SE (1970) Isolation of a sialogogic peptide from bovine hypothalamic tissue and its characterization as substance P. J Biol Chem 245(18):4784–4790
52. Cascieri MA, Huang RR, Fong TM, Cheung AH, Sadowski S, Ber E, Strader CD (1992) Determination of the amino acid residues in substance P conferring selectivity and specificity for the rat neurokinin receptors. Mol Pharmacol 41(6):1096–1099
53. Yokota Y, Sasai Y, Tanaka K, Fujiwara T, Tsuchida K, Shigemoto R, Kakizuka A, Ohkubo H, Nakanishi S (1989) Molecular characterization of a functional cDNA for rat substance P receptor. J Biol Chem 264(30):17649–17652
54. Amadesi S, Moreau J, Tognetto M, Springer J, Trevisani M, Naline E, Advenier C, Fisher A, Vinci D, Mapp C, Miotto D, Cavallesco G, Geppetti P (2001) NK1 receptor stimulation causes contraction and inositol phosphate increase in medium-size human isolated bronchi. Am J Respir Crit Care Med 163(5):1206–1211
55. Lin YR, Kao PC, Chan MH (2005) Involvement of Ca2+ signaling in tachykinin-mediated contractile responses in swine trachea. J Biomed Sci 12(3):547–558
56. Okamoto A, Lovett M, Payan DG, Bunnett NW (1994) Interactions between neutral endopeptidase (EC 3.4.24.11) and the substance P (NK1) receptor expressed in mammalian cells. Biochem J 299(Pt 3):683–693
57. Caterina MJ, Julius D (2001) The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 24:487–517
58. Bayliss WM (1901) On the origin of the vasodilator fibres of the hind limb, and on the nature of these fibers. J Physiol 26:173–209
59. Lembeck F, Holzer P (1979) Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma extravasation. Naunyn Schmiedeberg's Arch Pharmacol 310(2):175–183
60. Escott KJ, Brain SD (1993) Effect of a calcitonin gene-related peptide antagonist (CGRP8-37) on skin vasodilatation and oedema induced by stimulation of the rat saphenous nerve. Br J Pharmacol 110(2):772–776
61. Brain SD, Williams TJ, Tippins JR, Morris HR, MacIntyre I (1985) Calcitonin gene-related peptide is a potent vasodilator. Nature 313(5997):54–56
62. Brain SD, Newbold P, Kajekar R (1995) Modulation of the release and activity of neuropeptides in the microcirculation. Can J Physiol Pharmacol 73(7):995–998
63. Wang H, Woolf CJ (2005) Pain TRPs. Neuron 46(1):9–12
64. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389(6653):816–824
65. Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D (1998) The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21(3):531–543
66. De Camilli P, Jahn R (1990) Pathways to regulated exocytosis in neurons. Annu Rev Physiol 52:625–645
67. Sauer SK, Bove GM, Averbeck B, Reeh PW (1999) Rat peripheral nerve components release calcitonin gene-related peptide and prostaglandin E2 in response to noxious stimuli: evidence that nervi nervorum are nociceptors. Neuroscience 92(1):319–325
68. Kilo S, Harding-Rose C, Hargreaves KM, Flores CM (1997) Peripheral CGRP release as a marker for neurogenic inflammation: a model system for the study of neuropeptide secretion in rat paw skin. Pain 73(2):201–207
69. Flores CM, Leong AS, Dussor GO, Hargreaves KM, Kilo S (2001) Capsaicin-evoked CGRP release from rat buccal mucosa: development of a model system for studying trigeminal mechanisms of neurogenic inflammation. Eur J Neurosci 14(7):1113–1120
70. Fischer MJ, Reeh PW, Sauer SK (2003) Proton-induced calcitonin gene-related peptide release from rat sciatic nerve axons, in vitro, involving TRPV1. Eur J Neurosci 18(4):803–810

71. Petho G, Izydorczyk I, Reeh PW (2004) Effects of TRPV1 receptor antagonists on stimulated iCGRP release from isolated skin of rats and TRPV1 mutant mice. Pain 109(3):284–290
72. Kessler F, Habelt C, Averbeck B, Reeh PW, Kress M (1999) Heat-induced release of CGRP from isolated rat skin and effects of bradykinin and the protein kinase C activator PMA. Pain 83(2):289–295
73. Sauer SK, Reeh PW, Bove GM (2001) Noxious heat-induced CGRP release from rat sciatic nerve axons in vitro. Eur J Neurosci 14(8):1203–1208
74. Guo A, Vulchanova L, Wang J, Li X, Elde R (1999) Immunocytochemical localization of the vanilloid receptor 1 (VR1): relationship to neuropeptides, the P2X(3) purinoceptor and IB4 binding sites. Eur J Neurosci 11(3):946–958
75. Smith GD, Gunthorpe MJ, Kelsell RE, Hayes PD, Reilly P, Facer P, Wright JE, Jerman JC, Walhin JP, Ooi L, Egerton J, Charles KJ, Smart D, Randall AD, Anand P, Davis JB (2002) TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 418(6894):186–190
76. Kedei N, Szabo T, Lile JD, Treanor JJ, Olah Z, Iadarola MJ, Blumberg PM (2001) Analysis of the native quaternary structure of vanilloid receptor 1. J Biol Chem 276(30):28613–28619
77. Lintschinger B, Balzer-Geldsetzer M, Baskaran T, Graier WF, Romanin C, Zhu MX, Groschner K (2000) Coassembly of Trp1 and Trp3 proteins generates diacylglycerol- and Ca2+−sensitive cation channels. J Biol Chem 275(36):27799–27805
78. Kichko TI, Reeh PW (2004) Why cooling is beneficial: non-linear temperature-dependency of stimulated iCGRP release from isolated rat skin. Pain 110(1–2):215–219
79. Franco-Cereceda A, Saria A, Lundberg JM (1989) Differential release of calcitonin gene-related peptide and neuropeptide Y from the isolated heart by capsaicin, ischaemia, nicotine, bradykinin and ouabain. Acta Physiol Scand 135(2):173–187
80. Hua XY, Yaksh TL (1993) Pharmacology of the effects of bradykinin, serotonin, and histamine on the release of calcitonin gene-related peptide from C-fiber terminals in the rat trachea. J Neurosci 13(5):1947–1953
81. Averbeck B, Reeh PW (2001) Interactions of inflammatory mediators stimulating release of calcitonin gene-related peptide, substance P and prostaglandin E2 from isolated rat skin. Neuropharmacology 40(3):416–423
82. Opree A, Kress M (2000) Involvement of the proinflammatory cytokines tumor necrosis Factor-alpha, IL-1beta, and IL-6 but not IL-8 in the development of heat hyperalgesia: effects on heat-evoked calcitonin gene-related peptide release from rat skin. J Neurosci 20(16):6289–6293
83. Kress M, Guthmann C, Averbeck B, Reeh PW (1999) Calcitonin gene-related peptide and prostaglandin E2 but not substance P release induced by antidromic nerve stimulation from rat skin in vitro. Neuroscience 89(1):303–310
84. Cesare P, McNaughton P (1996) A novel heat-activated current in nociceptive neurons and its sensitization by bradykinin. PNAS 93(26):15435–15439
85. Nemeth J, Helyes Z, Oroszi G, Than M, Pinter E, Szolcsanyi J (1998) Inhibition of nociceptin on sensory neuropeptide release and mast cell-mediated plasma extravasation in rats. Eur J Pharmacol 347(1):101–104
86. Faisy C, Naline E, Rouget CL, Risse PA, Guerot E, Fagon JY, Chinet T, Roche N, Advenier C (2004) Nociceptin inhibits vanilloid TRPV-1-mediated neurosensitization induced by fenoterol in human isolated bronchi. Naunyn Schmiedeberg's Arch Pharmacol 370(3):167–175
87. Corboz MR, Rivelli MA, Egan RW, Tulshian D, Matasi J, Fawzi AB, Benbow L, Smith-Torhan A, Zhang H, Hey JA (2000) Nociceptin inhibits capsaicin-induced bronchoconstriction in isolated guinea pig lung. Eur J Pharmacol 402(1–2):171–179
88. Giuliani S, Maggi CA (1997) Prejunctional modulation by nociceptin of nerve-mediated inotropic responses in guinea-pig left atrium. Eur J Pharmacol 332(3):231–236
89. Helyes Z, Nemeth J, Pinter E, Szolcsanyi J (1997) Inhibition by nociceptin of neurogenic inflammation and the release of SP and CGRP from sensory nerve terminals. Br J Pharmacol 121(4):613–615
90. Bartho L, Ernst R, Pierau FK, Sann H, Faulstroh K, Petho G (1992) An opioid peptide inhibits capsaicin-sensitive vasodilatation in the pig's skin. Neuropeptides 23(4):227–237
91. Ray NJ, Jones AJ, Keen P (1991) Morphine, but not sodium cromoglycate, modulates the release of substance P from capsaicin-sensitive neurons in the rat trachea in vitro. Br J Pharmacol 102(4):797–800
92. Yaksh TL (1988) Substance P release from knee joint afferent terminals: modulation by opioids. Brain Res 458(2):319–324
93. Yonehara N, Imai Y, Chen JQ, Takiuchi S, Inoki R (1992) Influence of opioids on substance P release evoked by antidromic stimulation of primary afferent fibers in the hind instep of rats. Regul Pept 38(1):13–22
94. Khalil Z, Townley SL, Grimbaldeston MA, Finlay-Jones JJ, Hart PH (2001) Cis-Urocanic acid stimulates neuropeptide release from peripheral sensory nerves. J Invest Dermatol 117(4):886–891
95. Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400(6743):452–457
96. Smart D, Gunthorpe MJ, Jerman JC, Nasir S, Gray J, Muir AI, Chambers JK, Randall AD, Davis JB (2000) The endogenous lipid anandamide is a full agonist at the human vanilloid receptor (hVR1). Br J Pharmacol 129(2):227–230

97. Willis WD (1999) Dorsal root potentials and dorsal root reflexes: a double-edged sword. Exp Brain Res 124(4):395–421
98. Lin Q, Wu J, Willis WD (1999) Dorsal root reflexes and cutaneous neurogenic inflammation after intradermal injection of capsaicin in rats. J Neurophysiol 82(5):2602–2611
99. Peng YB, Wu J, Willis WD, Kenshalo DR (2001) GABAA and 5-HT3 receptors are involved in dorsal root reflexes: possible role in periaqueductal gray descending inhibition. J Neurophysiol 86(1):49–58
100. Silverman JD, Kruger L (1987) An interpretation of dental innervation based upon the pattern of calcitonin gene-related peptide (CGRP)-immunoreactive thin sensory axons. Somatosens Res 5(2):157–175
101. Fundin BT, Arvidsson J, Aldskogius H, Johansson O, Rice SN, Rice FL (1997) Comprehensive immunofluorescence and lectin binding analysis of intervibrissal fur innervation in the mystacial pad of the rat. J Comp Neurol 385(2):185–206
102. Rice FL, Fundin BT, Arvidsson J, Aldskogius H, Johansson O (1997) Comprehensive immunofluorescence and lectin binding analysis of vibrissal follicle sinus complex innervation in the mystacial pad of the rat. J Comp Neurol 385(2):149–184
103. Fundin BT, Pfaller K, Rice FL (1997) Different distributions of the sensory and autonomic innervation among the microvasculature of the rat mystacial pad. J Comp Neurol 389(4):545–568
104. Ruocco I, Cuello AC, Shigemoto R, Ribeiro-da-Silva A (2001) Light and electron microscopic study of the distribution of substance P-immunoreactive fibers and neurokinin-1 receptors in the skin of the rat lower lip. J Comp Neurol 432(4):466–480
105. Burbach GJ, Kim KH, Zivony AS, Kim A, Aranda J, Wright S, Naik SM, Caughman SW, Ansel JC, Armstrong CA (2001) The neurosensory tachykinins substance P and neurokinin A directly induce keratinocyte nerve growth factor. J Invest Dermatol 117(5):1075–1082
106. Thompson PD, Thomas PK (2005) Clinical patterns of peripheral neuropathy. In: Dyck PJ, Thomas PK (eds) Peripheral neuropathy, 4th edn. Elsevier Saunders, Philadelphia, pp 1137–1161
107. Paus R (2016) Exploring the "brain-skin connection": leads and lessons from the hair follicle. Curr Res Transl Med 64(4):207–214
108. Hsieh ST, Choi S, Lin WM, Chang YC, McArthur JC, Griffin JW (1996) Epidermal denervation and its effects on keratinocytes and Langerhans cells. J Neurocytol 25(9):513–524
109. Stankovic N, Johansson O, Oqvist G, Hildebrand C (1999) Indirect effect of sciatic nerve injury on the epidermal thickness of plantar glabrous skin in rats. Scand J Plast Reconstr Surg Hand Surg 33(3):273–279
110. Hsieh ST, Lin WM (1999) Modulation of keratinocyte proliferation by skin innervation. J Invest Dermatol 113(4):579–586
111. Huang IT, Lin WM, Shun CT, Hsieh ST (1999) Influence of cutaneous nerves on keratinocyte proliferation and epidermal thickness in mice. Neuroscience 94(3):965–973
112. Burgess PR, English KB, Horch KW, Stensaas LJ (1974) Patterning in the regeneration of type I cutaneous receptors. J Physiol 236(1):57–82
113. Nurse CA, Macintyre L, Diamond J (1984) A quantitative study of the time course of the reduction in Merkel cell number within denervated rat touch domes. Neuroscience 11(2):521–533
114. Tanaka T, Danno K, Ikai K, Imamura S (1988) Effects of substance P and substance K on the growth of cultured keratinocytes. J Invest Dermatol 90(3):399–401
115. Kahler CM, Herold M, Reinisch N, Wiedermann CJ (1996) Interaction of substance P with epidermal growth factor and fibroblast growth factor in cyclooxygenase-dependent proliferation of human skin fibroblasts. J Cell Physiol 166(3):601–608
116. Peters EM, Botchkarev VA, Muller-Rover S, Moll I, Rice FL, Paus R (2002) Developmental timing of hair follicle and dorsal skin innervation in mice. J Comp Neurol 448(1):28–52
117. Botchkarev VA, Eichmuller S, Johansson O, Paus R (1997) Hair cycle-dependent plasticity of skin and hair follicle innervation in normal murine skin. J Comp Neurol 386(3):379–395
118. Peters EM, Botchkarev VA, Botchkareva NV, Tobin DJ, Paus R (2001) Hair-cycle-associated remodeling of the peptidergic innervation of murine skin, and hair growth modulation by neuropeptides. J Invest Dermatol 116(2):236–245
119. Paus R, Heinzelmann T, Schultz KD, Furkert J, Fechner K, Czarnetzki BM (1994) Hair growth induction by substance P. Lab Investig 71(1):134–140
120. Maurer M, Fischer E, Handjiski B, von Stebut E, Algermissen B, Bavandi A, Paus R (1997) Activated skin mast cells are involved in murine hair follicle regression (catagen). Lab Investig 77(4):319–332
121. Chan J, Smoller BR, Raychauduri SP, Jiang WY, Farber EM (1997) Intraepidermal nerve fiber expression of calcitonin gene-related peptide, vasoactive intestinal peptide and substance P in psoriasis. Arch Dermatol Res 289(11):611–616
122. Lazarova R, Hristakieva E, Lazarov N, Shani J (2000) Vitiligo-related neuropeptides in nerve fibers of the skin. Arch Physiol Biochem 108(3):262–267
123. Urashima R, Mihara M (1998) Cutaneous nerves in atopic dermatitis. Virchows Arch 432(4):363–370
124. Naukkarinen A, Jarvikallio A, Lakkakorpi J, Harvima IT, Harvima RJ, Horsmanheimo M (1996) Quantitative histochemical analysis of mast cells and sensory nerves in psoriatic skin. J Pathol 180(2):200–205
125. Jarvikallio A, Harvima IT, Naukkarinen A (2003) Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. Arch Dermatol Res 295(1):2–7

126. Zenz R, Eferl R, Kenner L, Florin L, Hummerich L, Mehic D, Scheuch H, Angel P, Tschachler E, Wagner EF (2005) Psoriasis-like skin disease and arthritis caused by inducible epidermal deletion of Jun proteins. Nature 437(7057):369–375
127. Raychaudhuri SP, Jiang WY, Farber EM (1998) Psoriatic keratinocytes express high levels of nerve growth factor. Acta Derm Venereol 78(2):84–86
128. Martinez-Martinez E, Galvan-Hernandez CI, Toscano-Marquez B, Gutierrez-Ospina G (2012) Modulatory role of sensory innervation on hair follicle stem cell progeny during wound healing of the rat skin. PLoS One 7(5):e36421
129. Martinez-Martinez E, Toscano-Marquez B, Gutierrez-Ospina G (2011) Long-term effects of neonatal capsaicin treatment on intraepidermal nerve fibers and keratinocyte proliferation in rat glabrous skin. Anat Rec (Hoboken) 294(1):173–184
130. Mouton PR (2002) Principles and practices of unbiased stereology: an introduction for bioscientists. Johns Hopkins University Press, Baltimore, p 214
131. Howard V, Reed MG (2005) Unbiased stereology: three-dimensional measurement in microscopy, 2nd edn. Garland Science, New York, NY, p 277
132. Martinez-Martinez E, Uribe-Querol E, Galvan-Hernandez CI, Gutierrez-Ospina G (2016) Stereological quantification of cell-cycle kinetics and mobilization of epithelial stem cells during wound healing. Methods Mol Biol 1453:93–107
133. Harris LW, Purves D (1989) Rapid remodeling of sensory endings in the corneas of living mice. J Neurosci 9(6):2210–2214
134. Carr RW, Delaney CA, Westerman RA, Roberts RG (1993) Denervation impairs cutaneous microvascular function and blister healing in the rat hind limb. Neuroreport 4(5):467–470
135. Harsum S, Clarke JD, Martin P (2001) A reciprocal relationship between cutaneous nerves and repairing skin wounds in the developing chick embryo. Dev Biol 238(1):27–39
136. Westerman RA, Carr RW, Delaney CA, Morris MJ, Roberts RG (1993) The role of skin nociceptive afferent nerves in blister healing. Clin Exp Neurol 30:39–60
137. Fukai T, Takeda A, Uchinuma E (2005) Wound healing in denervated rat skin. Wound Repair Regen 13(2):175–180
138. Maggi CA, Borsini F, Santicioli P, Geppetti P, Abelli L, Evangelista S, Manzini S, Theodorsson-Norheim E, Somma V, Amenta F et al (1987) Cutaneous lesions in capsaicin-pretreated rats. A trophic role of capsaicin-sensitive afferents? Naunyn Schmiedeberg's Arch Pharmacol 336(5):538–545
139. Langton AK, Herrick SE, Headon DJ (2008) An extended epidermal response heals cutaneous wounds in the absence of a hair follicle stem cell contribution. J Invest Dermatol 128(5): 1311–1318
140. Garcin CL, Ansell DM, Headon DJ, Paus R, Hardman MJ (2016) Hair follicle bulge stem cells appear dispensable for the acute phase of wound re-epithelialization. Stem Cells 34(5):1377–1385
141. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, Frenette PS (2006) Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. Cell 124(2):407–421
142. Brownell I, Guevara E, Bai CB, Loomis CA, Joyner AL (2011) Nerve-derived sonic hedgehog defines a niche for hair follicle stem cells capable of becoming epidermal stem cells. Cell Stem Cell 8(5): 552–565

Part VI

Wound Repair

Reconstructive Options of Abdominal Wounds in the Setting of Abdominal Wall Defects and Hernias

James Gatherwright, Rebecca Knackstedt, Rachel Aliotta, and Raffi Gurunluoglu

1 Abdominal Wall Defects

The abdominal wall is an integral, yet often ignored, functional unit required to protect and maintain vital internal organs while simultaneously providing an integral aesthetic role. Perhaps even less appreciated is the contribution the abdominal wall makes to allowing for physical activity and its biomechanically, vital importance to all levels of activity, from sitting to complex sports.

Abdominal wall defects are generally divided into two major categories: congenitally acquired versus non-congenitally acquired. This chapter will focus mainly on non-congenitally acquired defects, as this comprises the majority of abdominal wounds that most reconstructive surgeons will encounter. Acquired defects can be due to traumatic injuries; due to postsurgical, secondary to oncologic resections; and due to necrotizing infections. This chapter will also review abdominal wall reconstruction in the context of chronic wounds in the settings of unstable scars, entero-cutaneous fistulas (ECFs), and mesh exposures.

1.1 Goals

The goals of abdominal wall reconstruction (AWR) are multiple, and include, providing a physiologic housing for internal viscera, supporting fascial integrity, and acting as a functional anatomic unit for physical activity. Producing a functional anatomic unit remains the most difficult goal to reliably achieve and is one of the most significant sources of postoperative physician and patient dissatisfaction. The difficulty in producing a functional abdominal unit is largely due to the inherent complexity involved in abdominal wall reconstruction due to the composite nature of tissues involved.

1.2 Anatomy

In order to truly understand the complex nature of composite defects of the abdominal wall and to appropriately plan for reconstruction, one must have a solid foundation of abdominal wall anatomy.

Areas of the abdominal wall that must be considered for reconstructive purposes include:

1. Soft tissue—skin, fat. Plastic surgeons have made significant contributions to understand-

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ing the skin's blood supply. Huger zones were originally described in the context of abdominoplasty surgery, but its implication in AWR cannot be understated. While the reconstructive surgeon is usually providing a contributory role in solving a much larger surgical issue, whenever a surgeon makes an abdominal incision, they must consider the blood supply and how incisions will interrupt blood flow to the skin. This is especially pertinent when developing laterally based skin flaps, as the lateral perforators must be preserved. Although wound necrosis and/or dehiscence in the presence of intact fascia can present a significant cosmetic concern, it rarely leads to a life-altering event. However, when fascia is compromised or when synthetic/biologic materials may be exposed following skin loss, the importance of an intact skin surface becomes paramount.

2. Fascia—the major fascial components consist of the muscular aponeuroses of the abdominal wall. Fascia is relatively avascular, which has implications both in primary reconstructive closure and in postoperative infections and recurrence. Primary fascial closure should be made with a monofilament, appropriately sized suture in a running technique 5–8 mm from the wound edge at 4–5 mm intervals [1–6].
3. Muscle—the muscles of the abdominal wall are composed of the paired rectus abdominis, external and internal obliques, and the transversus abdominis.
4. Peritoneum and viscera—these units are not typically discussed in the context of AWR, but they must be considered as they contribute to compartment pressures and are involved in ECF fistulas, enterotomies, and stomas.

The aforementioned components of the abdominal wall must be individually, as well as collectively, addressed when reconstructing abdominal wall defects. Every complex abdominal defect is unique, and there are often multiple appropriate options for reconstruction. However, by adhering to a specific construction algorithm, the surgeon will be able to successfully apply the necessary surgical principles and techniques to a wide variety of defects.

2 Preoperative Examination

A thorough physical exam should be performed with a focus on assessing the patient's skin quality, overall health, existing scars and/or incisions, presence of a stoma, abdominal wall fascial integrity, and overall abdominal musculature.

Preoperative computed tomography (CT) imaging of the abdomen and pelvis can prove invaluable in surgical planning as it can provide information on the size, number, and relationship of abdominal wall defect(s). It can also elucidate the integrity of the abdominal wall musculature and demonstrate any previously placed prosthetic mesh. The extent of the loss of domain as measured on CT can also serve as a predictor of postoperative abdominal compartment syndrome and/or respiratory compromise.

3 Preoperative Optimization

The importance of preoperative optimization cannot be underscored. Complex abdominal wall defects often result from complicated underlying disease states or suboptimal patient conditions.

In order to even attempt successful wound healing, nutrition must be appropriately addressed. While there are several laboratory indicators of patient nutrition, perhaps one of the most reliable is clinical evidence of healing. A patient who is not able to heal small wounds (e.g., port/IV sites) and does not demonstrate evidence of healing (granulation tissue), even with adequate laboratory values, should not be operated on until they are nutritionally optimized. A large prospective trial from the VA found that an albumin level less than 3 g/dL was an independent risk factor for morbidity and mortality. While multiple studies suggest nutritional interventions, such as carbohydrate

loading or amino acid supplementation, may have some benefit, a direct relationship with improved surgical wound healing has yet to be established. Increased BMI has been associated with surgical site infections and hernia recurrence [7, 8]. Similarly, glycemic control pre-, peri-, and postoperatively has been shown to have an impact on surgical site infections and morbidity. While originally, a tight glucose regimen was suggested, this was found to be associated with the unacceptable risk of severe hypoglycemia, and, therefore, recommendations have been made for to the target glucose to be 140–160 mg/dL [9–13]. What is likely most important is correctly identifying high-risk patients preoperatively in order to prioritize nutritional optimization pre-, peri-, and postoperatively [14–16].

4 Contamination/Infection

When determining the role of antibiotics in abdominal wall reconstruction, the classification of the case must be considered. Skin preparation should be performed with an alcohol-containing solution [17–21], and if there is known or suspected MRSA colonization, preoperative treatment with mupirocin and chlorhexidine showers should be considered [22, 23]. Prophylactic antibiotics have shown to decrease the risk of postoperative infection in clean cases. Typically, a cephalosporin, such as Ancef, should be given at least 30 min prior to incision and dosed according to BMI (1 g < 30 BMI, 2 g > 30 BMI). In high-risk or allergic patients, vancomycin should be administered according to weight and creatinine clearance. Regardless of antibiotic choice, the antibiotic must be redosed based on its half-life, an idea which becomes especially pertinent during long AWR cases. As the reconstructive surgeon may perform his or her role toward the end of the case, this is a conversation that must be had with anesthesia and the other operative teams to ensure adequate coverage. There is no proven benefit of continuing antibiotics beyond incisional closure [24–28].

The guidelines are mainly based on clean cases, absent of infection. If possible, certain patient factors should be reversed, and/or adequately treated, to ensure the greatest chance of preventing infection. Similarly, the presence of an acute or subacute infection or contamination will dictate what type of mesh should be used. For example, the presence of a stoma may preclude the use of synthetic mesh, as bioprosthetic materials have been shown to be more resistant to infection. Other conditions, such as exposed infected mesh and/or ECF fistulas, should be managed appropriately to minimize any potential untoward effects that they may have on AWR. Some authors suggest a preoperative bowel preparation to decrease risk of contamination following an inadvertent enterotomy; however this is somewhat controversial [29]. Chronic wounds present a unique problem of unstable soft tissue coverage in the context of contamination/infection and should be treated according to surgical principles of adequate debridement. Following adequate washouts and debridement, these wounds can also be bridged to definitive repair utilizing lower rungs of the reconstructive ladder (e.g., skin grafting, NPWT). The importance of adequate infection/contamination treatment is paramount as there is a significant increase in the incidence of wound complications (e.g., dehiscence, infection) and a threefold increase in hernia recurrence rates in these settings compared to "clean" cases [30].

In addition to optimizing patient factors, two key surgical aspects that should be optimized during any abdominal surgery to reduce infection risk are the blood supply to the skin and the fascial closure. These topics have been addressed to some extent above but they deserve to be highlighted again.

5 Reconstruction

5.1 Open Hernia Repair

In order to discuss AWR, one must address the reduction of visceral contents and hernia repair.

Techniques for simple fascial closure have been previously discussed and rarely require a specialized AWR. When the fascia cannot be closed primarily, more advanced techniques are required, such as the utilization of mesh or component separation, with or without mesh. The anterior component separation as described by Ramirez [31] is one such technique, which has, since its original description, undergone numerous refinements. This approach involves the raising of lipocutaneous flaps to allow access to the lateral abdominal wall. The external oblique fascia is released laterally at the semilunar line, thus allowing for advancement of the rectus abdominis, internal oblique and transversus abdominis muscle, and fascia as a single unit. Advancement has been cited to be as great as 10 cm at the midline with less advancement available at the cephalic and caudal ends of the abdomen [31]. This technique can obviate the necessity of mesh in some cases but is often utilized with mesh to repair larger hernias. The raising of these large flaps generally results in the loss of the main blood supply from the deep inferior epigastric vessels, resulting in infection and/or necrosis in upward of 25% of cases. With perforator preservation modifications, these risks have been decreased significantly [32, 33].

The most successful approach to circumvent skin necrosis would be to avoid dissections that disrupt the skin's blood supply. Therefore, the originally described component separation was modified to have a posterior approach [33]. This involves the creation of a retro-rectus plane of dissection, taking care to avoid injury to the epigastric vessels both as they enter the rectus muscle and at their take off from external iliac vessels. This also allows for the preservation of the innervation to the rectus. The dissection is carried lateral to the semilunar line at which the transversus abdominis muscle is released to allow for advancement and closure of the defect. Similar to the originally described component separation, the posterior approach is often combined with mesh reinforcement. Laparoscopic techniques have been described to allow for release in a less invasive fashion while serving to preserve skin perforators [34].

5.2 Mesh

In general, mesh can be used either as reinforcement or as a primary form of ventral hernia repair. If fascial edges are able to be approximated either directly or via some sort of component separation, mesh can be used as a reinforcement. There is substantial evidence that except in very small hernias, even less than 2 cm in diameter, the use of mesh as a reinforcement, either in an underlay or overlay fashion, decreases the risk of hernia recurrence [35, 36]. Thus, the use of mesh should be used to reinforce the majority of hernia repairs and all incisional ventral hernia repairs.

Mesh can be positioned at different anatomic layers (overlay, inlay, underlay, retro-rectus, retroperitoneal, etc.). Mesh can be placed in either an underlay (underneath fascia) or overlay (on top of the fascia) when fascial edges can be approximated. The overlay technique minimizes the risk of bowel adhesions and ECF fistulas but increases the risk of infection and associated issues with exposure if not adequately covered with soft tissue. The underlay technique can potentially lead to bowel erosion, adhesions, and/or ECF fistulas but may provide a repair that is more resistant to infection [37]. When the fascial edges are unable to be coapted, mesh can be used in a bridging technique. In general, regardless of choice of mesh material, it is generally recommended to use an underlay approach, and all attempts at fascial closure should be exhausted before considering a bridging technique. Regardless of position, mesh should have three to 5 cm overlap with the fascial edges [38–43].

5.3 Mesh Material

Materials for mesh hernia reconstruction are typically divided into two categories: synthetic and bioprosthetic. Synthetic materials can be further divided into macroporous and microporous. Macroporous materials are associated with higher rates of bowel erosion and ECF fistulas, while microporous materials tend to exhibit less integration and are associated with an increased risk of infection. There are several mesh products

available that attempt to combine the properties of each, but a full discussion of all available products is beyond the scope of this chapter. Bioprosthetic materials can generally be divided into either human or animal derived. The most common biologics include chemically treated human or porcine dermis (e.g., Alloderm and Strattice). Alloderm is thought to undergo better incorporation following repair and demonstrates increased resistance to infection but has questionably increased rates of hernia recurrence as compared to porcine materials [44–46]. The main advantage of prosthetic materials is their strength and associated decreased risk of hernia recurrence. However, the main disadvantages are the increased risk of contamination, infection, bowel adhesions, and ECF fistula development.

Historically, it was not recommended to use prosthetic materials in the setting of infection and/or contamination due to an increased risk of complications. Biologic materials, due to their ability to be vascularized, were thought to have an increased ability to clear infection and were therefore recommended in grossly contaminated wounds [44–66]. However, there is some controversy over whether this still holds true in light of newly available materials, provided there is adequate soft tissue coverage [67]. Therefore, the use of prosthetic mesh in contaminated fields (ECF, chronic wounds, etc.) should be done in a patient specific manner context [30, 68–70].

5.4 Soft Tissue Coverage

The success of abdominal wall reconstruction relies heavily on the presence of healthy, well-vascularized, pliable soft tissue coverage. The complexity of AWR runs the full gamut of the reconstructive ladder and will be addressed in a stepwise fashion with a particular focus on locoregional and free flap reconstruction.

The lowest rung of the reconstructive ladder is local wound care and had been the mainstay for a significant proportion of abdominal wounds. Local wound care had been advanced significantly with the introduction of negative pressure wound (NPW) therapies which are able to create smaller wounds with a decreased bacterial burden [71]. Thus, NPW therapy has increased the number of wounds that are able to be subsequently skin grafted or closed in a delayed primary fashion. However, by using NPW therapy, there is a protracted treatment course with associated costs and a potentially negative impact on the patient's experience. With an increased understanding of anatomy and options for perforator and free flap reconstruction, there has been a trend toward early flap repair.

When utilizing a flap, in general, reconstructive options are dictated by the location of the defect on the abdominal wall. Lower composite defects are more amenable to local and/or locoregional pedicled flaps, while central and upper abdominal defects typically require free flap reconstruction. While flaps may provide additional fascia, it is typically recommended that this approach is not used in bridging defects, and instead, mesh reconstruction is performed, with or without component separation, to reduce hernia recurrence [72].

5.5 Tissue Expansion

Tissue expansion of the abdominal wall has the advantage of providing well-vascularized autologous skin, subcutaneous tissue, and/or abdominal fascia for the repair of large defects [73]. Tissue expanders can be placed in either a subcutaneous or submuscular plane [74]. Disadvantages of tissue expansion include the risks of expander rupture or extrusion, infection, patient intolerance, and expander failure. It also increases the duration of treatment and requires multiple, repeated patient visits to complete. Recently published data from our institution demonstrated favorable long-term outcomes with improved patient reported outcomes and low rates of hernia recurrence in a series of twelve patients who underwent two-stage abdominal wall reconstruction with tissue expansion and an anterior component separation [75]. A sample patient reconstructed with subcutaneous tissue expanders is depicted in Fig. 1.

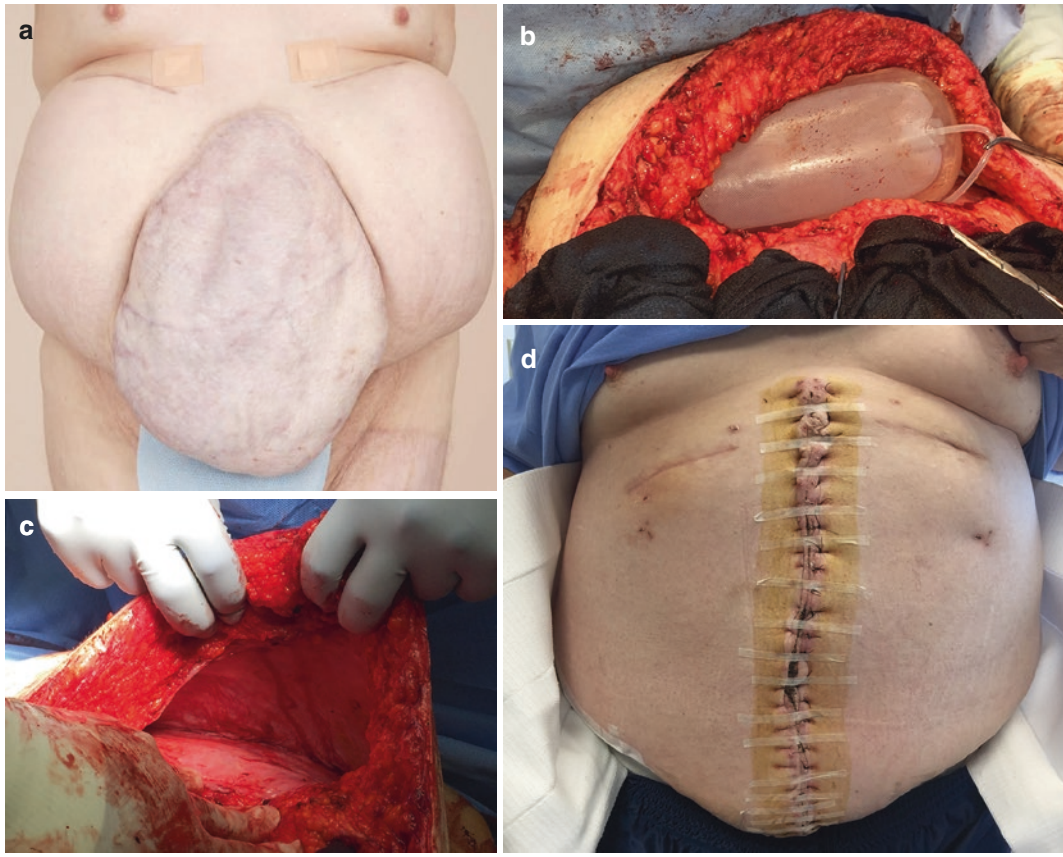


Fig. 1 Patient with large ventral hernia repaired with preoperative tissue expansion. (**a**) Preoperative. (**b**) Removal of tissue expanders after being placed in the Scarpa's tissue plane. (**c**) Pocket created after tissue expansion. Note the pre-expanded skin and subcutaneous tissues. (**d**) Postoperative showing hernia repair posterior component separation and transverse rectus abdominis release with mesh

This patient had a large ventral hernia with a paucity of soft tissue available. The tissue expanders were placed bilaterally between the external and internal oblique muscles to allow for expansion of both sets of muscles. This patient then underwent posterior component separation with success.

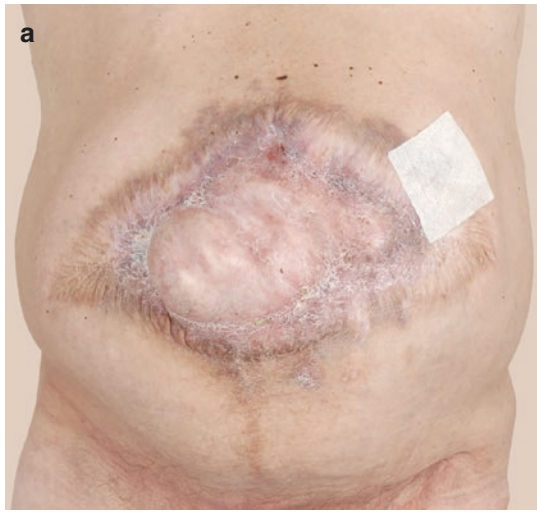
5.6 Lower Abdominal Defects

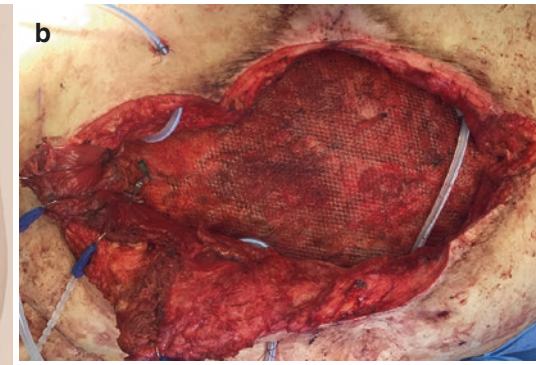
Lower abdominal defects, due to their location, are usually amenable to thigh flap reconstruction. These flaps include the anterolateral thigh (ALT), tensor fascia lata (TFL), and rectus femoris(RF) flaps. All three flaps are based off the lateral femoral circumflex vessels, which allows for combinations of these flaps to be utilized.

5.6.1 ALT Flap

With the advent of fasciocutaneous flaps, the ALT flap has become a workhorse in AWR. It provides a large surface area, up to eight by 25 cm, with a reliable, relatively long, greater than 7 cm in some patients, vascular supply based on the descending branch of the lateral femoral circumflex. The ALT flap has been reported to be able to reach defects up to the costal margin cephalically [76], to the ipsilateral posterior superior iliac spine posteriorly, and to the contralateral fossa laterally. There is minimal donor-site morbidity as the vastus lateralis is, for the most part, preserved and other extensor muscles are able to compensate adequately.

A sample patient reconstructed with Bard mesh, Davol Inc requiring a free ALT for coverage is depicted in Fig. 2.





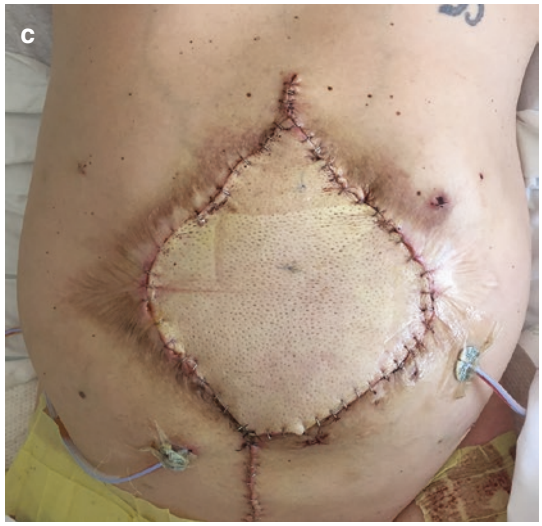


Fig. 2 Patient with large ventral hernia repaired with posterior component separation and transverse rectus abdominis release with ALT and mesh. (**a**) Preoperative. (**b**) Intraoperative demonstrating soft tissue deficit. (**c**) Soft tissue deficit reconstructed using ALT with left inferior epigastric vessels as recipient vessels

5.6.2 TFL

The TFL flap, based off the ascending branch of the lateral femoral circumflex, can also provide a large skin paddle up to 15/40 cm. Its range is extensive and it can reliably be brought to the periumbilical area. However, it can be associated with distal skin necrosis when combined with the RF flap [77–79].

5.6.3 RF

The RF flap can be transferred as a muscle, musculocutaneous, or as part of a combined thigh flap based on the lateral circumflex femoral vessels. It provides a long cylindrical muscle, approximately 6 cm wide and can support a 12/20 cm skin island.

5.6.4 Combined Thigh Flaps

Combination thigh flaps take advantage of the versatile lateral circumflex femoral vessel system and can include the RF, TFL, and ALT flaps. Near complete AWR has been described using bilateral pedicled subtotal thigh flaps [72].

5.7 Lateral and Central Defects

Pedicled options for lateral and central defects are limited to the latissimus dorsi (LD) and rectus abdominis (RA) flaps, respectively. However,

most large defects in these areas will generally require free flap reconstruction.

5.7.1 LD

The LD flap can be used for coverage of the superolateral abdominal wall due to its arc of rotation. It can be transposed either with a skin paddle, as a musculocutaneous flap, or as a muscle flap alone.

5.7.2 RA

The RA flap is useful for peripheral defects but is counterproductive for large defects, as the donor site creates a second large abdominal wound. The skin paddle can be oriented vertically, horizontally, as an extended deep inferior epigastric artery flap (musculocutaneous flap with.

lateral skin extension based on the periumbilical perforators), or as a flag flap (skin from the upper abdomen with extensions to the inframammary fold and the anterior axillary line) [80–83].

5.7.3 Free Flap Reconstruction

Free flap reconstruction is usually required for larger, upper/central defects where either pedicled flap size and/or reach are inadequate to cover the defect. The choice of flaps is similar to pedicled options and includes the TFL, ALT, and combination flaps [78, 84, 85]. Free flap reconstruction is complicated by its dependency on suitable recipient vessels. A recent systematic review found that the inferior epigastric artery was the most commonly utilized recipient vessel [86]. Other utilized vessels included the internal mammary, epigastric, and gastroepiploic vessels. If femoral vessels were used, they often required a vein graft to ensure adequate length. In fact, in this review of literature, 30.8% of patients undergoing free flap abdominal wall reconstruction required either a vein graft or an AV loop to supply the transferred flap [86]. Gastroepiploic vessels are typically avoided as they require an intra-abdominal procedure and predispose the patient to an additional hernia or bulge, as well as present a risk for pedicle compression and associated thrombosis. The status of the internal mammary and epigastric vessels can be a significant impediment in AWR as they may not be able for use due to inherent anatomical issues and/or iatrogenic sacrifice from trauma or previous surgeries. In addition, vessels may have been radiated as part of adjuvant or neo-adjuvant oncologic treatment. The lack of suitable vessels generally requires either vein grafting or arteriovenous loop creation based off one of the following vessels: femoral vessels, branches of the internal mammary, superior epigastric, inferior epigastric, thoracodorsal, superficial femoral, and gastroepiploic vessels [86–90]. Free flap reconstruction with anastomosis to abdominal wall perforators has been described but is generally not feasible or recommended given variability, technical difficulty, and size.

6 Perioperative Management

The operative room should be warmed to maintain patient temperature. Prior to induction, preoxygenation should be performed as well. A decompressive gastric tube, as well as a urinary catheter, should be placed. Antibiotics should be given at least a half-hour before skin incision. As previously discussed, the patient should be prepared appropriately.

7 Postoperative Management

7.1 Airway Management

In cases with prolonged operative times, patients with underlying pulmonary disease, and/or if peak airway pressures increase more than 6 cm H20, the patient should be kept intubated overnight. For significant rises in plateau pressure, 9 cm H20 or greater, the patient may benefit from remaining intubated up to 48 h, and chemical paralysis may be required [91].

7.2 Pain Management

Placement of an epidural catheter is a useful adjunct and can serve to reduce adverse effects related to narcotics. If an epidural catheter cannot be placed, or is contraindicated, an intravenous patient-controlled analgesia device is used, and the patient is transitioned to oral narcotics when tolerating a diet.

7.3 Diet

There is a delicate balance between avoiding increased abdominal pressure, retching, and emesis while optimizing nutrition. The patient's diet should not be advanced until passing flatus, and the patient must be monitored for nausea and abdominal distension. Nasogastric tube decompression is strongly recommended in patients whom have had extensive adhesiolysis or a small bowel resection.

7.4 Flap Monitoring/Care

Routine flap checks should be performed with particular emphasis on the first 48 h. Given the location of the anastomosis and pedicle, care should be made to avoid any potential for compression or kinking (e.g., hip flexion at the waist for pedicled ALT). Drains are typically placed and kept until output is less than 30 mL per day.

Conclusions

Abdominal wounds are some of the most challenging problems reconstructive surgeons face, as the defects are typically large and composite in nature in the setting of complex patient comorbidities and often require the utilization of foreign bodies, such as mesh. The wounds are associated with significant physical, functional, and psychological morbidity. In order to achieve a successful AWR, all parameters need to be optimized and a multidisciplinary team assembled. Even under the best of circumstances, reconstructive failure is a known consequence and limits future options. The ability to provide definitive, well-vascularized soft tissue has significantly improved the chances of performing a successful reconstruction. While this chapter has examined the general reconstructive options of abdominal wounds, treatment plans should be individualized to the specifics of the patient and wound presentation addressing all components of the defect.

References

1. Israelsson LA, Jonsson T (1996) Knutsson Suture technique and wound healing in midline laparotomy incisions. Eur J Surg 162(8):605–609
2. Millbourn D, Israelsson LA (2004) Wound complications and stitch length. Hernia 8(1):39–41
3. Cengiz Y, Gislason H, Svanes K, Israelsson LA (2001) Mass closure technique: an experimental study on separation of wound edge. Eur J Surg 167(1):60–63
4. Harlaar JJ, van Ramshorst GH, Nieuwenhuizen J, Ten Brinke JG, Hop WC, Kleinrensink GJ, Jeekel H, Lange JF (2009) Small stitches with small suture distances increase laparotomy closure strength. Am J Surg 198(3):392–395
5. Millbourn D, Cengiz Y, Israelsson LA (2009) Effect of stitch length on wound complications after closure of midline incisions: a randomized controlled trial. Arch Surg 144(11):1056–1059
6. Israelsson LA, Jonsson T (1997) Overweight and healing of midline incisions: the importance of suture technique. Eur J Surg 163(3):175–180
7. Sugerman HJ, Kellum JM Jr, Reines HD, DeMaria EJ, Newsome HH, Lowry JW (1996) Greater risk of incisional hernia with morbidly obese than steroid-dependent patients and low recurrence with prefascial polypropylene mesh. Am J Surg 171(1):80–84
8. Puzziferri N, Austrheim-Smith IT, Wolfe BM, Wilson SE, Nguyen NT (2006) Three-year follow-up of a prospective randomized trial comparing laparoscopic versus open gastric bypass. Ann Surg 243(2):181–188
9. Dronge AS, Perkal MF, Kancir S, Concato J, Aslan M, Rosenthal RA (2006) Long-term glycemic control and postoperative infectious complications. Arch Surg 141(4):375–380
10. Turina M, Fry DE, Polk HC Jr (2005) Acute hyperglycemia and the innate immune system: clinical, cellular, and molecular aspects. Crit Care Med 33(7):1624–1633
11. van den Berghe G, Wouters P, Weekers F, Verwaest C, Bruyninckx F, Schetz M, Vlasselaers D, Ferdinande P, Lauwers P, Bouillon R (2001) Intensive insulin therapy in critically ill patients. N Engl J Med 345(19):1359–1367
12. The NICE-SUGAR Study Investigators (2012) Hypoglycemia and risk of death in critically ill patients. N Engl J Med 367(12):1108–1118
13. Ramos M, Khalpey Z, Lipsitz S, Steinberg J, Panizales MT, Zinner M, Rogers SO (2008) Relationship of perioperative hyperglycemia and postoperative infections in patients who undergo general and vascular surgery. Ann Surg 248(4):585–591
14. Gianotti L, Braga M, Nespoli L, Radaelli G, Beneduce A, Di Carlo V (2002) A randomized controlled trial of preoperative oral supplementation with a specialized diet in patients with gastrointestinal cancer. Gastroenterology 122(7):1763–1770
15. Braga M, Gianotti L, Vignali A, Schmid A, Nespoli L, Di Carlo V (2005) Hospital resources consumed for surgical morbidity: effects of preoperative arginine and omega-3 fatty acid supplementation on costs. Nutrition 21(11–12):1078–1086
16. Kondrup J, Rasmussen HH, Hamberg O, Stanga Z, Ad Hoc ESPEN Working Group (2003) Nutritional risk screening (NRS 2002): a new method based on an analysis of controlled clinical trials. Clin Nutr 22(3):321–336

17. Darouiche RO, Wall MJ Jr, Itani KM, Otterson MF, Webb AL, Carrick MM, Miller HJ, Awad SS, Crosby CT, Mosier MC, Alsharif A, Berger DH (2010) Chlorhexidine-alcohol versus povidone-iodine for surgical-site antisepsis. N Engl J Med 362(1):18–26
18. Swenson BR, Sawyer RG (2010) Importance of alcohol in skin preparation protocols. Infect Control Hosp Epidemiol 31(9):977
19. Tanner J, Norrie P, Melen K (2011) Preoperative hair removal to reduce surgical site infection. Cochrane Database Syst Rev 11:CD004122
20. Dumville JC, McFarlane E, Edwards P, Lipp A, Holmes A (2013) Preoperative skin antiseptics for preventing surgical wound infections after clean surgery. Cochrane Database Syst Rev (3):CD003949
21. Edmiston CE Jr, Okoli O, Graham MB, Sinski S, Seabrook GR (2010) Evidence for using chlorhexidine gluconate preoperative cleansing to reduce the risk of surgical site infection. AORN J 92(5): 509–518
22. Bode LG, Kluytmans JA, Wertheim HF, Bogaers D, Vandenbroucke-Grauls CM, Roosendaal R, Troelstra A, Box AT, Voss A, van der Tweel I, van Belkum A, Verbrugh HA, Vos MC (2010) Preventing surgical-site infections in nasal carriers of Staphylococcus aureus. N Engl J Med 362(1):9–17
23. Kim DH, Spencer M, Davidson SM, Li L, Shaw JD, Gulczynski D, Hunter DJ, Martha JF, Miley GB, Parazin SJ, Dejoie P, Richmond JC (2010) Institutional prescreening for detection and eradication of methicillin-resistant Staphylococcus aureus in patients undergoing elective orthopaedic surgery. J Bone Joint Surg Am 92(9):1820–1826
24. Enzler MJ, Berbari E, Osmon DR (2011) Antimicrobial prophylaxis in adults. Mayo Clin Proc 86(7):686–701
25. Berbari EF, Osmon DR, Lahr B, Eckel-Passow JE, Tsaras G, Hanssen AD, Mabry T, Steckelberg J, Thompson R (2012) The Mayo prosthetic joint infection risk score: implication for surgical site infection reporting and risk stratification. Infect Control Hosp Epidemiol 33(8):774–781
26. Bratzler DW, Houck PM, Surgical Infection Prevention Guidelines Writers Workgroup.; American Academy of Orthopaedic Surgeons.; American Association of Critical Care Nurses et al (2004) Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. Clin Infect Dis 38(12):1706–1715
27. Suehiro T, Hirashita T, Araki S, Matsumata T, Tsutsumi S, Mochiki E, Kato H, Asao T, Kuwano H (2008) Prolonged antibiotic prophylaxis longer than 24 hours does not decrease surgical site infection after elective gastric and colorectal surgery. Hepatogastroenterology 55(86–87):1636–1639
28. Fonseca SN, Kunzle SR, Junqueira MJ, Nascimento RT, de Andrade JI, Levin AS (2006) Implementing 1-dose antibiotic prophylaxis for prevention of surgical site infection. Arch Surg 141(11):1109–1113
29. Krpata DM, Haskins IN, Phillips S, Prabhu AS, Rosenblatt S, Poulose BK, Rosen MJ (2017) Does preoperative bowel preparation reduce surgical site infections during elective ventral hernia repair? J Am Coll Surg 224(2):204–211
30. Atema JJ, de Vries FE, Boermeester MA (2016) Systematic review and meta-analysis of the repair of potentially contaminated and contaminated abdominal wall defects. Am J Surg 212(5):982–995. e1
31. Ramirez OM, Ruas E, Dellon AL (1990) "Components separation" method for closure of abdominal-wall defects: an anatomic and clinical study. Plast Reconstr Surg 86(3):519–526
32. Nguyen V, Shestak KC (2006) Separation of anatomic components method of abdominal wall reconstruction—clinical outcome analysis and an update of surgical modifications using the technique. Clin Plast Surg 33(2):247–257
33. Punjani R, Shaikh I, Soni V (2015) Component separation technique: an effective way of treating large ventral hernia. Indian J Surg 77(Suppl 3): 1476–1479
34. Malik K, Bowers SP, Smith CD, Asbun H, Preissler S (2009) A case series of laparoscopic components separation and rectus medialization with laparoscopic ventral hernia repair. J Laparoendosc Adv Surg Tech A 19(5):607–610
35. Burger JW, Luijendijk RW, Hop WC, Halm JA, Verdaasdonk EG, Jeekel J (2004) Long-term follow-up of a randomized controlled trial of suture versus mesh repair of incisional hernia. Ann Surg 240(4):578–583
36. Ventral Hernia Working Group, Breuing K, Butler CE, Ferzoco S, Franz M, Hultman CS, Kilbridge JF, Rosen M, Silverman RP, Vargo D (2010) Incisional ventral hernias: review of the literature and recommendations regarding the grading and technique of repair. Surgery 148(3):544–558
37. Turner PL, Park AE (2008) Laparoscopic repair of ventral incisional hernias: pros and cons. Surg Clin North Am 88(1):85–100
38. Wassenaar EB, Raymakers JT, Rakic S (2008) Impact of the mesh fixation technique on operation time in laparoscopic repair of ventral hernias. Hernia 12(1):23–25
39. Tsimoyiannis EC, Tsimogiannis KE, Pappas-Gogos G, Nikas K, Karfis E, Sioziou H (2008) Seroma and recurrence in laparoscopic ventral hernioplasty. JSLS 12(1):51–57
40. Klinge U, Conze J, Krones CJ, Schumpelick V (2005) Incisional hernia: open techniques. World J Surg 29(8):1066–1072
41. LeBlanc KA (2004) Laparoscopic incisional and ventral hernia repair: complications-how to avoid and handle. Hernia 8(4):323–331
42. Schumpelick V, Klinge U, Junge K, Stumpf M (2004) Incisional abdominal hernia: the open mesh repair. Langenbecks Arch Surg 389(1):1–5
43. Tagaya N, Mikami H, Aoki H, Kubota K (2004) Long-term complications of laparoscopic ventral

and incisional hernia repair. Surg Laparosc Endosc Percutan Tech 14(1):5–8
44. Ko JH, Wang EC, Salvay DM, Paul BC, Dumanian GA (2009) Abdominal wall reconstruction: lessons learned from 200 "components separation" procedures. Arch Surg 144(11):1047–1055
45. Patel KM, Nahabedian MY, Gatti M, Bhanot P (2012) Indications and outcomes following complex abdominal reconstruction with component separation combined with porcine acellular dermal matrix reinforcement. Ann Plast Surg 69(4):394–398
46. Jin J, Rosen MJ, Blatnik J, McGee MF, Williams CP, Marks J, Ponsky J (2007) Use of acellular dermal matrix for complicated ventral hernia repair: does technique affect outcomes? J Am Coll Surg 205(5):654–660
47. Voyles CR, Richardson JD, Bland KI, Tobin GR, Flint LM, Polk HC Jr (1981) Emergency abdominal wall reconstruction with polypropylene mesh: short-term benefits versus long-term complications. Ann Surg 194(2):219–223
48. Bauer JJ, Harris MT, Kreel I, Gelernt IM (1999) Twelve-year experience with expanded polytetrafluoroethylene in the repair of abdominal wall defects. Mt Sinai J Med 66(1):20–25
49. Miller SH, Rudolph R (1990) Healing in the irradiated wound. Clin Plast Surg 17(3):503–508
50. Mathes SJ, Alexander J (1996) Radiation injury. Surg Oncol Clin N Am 5(4):809–824
51. Sarikaya A, Record R, CC W, Tullius B, Badylak S, Ladisch M (2002) Antimicrobial activity associated with extracellular matrices. Tissue Eng 8(1):63–71
52. Harth KC, Broome AM, Jacobs MR, Blatnik JA, Zeinali F, Bajaksouzian S, Rosen MJ (2011) Bacterial clearance of biologic grafts used in hernia repair: an experimental study. Surg Endosc 25(7):2224–2229
53. Badylak SF, Coffey AC, Lantz GC, Tacker WA, Geddes LA (1994) Comparison of the resistance to infection of intestinal submucosa arterial autografts versus polytetrafluoroethylene arterial prostheses in a dog model. J Vasc Surg 19(3):465–472
54. Menon NG, Rodriguez ED, Byrnes CK, Girotto JA, Goldberg NH, Silverman RP (2003) Revascularization of human acellular dermis in full-thickness abdominal wall reconstruction in the rabbit model. Ann Plast Surg 50(5):523–527
55. Kim H, Bruen K, Vargo D (2006) Acellular dermal matrix in the management of high-risk abdominal wall defects. Am J Surg 192(6):705–709
56. Itani KM, Rosen M, Vargo D, Awad SS, Denoto G 3rd, Butler CE, RICH Study Group (2012) Prospective study of single-stage repair of contaminated hernias using a biologic porcine tissue matrix: the RICH Study. Surgery 152(3):498–505
57. Butler CE, Prieto VG (2004) Reduction of adhesions with composite AlloDerm/polypropylene mesh implants for abdominal wall reconstruction. Plast Reconstr Surg 114(2):464–473
58. Burns NK, Jaffari MV, Rios CN, Mathur AB, Butler CE (2010) Non-cross-linked porcine acellular dermal matrices for abdominal wall reconstruction. Plast Reconstr Surg 125(1):167–176
59. Patton JH Jr, Berry S, Kralovich KA (2007) Use of human acellular dermal matrix in complex and contaminated abdominal wall reconstructions. Am J Surg 193(3):360–363
60. Butler CE, Langstein HN, Kronowitz SJ (2005) Pelvic, abdominal, and chest wall reconstruction with AlloDerm in patients at increased risk for mesh-related complications. Plast Reconstr Surg 116(5):1263–1275
61. Blatnik J, Jin J, Rosen M (2008) Abdominal hernia repair with bridging acellular dermal matrix--an expensive hernia sac. Am J Surg 196(1):47–50
62. Boehmler JH 4th, Butler CE, Ensor J, Kronowitz SJ (2009) Outcomes of various techniques of abdominal fascia closure after TRAM flap breast reconstruction. Plast Reconstr Surg 123(3):773–781
63. Ko JH, Salvay DM, Paul BC, Wang EC, Dumanian GA (2009) Soft polypropylene mesh, but not cadaveric dermis, significantly improves outcomes in midline hernia repairs using the components separation technique. Plast Reconstr Surg 124(3):836–847
64. Sandor M, Xu H, Connor J, Lombardi J, Harper JR, Silverman RP, McQuillan DJ (2008) Host response to implanted porcine-derived biologic materials in a primate model of abdominal wall repair. Tissue Eng Part A 14(12):2021–2031
65. Gupta A, Zahriya K, Mullens PL, Salmassi S, Keshishian A (2006) Ventral herniorrhaphy: experience with two different biosynthetic mesh materials, Surgisis and Alloderm. Hernia 10(5):419–425
66. Espinosa-de-los-Monteros A, de la Torre JI, Marrero I, Andrades P, Davis MR, Vásconez LO (2007) Utilization of human cadaveric acellular dermis for abdominal hernia reconstruction. Ann Plast Surg 58(3):264–267
67. Carbonell AM, Cobb WS (2013) Safety of prosthetic mesh hernia repair in contaminated fields. Surg Clin North Am 93(5):1227–1239
68. Krpata DM, Stein SL, Eston M, Ermlich B, Blatnik JA, Novitsky YW, Rosen MJ (2013) Outcomes of simultaneous large complex abdominal wall reconstruction and enterocutaneous fistula takedown. Am J Surg 205(3):354–358
69. Diaz-Godoy A, García-Ureña MA, López-Monclús J, Vega Ruíz V, Melero Montes D, Erquinigo Agurto N (2011) Searching for the best polypropylene mesh to be used in bowel contamination. Hernia 15(2):173–179
70. Blatnik JA, Krpata DM, Jacobs MR, Gao Y, Novitsky YW, Rosen MJ (2012) In vivo analysis of the morphologic characteristics of synthetic mesh to resist MRSA adherence. J Gastrointest Surg 16(11):2139–2144
71. Heller L, Levin SL, Butler CE (2006) Management of abdominal wound dehiscence using vacuum assisted closure in patients with compromised healing. Am J Surg 191(2):165–172

72. Lin SJ, Butler CE (2010) Subtotal thigh flap and bioprosthetic mesh reconstruction for large, composite abdominal wall defects. Plast Reconstr Surg 125(4):1146–1156
73. Carlson GW, Elwood E, Losken A, Galloway JR (2000) The role of tissue expansion in abdominal wall reconstruction. Ann Plast Surg 44(2):147–153
74. Wooten KE, Ozturk CN, Ozturk C, Laub P, Aronoff N, Gurunluoglu R (2017) Role of tissue expansion in abdominal wall reconstruction: a systematic evidence-based review. J Plast Reconstr Aesthet Surg 70(6):741–751
75. Alleyne B, Ozturk CN, Rampazzo A, Johnson J, Gurunluoglu R (2017) Combined submuscular tissue expansion and anterior component separation technique for abdominal wall reconstruction: Long-term outcome analysis. J Plast Reconstr Aesthet Surg 70(6):752–758
76. Lannon DA, Ross GL, Addison PD, Novak CB, Lipa JE, Neligan PC (2011) Versatility of the proximally pedicled anterolateral thigh flap and its use in complex abdominal and pelvic reconstruction. Plast Reconstr Surg 127(2):677–688
77. Nahai F, Hill L, Hester TR (1979) Experiences with the tensor fascia lata flap. Plast Reconstr Surg 63(6):788–799
78. Williams JK, Carlson GW, deChalain T, Howell R, Coleman JJ (1998) Role of tensor fasciae latae in abdominal wall reconstruction. Plast Reconstr Surg 101(3):713–718
79. Silberman DA, Elliott LF 2nd, Hoffman GW (1985) Extended myofascial thigh flaps for repair of large full-thickness abdominal wall defects. Ann Plast Surg 5(2):170–176
80. De la Plaza R, Arroyo JM, Vasconez LO (1984) Upper transverse rectus abdominis flap: the flag flap. Ann Plast Surg 12(5):410–418
81. Gottlieb ME, Chandrasekhar B, Terz JJ, Sherman R (1986) Clinical applications of the extended deep inferior epigastric flap. Plast Reconstr Surg 78(6):782–792
82. Taylor GI, Corlett R, Boyd JB (1983) The extended deep inferior epigastric flap: a clinical technique. Plast Reconstr Surg 72(6):751–765
83. Mathes SJ, Bostwick J 3rd (1977) A rectus abdominis myocutaneous flap to reconstruct abdominal wall defects. Br J Plast Surg 30(4):282–283
84. Ninkovic M, Kronberger P, Harpf C, Rumer A, Anderl H (1998) Free innervated latissimus dorsi muscle flap for reconstruction of full-thickness abdominal wall defects. Plast Reconstr Surg 101(4):971–978
85. Cooper TM, Lewis N, Baldwin MA (1999) Free groin flap revisited. Plast Reconstr Surg 103(3):918–924
86. Gurunluoglu R, Rosen MJ (2017) Recipient vessels for microsurgical flaps to the abdomen: a systematic review. Microsurgery 37(6):707–716
87. Clement RW, Young VL, Marsh JKL (1984) Use of the greater omentum as a vascular supply for free-flap transfer. Plast Reconstr Surg 74(1):131–132
88. Baumann DP, Butler CE (2013) Soft tissue coverage in abdominal wall reconstruction. Surg Clin North Am 93(5):1199–1209
89. Earle AS, Feng LJ, Jordan RB (1990) Long saphenous vein grafts as an aid to microsurgical reconstruction of the trunk. J Reconstr Microsurg 6(2):165–169
90. Gurunluoglu R, Ghaznavi A, Krpata D, Zins J, Rosen MJ (2016) Arteriovenous loop graft in abdominal wall reconstruction using free tissue transfer. J Plast Reconstr Aesthet Surg 69(11):1513–1515
91. Blatnik JA, Krpata DM, Pesa NL, Will P, Harth KC, Novitsky YW, Rowbottom JR, Rosen MJ (2012) Predicting severe postoperative respiratory complications following abdominal wall reconstruction. Plast Reconstr Surg 130(4):836–841

Adipose Tissue for Wound Repair

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1 Introduction

In 1676 Antoine van Leeuwenhoek made history with a new microscopic lens powerful enough to view bacteria and confirm the suspicion that bacterial contamination strongly affected the wound healing [1]. He informed the Royal Society of London of his findings [2–4]. Greater understanding of the physiology of the healing of acute and chronic wounds permitted the scientist to implement new treatment technologies in the late twentieth century [5, 6].

Wound healing is a complex process whereby the skin repairs according to a specific sequence: haemostasis, inflammation, proliferation and remodelling. A set of biochemical events take place in a closely orchestrated cascade to repair the damage [7].

Chronic wounds are those that fail to heal normally [5, 8, 9]. Factors such as hypoxia of the tissues, infection, exudates or an excess in the level of inflammatory cytokines extend one or more of the states of wound healing making the wound chronic [10].

Chronic wounds cannot heal successfully and do not respond to standard treatment [9]. Co-morbid clinical conditions like ageing, poor tissue perfusion, malnutrition, unrelieved pressure to the surface of the wound, immune suppression, malignancy, infection, obesity and certain medication can also affect healing. Tissue reconstruction in patients with loss of fat, bone and muscle due to trauma, tumour resection or vascular damage is also challenging [11].

The social impact of this pathology is important. Chronic wounds affect around 120 of 100,000 people aged between 45 and 65 and 800 per 100,000 people over 75 [5, 8]. Over 4 million patients in the United States have chronic wounds, and nearly US$ 50 billion is spent every year on their treatment [12, 13]. When an acute wound cannot be closed normally due to extended cutaneous loss and traumatic tissue damage other procedures are attempted. One possibility is to wait for the granulation of the wound and perform a cutaneous graft.

The first recorded skin graft to cover a skin defect was performed in India over 2500 years ago for reconstruction of noses, which had been cut as a sentence of justice [14]. In 1869 Davis described the first pinch graft to cover the skin lost and in 1872 Thiersch described the split-thickness skin graft. Three years later Wolfe described the use of total skin grafting [15–18].

The cutaneous graft is used when other methods of reconstruction such as primary closure, secondary intention healing, local cutaneous flaps or free flaps cannot be used. Skin grafts do not have intact blood supplies and are therefore based on the growth of new blood vessels in the recipient bed.

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Flaps are taken from the donor site to the wound with their own vascularisation [19]. Flaps can be local, regional or distant. Pedicle flaps are restricted by their arc of rotation; surgeons must be trained in the management of tissues and have a good knowledge of angiosomes to insert them. An angiosome is a three-dimensional anatomical region that is irrigated by a single artery and can include skin, soft tissue and bone [20]. Free flaps require microsurgery with artery and vein anastomosis [19].

Regenerative medicine is a multidisciplinary science that has evolved with recent biotechnological advances [21].

Cell therapy can potentially improve wound healing conditions without major surgical procedures and donor-site morbidity, and can be applied to both acute and chronic wounds. Autologous adipose graft is often used to heal soft tissues in plastic surgery. Its efficacy and safety are widely accepted [22]. Small amounts of adipose tissue contain thousands of stem cells [23]. Mesenchymal stem cells (MSC) have proven to be beneficial and have safe immunomodulatory effects in several fields [24].

Adipose tissue is highly complex and is composed of mature adipocytes, pre-adipocytes, fibroblasts, vascular smooth muscle cells, endothelial cells, monocytes, macrophages, lymphocytes and other cell types, such as adipose stem cells (AdSC) and vascular endothelial cells [25]. Each cubic centimetre of human adipose tissue contains one million AdSC, one million endothelial cells and one to two millions other cells, such as blood-derived cells, per cubic centimetre of human adipose tissue. Adipose-derived stem cells play pivotal roles not only in adipose tissue homeostasis, but also in adipose wound healing. They may also help adipose tissue become adequate to ischemia [26].

Adipose-derived stem cells do not undergo apoptosis and therefore play key roles in the repair process. Vascular endothelial cells proliferate and appear to substantially contribute to adipogenesis and angiogenesis in response to ischemia. In contrast, adipocytes and vascular endothelial cells undergo apoptosis after exposure to severe ischemia. Macrophages digest the lipid material and replace all of the adipose tissue in the graft (replacement theory) [27].

Adipose-derived stem cells survive and are activated, contributing to adipogenesis and angiogenesis. Immediately after implantation, adipose stem cells live by diffusion of plasma and nutrients [28]. Neovascularisation occurs after 48 h [29]. The adipocytes die due to ischemia/hypoxia and are replaced by new adipocytes derived from the stem cells contained in the graft. Adipose-derived stem cells survive and contribute to adipose regeneration [26, 30].

In this chapter we show our clinical experience in treating patients with acute and chronic wounds by using autologous adipose tissue cells.

2 Surgical Technique

We define wounds as acute when the lesion is less than 30 days old and chronic when it is over than 30 days old [31]. Treatment of chronic wounds requires some additional steps.

The tumescent liposuction technique is a method that provides local anaesthesia to large volumes of subcutaneous fat. This technique involves subcutaneous infiltration of large volumes of crystalloid fluid called Klein's solution, which contains low concentrations of lidocaine (200–400 mL of physiological solution + 20 mL of lidocaine at 2% + 20 drops of adrenaline) and epinephrine (capillary constrictor).

Small incision is made in the skin, and a micro-cannula is inserted to perform suction-assisted aspiration of fat [32]. Suction is used to obtain 80 mL of autologous adipose graft from subcutaneous abdominal tissue. A 3 mm blunt cannula is connected to a 60 mL syringe with vacuum locks and the adipose graft is placed in sterile conical tubes. The graft is centrifuged for 3 min at 13331 RCF or G force (Hettich centrifuge, rotofix 32 A) [22, 33, 34]. This technique reduces bleeding. However, blood loss can still occur during tumescent liposuction, especially in patients with coagulation disorders [35].

The adipose tissue is injected within 1 cm of the lesion along the complete perimeter of the wound. Additional adipose tissue is applied to

the wound and covered with physiological solution dressing for 4–5 days. Chronic wounds require rigotomies, before the application of the adipose concentrate. A needle (16G) is inserted in the peripheral area of the lesion, and moved laterally to break up the fibrosis in the subcutaneous tissue [34].

Incisions are made 2–3 cm from the edge of the wound surrounding it with a network of tunnels radiating from four sides. In wounds larger than 10 × 10 cm, the tunnel network is made to radiate from six lateral points instead of four (Fig. 1).

Leftover centrifuged adipose tissue is refrigerated at 4 °C to be used on the same patient if again needed. Adipose tissue may be refrigerated for up to 2 weeks.

2.1 Patients

Patients were divided into groups according to the characteristics of wound bed.

Group I: Two patients with healthy skin and well-vascularised acute posttraumatic wound bed. A 17-year-old patient had a 3-day-old scrape wound on his middle finger wound. The surface was 8 cm^2 (2 cm × 4 cm). A 35-year-old patient had a 7-day-old injury of the first

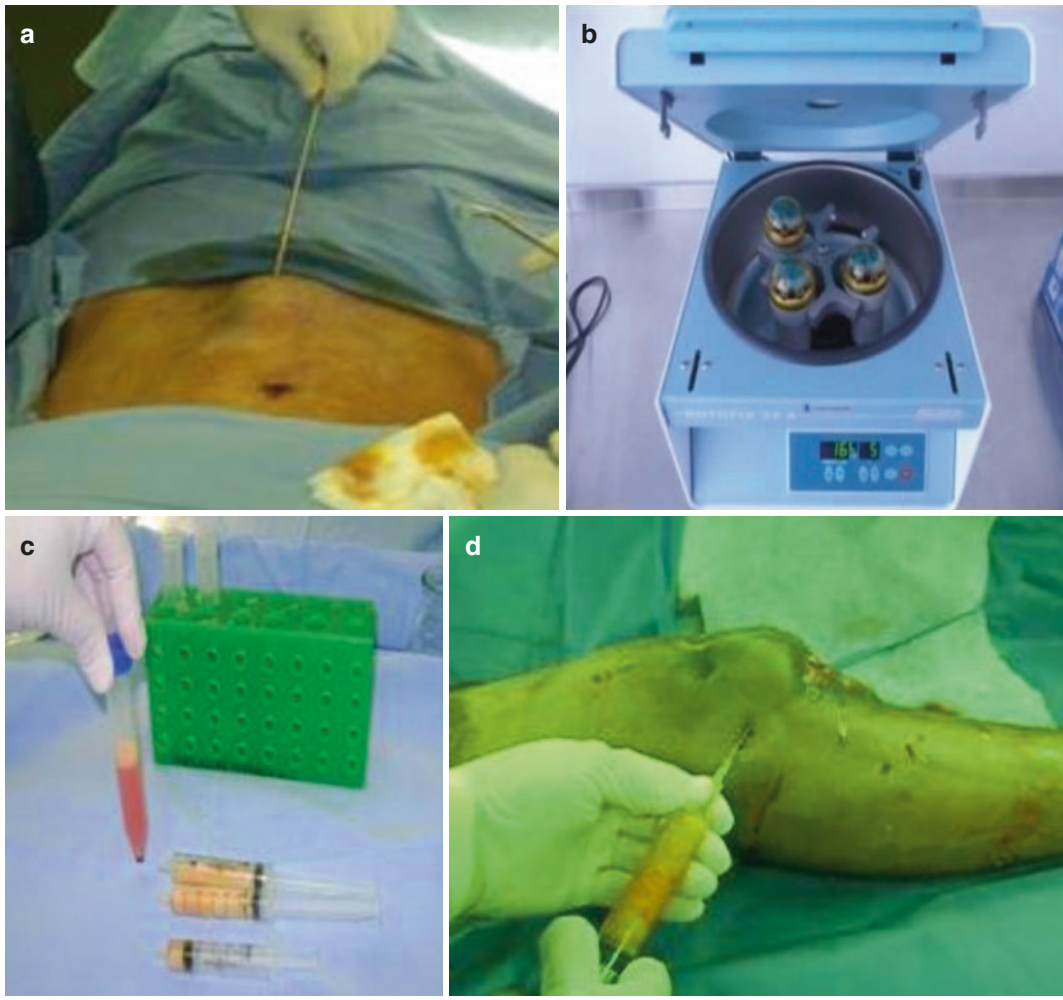

Fig. 1 (**a**) Aspiration of adipose tissue, (**b**) Centrifugation, (**c**) Isolation, (**d**) Injection of adipose tissue around the lesion

phalanx of the thumb with exposure of the extensor tendon. The wound surface area was 3 cm^2 (1.5 cm × 1.5 cm).

Wounds were an average of 5 days old. The average age of patients was 26 years old. The average wound surface area was 5 cm^2. Both patients required two applications.

Patients with chronic lesions were divided into four groups according to the characteristics of their wound bed:

Group II: Five patients with integumentary ischemia. One was a patient with haemophilia who had flap necrosis after a knee replacement. The second patient had necrosis in the anterolateral flap in the proximal tibia after tibial plate fracture. The third patient had a contusion after exposed limb fracture in a trauma accident. The last two patients had foot trauma with hallux amputation. All were men. The average age was 39.4 years old (21–49). The average age of wounds evolution was 107 days (36–180) and the average surface area was 12 cm^2 (6–16 cm^2). Four patients received two applications and one (with the exposed fracture) received four applications. The average of adipose applications was two.

Group III: Two patients with ulcers and chronic wounds with fibrous beds with exposed osteosynthesis plates. A 25-year-old patient had two ulcers. Wound surfaces were 15 cm^2 and 10.5 cm^2. Wounds were 270 days old. The patient also had a Henoch-Schonlein purpura.

A 39-year-old patient with a 1200-day-old wound exposing a forearm osteosynthesis plate. The average surface area was 48 cm^2 (12 cm × 4 cm).

The average age in the pair was 32 years old and the average evolution time was 720 days. Both patients needed three applications.

Group IV: Patients with vascular deficit in the recipient wound bed. Two patients with three lesions. A 77-year-old woman with Leriche disease had a 180-day-old ulcer on the leg, with a wound surface area of 50 cm^2 (10 cm × 5 cm). The second was a 67-year-old woman with a diabetic microangioplasty with two 70-day-old cutaneous lesions: one in the leg (7 cm × 4.5 cm) and the other on the talus (4 cm × 3 cm). The first woman received two applications and the second one three applications. The patients' average age was 72 years old. Average evolution time was 107 days and the average number of applications was two.

Group V: Two patients with bleeding disorders developed pelvis fistula after pseudotumour of the pelvis. These chronic fistulas are extremely difficult to heal since pelvic pseudotumour is difficult to control. The first patient was a 62-year-old man, who had haemophilia A without inhibitor. He had a bleeding fistula in the posterior region of the pelvis after a pseudotumour of the psoas iliacus muscle.

The second patient, a 43-year-old man, had von Willebrand disease. He had a pseudotumour in the iliac muscle, after extraction of iliac crest bone graft, to provide bone treatment of an exposed leg fracture.

In both patients pseudotumours were resected. Adipose tissue was extracted from abdominal tissues, centrifuged and injected around the fistula. Both patients received only one application.

2.2 Statistical Analysis

Frequencies and percentages were calculated to describe qualitative variables. Average and standard deviations were calculated for quantitative variables. Level of significance less than 5% will be used to reject the null hypothesis.

2.3 Results

All patients' wounds healed (acute and chronic) without any complications. A faster healing process was noted in wound beds without hypoxia ($p = 0.02$). Hypoxic wounds were larger (27.8 cm^2; SD: 18.6 cm^2) than those without hypoxia (9.4 cm^2; SD: 5.3 cm^2), and this difference was statistically significant ($p = 0.03$).

Healing took an average of 60 (30–90 days) days. Groups showed:

Group I: The vascular wound bed was not involved. Wounds were treated within an average of 5 days (3–7 days) and had an

Table 1 Groups results

Groups	Number	Average wound age (days)	Average healing time (days)	Average surface area of the wound (cm^2)	Injections
Group I	2	5	30	5	2
Group II	5	107	56	12	2
Group III	3	560	85	25	3
Group IV	3	107	90	36	3
Group V	2	246	40	2	1
Total	15	273	60	16	2.2

average surface area of 5 cm^2 (2.25–8 cm^2). Both wounds healed in 30 days after two applications.

Group II: Patients with integumentary vascular wound bed ischemia were treated at 107.2 days (6–260 days). Average wound surface area was 12 cm^2 (6–16 cm^2). Wound healed in an average of 56 days (30–90 days), and required an average of two (2–4) applications.

Group III: Patients had fibrosis of the vascular wound bed. Wounds were an average of 560 days old (240–1200 days), with an average surface area of 25 cm^2 (10.5–48 cm^2), healed after an average of 85 days (75–90 days), and required three treatment applications.

Group IV: Vascular ischemia wound bed was an average 107 days old (70–180 days). The wounds healed at 90 days and average surface area was 36 cm^2 (12–50 cm^2), and required an average of two applications (2–3).

Group V: Patients with pelvis fistula after bleeding disorders. This group of patients did not have ischemia of the tissues and had chronic fistula due to pseudotumours. It should be noted that fistulas are a gateway to infections.

Wounds were an average 246 days old (94–398 days), and healed in an average of 40 days. Average surface area was 2 cm^2 (1.8–2.2 cm^2), and required one treatment application (Table 1).

3 Discussion

This study shows that the injection of centrifuged subcutaneous adipose tissue obtained by aspiration is effective for the healing of acute and chronic wounds. The properties of AdSC are affected by changing environmental conditions such as hypoxia. Hypoxia of the recipient bed plays an important role in the healing time of the wounds and is known to stimulate the pro-angiogenic effects of AdSC. In our study, patients with hypoxic wound bed showed longer healing time. The difference was statistically significant ($p < 0.002$).

The wounds of patients in group I (without hypoxia) healed in an average of 30 days. Those of patients in group V (fistulas related to bleeding disorders) healed in an average of 40 days. The wounds of patients in group II (with hypoxia) healed in an average of 56 days, almost double the average healing time compared to non-hypoxic acute injury tissue. In group IV, wounds with scarring surgical fibrosis or circulatory disorders of the wound bed had a healing time of 90 days, triple the healing time for group I (acute injury). Due to the small number of patients studied, the significance of these differences could not be calculated.

Roemeling-Van Rhijn [36] demonstrated, in cell culture, that hypoxia affected the inflammatory phase, due to cellular recruitment, secretion of immunomodulatory molecules and programmed death.

Furthermore, increased secretion of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in the hypoxic environment strongly suggests that the proliferative wound-healing stage is affected by increase in neo-angiogenesis independently of the MSC source.

Cequeira et al. [37] report that signalling inhibition might depend on both the O_2 concentration and the cell type since transforming growth

factor (TGF-b1 and TGF-b3) must be balanced to achieve skin regeneration and avoid fibrosis and scarring.

Yoshimura et al. [28] showed that implanted adipose cells receive nutrition and plasma by diffusion. Chan et al. [29] show that the cells begin to nourish themselves from the receptor bed 48 h after implantation.

Eto et al. [30] showed in an immunohistochemical analysis of the viability of adipose tissue graft obtained from mice that only the cells in the periphery of the tissue remain alive and are responsible for tissue regeneration. Suga et al. [26] have shown that when free fat graft is used in wounds, stem cells survive and adipose cells die. A previous study by Suga et al. had shown that human adipose-derived stem/progenitor cells remained viable after 1 day of storage at 4 °C, but the number of adipose-derived stem/progenitor cells was significantly reduced after more than 2 days of storage. This may explain why the cells live on the surface of the wound.

All the patients regenerated the normal skin but without hair follicles. This is probably because hair follicles and the epidermis have different embryological origins [38]. The application of autologous adipose graft is a simple and safe treatment for wound repair and is also effective in patients with coagulation disorders.

Patients with coagulation disorders did not have any complications during extraction or injection. Normal healing requires adequate haemostatic function. Coagulation disorders affect the natural evolution of wounds [39].

Cell therapy using autologous adipose graft promotes and accelerates the wound-healing process by reducing the time needed for host cells to invade wound tissue and synthesise new skin.

Conclusions

The application of autologous adipose graft is a simple and safe treatment for wound repair and is also affective in patients with coagulation disorders. Patients' wound heals, and they are able to perform their daily activities, improving their quality of life.

References

1. Porter JR (1976) Antony van Leeuwenhoek: tercentenary of his discovery of bacteria. Bacteriol Rev 40(2):260–269
2. van Leeuwenhoek A (1684) An abstract of a letter from Mr. Anthony Leevvenhoek at delft, dated Sep. 17, 1683, containing some microscopical observations, about animals in the scurf of the teeth, the substance Call'd worms in the nose, the cuticula consisting of scales. Philos Trans (1683–1775) 14(155–166):568–574
3. van Leeuwenhoek A (1700) Part of a letter from Mr Antony van Leeuwenhoek, concerning the worms in sheeps livers, gnats, and animalcula in the excrements of frogs. Philos Trans (1683–1775) 22(260–276):509–508
4. van Leeuwenhoek A (1702) Part of a letter from Mr Antony van Leeuwenhoek, F. R. S. Concerning green weeds growing in water, and some animalcula found about them. Philos Trans (1683–1775) 23(277–288):1304–1311
5. Robson MC, Steed DL, Franz MG (2001) Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg 38:72–140
6. Szycher M, Lee SJ (1992) Modern wound dressings: a systematic approach to wound healing. J Biomater Appl 7:142–213
7. You HJ, Han SK (2014) Cell therapy for wound healing. J Korean Med Sci 29:311–319
8. Natarajan S, Williamson D, Stiltz AJ et al (2000) Advances in wound care and healing technology. Am J Clin Dermatol 1:269–275
9. Vanwijck R (2001) Surgical biology of wound healing. Bull Mem Acad R Med Belg 115:175–184
10. Velnar T, Baileyt T, Smrkolj V (2009) The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res 37:1528–1542
11. Maxson S, Lopez EA, Yoo D, Danlkovitch-Miagkova A, Leroux MA (2012) Concise review: role of mesenchymal stem cells in wound repair. Stem Cells Transl Med 1:142–149
12. Frykberg RG, Banks J (2015) Challenges in the treatment of chronic wounds. Adv Wound Care (New Rochelle) 4:560–582
13. Fife CE, Carter MJ (2012) Wound care outcomes and associated cost among patients treated in US outpatient wound centers: data from the US Wound Registry. Wounds 24:10–17
14. Hauben DJ, Baruchin A, Mahler A (1982) On the history of the free skin graft. Ann Plast Surg 9(3):242–245
15. Davis JS (1994) The story of plastic surgery. Ann Surg 113:641
16. Pollack SV (1982) Wound healing: a review. IV. Systemic medications affecting wound healing. J Dermatol Surg Oncol 8(8):667–672
17. Brady JG, Grande DJ, Katz AE (1992) The purse-string suture in facial reconstruction. J Dermatol Surg Oncol 18(9):812–816

18. Hill TG (1987) Contouring of donor skin in full-thickness skin grafting. J Dermatol Surg Oncol 13(8):883–888
19. Guyuron B, Eriksson E, Persing JA, Chung KC, Disa J, Gosain A (2009) In: Kinney BM, Rubin JP (eds) Plastic surgery: indications and practice. Saunders Elsevier, Edinburgh
20. Houseman ND, Taylor GI, Pan WR (2000) The angiosomes of the head and neck: anatomic study and clinical applications. Plast Reconstr Surg 105(7):2287–2313
21. Gimble JM, Katz AJ, Bunnell BA (2007) Adipose derived stem cells for regenerative medicine. Circ Res 100:1249–1260
22. Kurita M, Matsumoto D, Shigeura T, Sato K, Gonda K, Harii K, Yoshimura K (2008) Influences of centrifugation on cells and tissues in liposuction aspirates: optimized centrifugation for lipotransfer and cell isolation. Plast Reconstr Surg 121:1033–1041
23. Orbay H, Tobita M, Mizuno H (2012) Mesenchymal stem cells isolated from adipose and other tissues: basic biological properties and clinical applications. Stem Cells Int 2012:461718
24. Grada A (2016) Novel stem cell therapies for applications to wound healing and tissue repair. Surg Technol Int XXIX:29–37
25. Caspar-Bauguil S, Cousin B, Galinier A, Segafredo C, Nibbelink M, André M, Casteilla L, Pénicaud L (2005) Adipose tissues as an ancestral immune organ: site-specific change in obesity. FEBS Lett 579(17):3487–3492
26. Suga H, Eto H, Aoi N, Kato H, Araki J, Doi H, Higashino T, Yoshimura K (2010) Adipose tissue remodeling under ischemia: death of adipocytes and activation of stem/ progenitor cells. Plast Reconstr Surg 126(6):1911–1923
27. Billings E, May JW (1989) Historical review and present status of free fat graft autotransplantation in plastic and reconstructive surgery. Plast Reconstr Surg 83(2):368–381
28. Yoshimura K, Sato K, Aoi N (2008) Cell-assisted lipotransfer for cosmetic breast augmentation: supportive use of adipose-derived stem/stromal cells. Aesthetic Plast Surg 32(1):48–55
29. Chan CW, McCulley SJ, Macmillan RD (2008) Autologous fat transfer—a review of the literature with a focus on breast cancer surgery. J Plast Reconstr Aesthet Surg 61(12):1438–1448
30. Eto H, Kato H, Suga H, Aoi N, Doi K, Kuno S, Yoshimura K (2012) The fate of adipocytes after nonvascularized fat grafting: evidence of early death and replacement of adipocytes. Plast Reconstr Surg 129(5):1081–1092
31. Braddock M (2005) Euro conference on tissue repair and ulcer/wound healing: molecular mechanisms, therapeutic targets and future directions. Expert Opin Investig Drugs 14(6):743–749
32. Venkataram J (2008) Tumescent liposuction: a review. J Cutan Aesthet Surg 1(2):49–57
33. Galiè M, Pignatti M, Scambi I, Sbarbati A, Rigotti G (2008) Comparison of different centrifugation protocols for the best yield of adipose-derived stromal cells from lipo - aspirates. Plast Reconstr Surg 122:e233–e234
34. Butala P, Sultan SM, Davidson EH, Crawford JL, Szpalski C, Knobel D, Allen RJ, Saadeh PB, Warren SM, Coleman S, Hazen A (2010) Augmentation of fat graft survival with progenitor cell mobilization. Plast Reconstr Surg 125(6):12
35. Klein JA (1987) The tumescent technique for liposuction surgery. Am J Cosmet Surg 4:263–267
36. Roemeling-van Rhijn M, Mensah FK, Korevaar SS, Leijs MJ, van Osch GJ, Ijzermans JN, Betjes MG, Baan CC, Weimar W, Hoogduijn MJ (2013) Effects of hypoxia on the immunomodulatory properties of adipose tissue-derived mesenchymal stem cells. Front Immunol 4:203
37. Cerqueira M, Pirraco R, Marques A (2016) Stem cells in skin wound healing: are we there yet? Adv Wound Care 5(4):164–175
38. Cerqueira MT, Pirraco RP, Santos TC, Rodrigues DB, Frias AM, Martins AR, Reis RL, Marques AP (2013) Human adipose stem cells cell sheet constructs impact epidermal morphogenesis in full-thickness excisional wounds. Biomacromolecules 14:3997–4008
39. Hoffman M, Monroe DM (2010) Wound healing in haemophilia-breaking the vicious cycle. Haemophilia 16:13–18

Prevascularized Stem Cell Sheet for Full-Thickness Skin Wound Repair

Daniel Radke, Lei Chen, Shaohai Qi, and Feng Zhao

1 Introduction

There are approximately 15,000–20,000 hospitalizations per year for acute burn injuries in the USA, as recorded by the American Burn Association [1]. Burn injuries are one of the leading causes of full-thickness skin wounds, for which the gold standard of treatment remains transplantation of an autologous full-thickness graft (FTG) or skin flap [2]. Alternative approaches and skin substitutes have been developed for burn treatment, but autologous FTG demonstrates better immunological acceptance and better match skin color and texture. Hence, it has been preferred as a safe and economically viable grafting method. However, the limited supply of donor skin and unavoidable donor-site injury restrict their ability to treat extensive and severe wounds.

One promising alternative is the application of an autologous split-thickness skin graft (STSG), in which only the epidermis and a portion of the dermis are harvested rather than the full thickness. STSG still requires re-epithelialization and ongoing wound therapy until the donor site is repaired, but these donor sites may be harvested repeatedly to resurface large wounds. STSG can be used under unfavorable conditions where FTG would fail, such as a recipient's wound having moderate infection or less vasculature [3]. However, STSG is more fragile than FTG and can contract significantly during the healing process, leading to poor cosmetic outcome, physical disability, and reduced pliability [4]. These disadvantages may be overcome by combining STSG with engineered dermal substitutes, but the insufficient blood supply at early stages of the transplantation causes these grafts to experience relatively long hypoxic and ischemic periods after surgery and suffer from degeneration and necrosis [5]. While bioengineered products such as natural substitutes—human allograft, Oasis wound matrix®; synthetic substitutes—Biobrane™; and permanent skin substitutes—Epicel®, and Integra® have been commercially available for years, major problems still exist with material sources, manufacturing techniques, material compatibility, and therapeutic effects associated with these skin grafts [6].

Cell-based therapies, especially those using stem cells, for improving the survival and therapeutic effect of skin grafts have emerged as a new approach in recent years [7]. Mesenchymal stem cells (MSCs) exhibit excellent potential for accelerating wound healing due to their self-renewal ability, secretion of paracrine factors, and ability to differentiate into different cell lin-

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eages [8]. Numerous studies reported positive results when utilizing MSCs for various wound regeneration applications [9–12]. Local delivery of rat adipose-derived MSCs to an excisional wound healing model showed enhanced epithelialization and granulation tissue deposition [13]. Human MSCs (hMSCs) grafted in impaired healing diabetic mice significantly improved healing by recruiting large amounts of host mouse MSCs to the wound bed, producing factors such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor receptor-α (PDGFR-α), which can stimulate angiogenesis [14]. Compared to traditional cell delivery strategies, such as cell injection or spraying, cell sheet engineering techniques enhance the cell delivery efficacy to injured tissue [15]. The cell sheet preserves cell-cell junctions, extracellular matrix (ECM), and cell-matrix connections, thus restricting the cells to the wound bed [16]. The rat adipose-derived MSC sheet demonstrated beneficial effects in diabetic wound healing when combined with artificial skin, where the cells accelerated wound healing directly by functioning as pericytes and indirectly through the secretion of paracrine signaling factors [17].

The primary factor that influences the quality of a transplanted STSG is the sufficient supply of blood to the skin graft [18]. During the first 48 h of transplantation the graft is engorged by plasmatic fluid, and a poorly vascularized bed hinders plasmatic diffusion [18]. In addition, newly formed blood vessels in the wound bed can deliver oxygen, nutrients, and essential growth factors to hypoxic and ischemic STSGs at early stages after placement [19]. A cell sheet with preformed microvessels may enhance angiogenesis as well as further improve tissue function by supporting cell survival and accelerating the integration of the graft with host tissues [20, 21].

Among all cell types used for angiogenesis and neovascularization, the role of endothelial cells (ECs) has been studied extensively. ECs are able to initiate postnatal neovascularization and express a series of growth factors and cytokines including platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β, granulocyte-macrophage colony-stimulating factor (GM-CSF), and interleukin-1 (IL-1), IL-5, and IL-6, which are beneficial for wound repair [22]. When co-cultured with hMSCs, the formation of a microvessel network is promoted due to the supporting and stabilizing functions of hMSCs [23]. Cross talk between ECs and hMSCs upregulates the expression of angiogenic genes such as von Willebrand factor (vWf), platelet/endothelial cell adhesion molecule-1 (PECAM-1), and cadherin 5 [24]. Furthermore, EC/hMSC cross talk has been shown to improve angiogenesis via synergistic effects [25]. Therefore, an hMSC cell sheet (HCS) co-cultured with ECs may facilitate neovasculature formation. It is anticipated that the prevascularized hMSC sheet (PHCS) will improve skin graft survival when combined with a STSG for full-thickness skin wound repair.

2 Technique

For cell sheet culture, the passage of three to five hMSCs were seeded on cover glasses coated with 20 μg/mL collagen I, and cultured under a hypoxic condition (2% O_2) for 4 weeks to obtain HCS. The HCS with a diameter of 2 cm was harvested by gently peeling the cell layers off the cover glass. To fabricate PHCS, ECs were seeded on top of hMSC sheets and cultured under a normoxic condition (20% O_2) for 1 week to obtain PHCS in endothelial cell growth medium (Fig. 1) [26].

Due to the importance of the immune system in wound healing [27, 28], an immune competent rat model was chosen for the purpose of simulating healing processes in human wound repair [29]. To begin with, Sprague-Dawley (SD) rats were anesthetized using gas anesthesia. The back of the rat was shaved and sterilized using alcohol wipes. A small part of SD rat's dorsum was cut off, shaping a round full-thickness wound with a diameter of 20 mm. The STSG was made by the removal of panniculus carnosus as well as deep partial dermis from the skin excision (Fig. 2) [26]. For the purpose of secretion drainage, fenestrations with equal spacing were created on STSG with a sharp-tipped scalpel. Three layers of cell sheets were grafted on the wound and then covered by STSG. The STSG transplantation

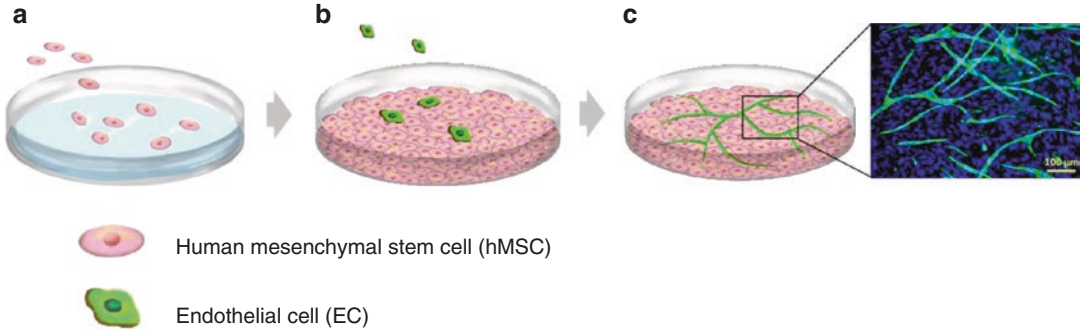

Fig. 1 Fabrication of PHCS via hypoxic hMSC culture (**a**), normoxic seeding of ECs on mature HCS (**b**), and development of microvasculature visualized with immunofluorescence (**c**)

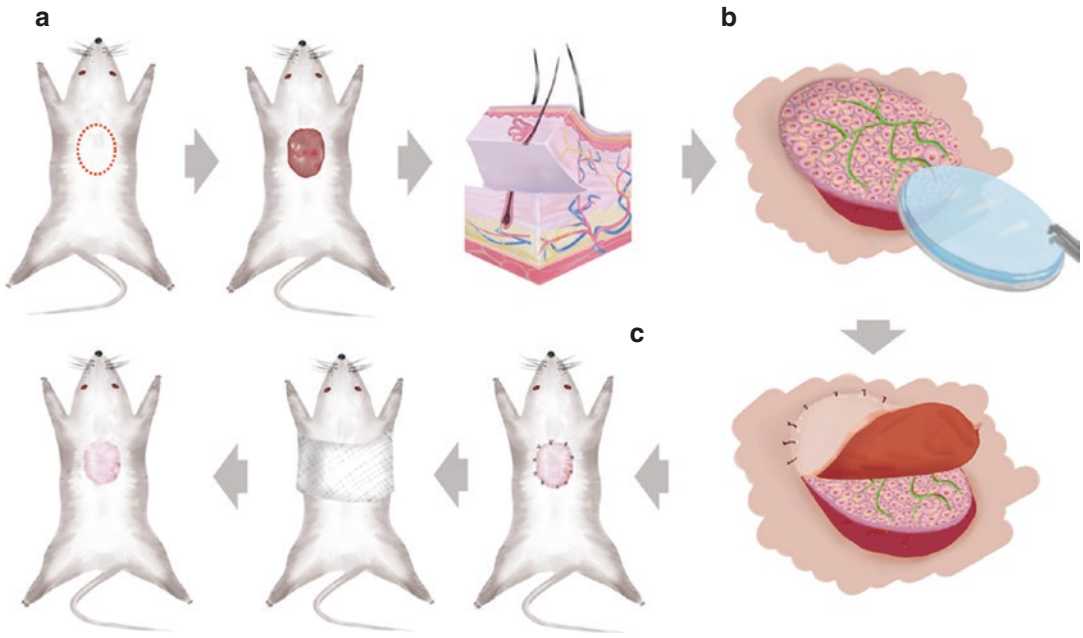

Fig. 2 Illustration of procedure including defect generation (**a**), PHCS grafting (**b**), and wound healing (**c**)

without cell sheets was served as control. The grafts as well as the adjacent wound margin were sutured with interrupted stitches, and then secured with a padded bolster (proper pressure provision and scratch prevention). The bolsters were changed on postoperative day 3 and removed on day 7. The implant contraction was observed over 28 days post-surgery. The relative size change of grafts was evaluated by gravitational planimetry and expressed as a percentage of the remaining skin graft size to its original wound size.

3 Discussion

The survival of skin grafts after implantation relies mainly on nutrient diffusion from the wound bed at the early stage of implantation [30, 31]. Engineered PHCSs transplanted in combination with an autologous STSG were found to promote vascularization and healing in a full-thickness wound model. The PHCS-STSG implants displayed no hemorrhage or necrosis after 3 days, preserved most skin appendages and connective tissues, and exhibited a controlled and

resolved inflammatory response by 14 days without incurring the fibrosis associated with prolonged immune response [32]. This resulted in significantly less contraction than STSG alone and morphology closer to the surrounding normal skin, indicating better performance in a sequence of overlapping wound-healing events including inflammatory response, neovascularization, proliferation, collagen deposition, and remodeling over the course of 28 days [33].

Hair follicles, skin glands, and dermal collagen are important components for reconstitution of skin structure and function. The number of skin appendages, especially sebaceous glands, significantly decreases in STSG transplantations. Simultaneously, collagen fibers degrade or undergo necrosis with slight hemorrhage. These phenomena are probably caused by surgical operation and insufficient blood supply [34]. Histological analysis after 28-day in vivo trials showed that PHCS significantly improved STSG performance in repairing full-thickness skin wounds, better maintaining the graft structure and components (hair follicles and sebaceous glands) from the beginning and remodeling collagen to integrate with host tissue without the occurrence of necrosis, hemorrhage, or fibrosis (Fig. 3) [26].

It has been highlighted that the optimal composition of skin substitute scaffolds should mimic that of normal skin in order to enhance clinical effectiveness [35]. Both HCS and PHCS contain large amounts of ECM molecules including collagen I, collagen III, collagen IV, elastin, fibronectin, and laminin [36]. The presence of these preexisting ECM components can aid in accelerating the regeneration of dermal tissue. Results from the in vivo evaluation suggest that the embedded cell sheets, especially the PHCS, may have also mitigated cellular processes associated with graft fibrosis and contraction.

Abnormal keratinocyte differentiation and their abnormal cytokine secretion are two factors demonstrated to contribute to tissue fibrosis and contraction by activating fibroblasts [37–39]. The epidermis of PHCS-STSG-implanted rats maintained its original appearance, and epidermal ulcers were soon covered with new, thin epidermis within 3 days in HCS-STSG rats, while the group with STSG alone showed less recovery. The thick-

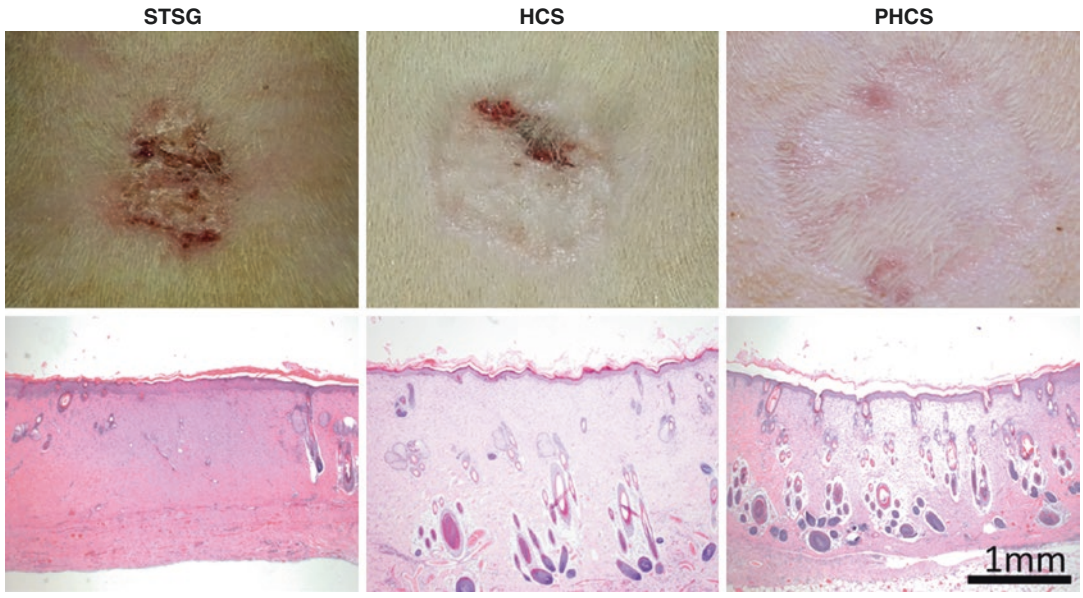

Fig. 3 Results of 28-day in vivo evaluation of capacity for full-thickness wound repair in immune-competent Sprague-Dawley rats of split-thickness skin graft alone (STSG), STSG with hMSC cell sheet (HCS), and STSG with prevascularized hMSC cell sheet (PHCS)

ness of the neoepidermis in the STSG group increased significantly over time due to epidermal hyperplasia, a common response of dermal wounds characterized by overdevelopment of the epithelial cell layer. In contrast, HCS and PHCS epidermis maintained a below-trend change and quickly returned to their normal morphology. Since the epidermis is the outmost layer of skin, a quick recovery of this layer can provide a barrier to infection from environmental pathogens and maintain water homeostasis inside the skin. However, excess epidermal hyperplasia results in dermal fibrosis and interferes with normal skin function [40]. Interestingly, it was found that the therapeutic result of different grafts (STSG alone, STSG with HSC, or STSG with PHSC) correlated with the growth factor levels in the preceding cell sheets, suggesting that the paracrine activity may dominate the healing process in the full-thickness wound model. PHCS were found to contain about 2.6 times more angiopoietin 1, a growth factor recognized in promoting vessel stabilization and tightness, compared to HCS.

Conclusion

STSG transplants are frequently used when simple nonsurgical wound care is not applicable; however STSG commonly has problems with graft necrosis due to inadequate nutrient supply at the early stages of wound healing, resulting in the formation of scar tissue that does not function the same as normal skin [33, 41, 42]. These problems can be alleviated through the use of hMSCs to promote early vascularization and mediate the wound-healing process. An in vivo evaluation with immune-competent animals demonstrated that the use of prevascularized stem cell sheets enhanced STSG by greatly reducing skin contraction during healing, preserving skin appendages, increasing the number and area of microvessels, mitigating inflammatory reactions, and resulting in a morphology that more closely resembled normal skin. Both HCS and PHCS combined with STSG exhibited markedly enhanced therapeutic value in the rat model and may be useful for facilitating the healing of full-thickness wounds in humans.

References

1. Mendez-Eastman S (2005) Burn injuries. Plast Surg Nurs 25:133–139
2. Dreifke MB, Jayasuriya AA, Jayasuriya AC (2015) Current wound healing procedures and potential care. Mater Sci Eng C 48:651–662
3. Shimizu R, Kishi K (2012) Skin graft. Plast Surg Int 2012:5
4. Wood FM (2014) Skin regeneration: the complexities of translation into clinical practise. Int J Biochem Cell Biol 56:133–140
5. Zografou A, Papadopoulos O, Tsigris C, Kavantzas N, Michalopoulos E, Chatzistamatiou T, Papassavas A, Stavropoulou-Gioka C, Dontas I, Perrea D (2013) Autologous transplantation of adipose-derived stem cells enhances skin graft survival and wound healing in diabetic rats. Ann Plast Surg 71:225–232
6. Halim AS, Khoo TL, Shah JMY (2010) Biologic and synthetic skin substitutes: an overview. Indian J Plast Surg 43:23
7. Jackson WM, Nesti LJ, Tuan RS (2012) Mesenchymal stem cell therapy for attenuation of scar formation during wound healing. Stem Cell Res Ther 3:20
8. Caplan AI (2007) Adult mesenchymal stem cells for tissue engineering versus regenerative medicine. J Cell Physiol 213:341–347
9. Balaji S, Keswani SG, Crombleholme TM (2012) The role of mesenchymal stem cells in the regenerative wound healing phenotype. Adv Wound Care 1:159–165
10. Deng W, Han Q, Liao L, Li C, Ge W, Zhao Z, You S, Deng H, Murad F, Zhao RC (2005) Engrafted bone marrow-derived Flk-1+ mesenchymal stem cells regenerate skin tissue. Tissue Eng 11:110–119
11. Fu X, Fang L, Li X, Cheng B, Sheng Z (2006) Enhanced wound-healing quality with bone marrow mesenchymal stem cells autografting after skin injury. Wound Repair Regen 14:325–335
12. Némos C, Basciano L, Dalloul A (2012) Biological effects and potential applications of mesenchymal stem cell culture under low oxygen pressure. Pathol Biol 60:193–198
13. Nie C, Yang D, Xu J, Si Z, Jin X, Zhang J (2011) Locally administered adipose-derived stem cells accelerate wound healing through differentiation and vasculogenesis. Cell Transplant 20:205–216
14. Shin L, Peterson DA (2013) Human mesenchymal stem cell grafts enhance normal and impaired wound healing by recruiting existing endogenous tissue stem/progenitor cells. Stem Cells Transl Med 2:33–42
15. Yang J, Yamato M, Nishida K, Ohki T, Kanzaki M, Sekine H, Shimizu T, Okano T (2006) Cell delivery in regenerative medicine: the cell sheet engineering approach. J Control Release 116:193–203
16. Yamato M, Utsumi M, Kushida A, Konno C, Kikuchi A, Okano T (2001) Thermo-responsive culture dishes allow the intact harvest of multilayered keratinocyte sheets without dispase by reducing temperature. Tissue Eng 7:473–480

17. Kato Y, Iwata T, Morikawa S, Yamato M, Okano T, Uchigata Y (2015) Allogeneic transplantation of an adipose-derived stem cell sheet combined with artificial skin accelerates wound healing in a rat wound model of type 2 diabetes and obesity. Diabetes 64:2723–2734
18. Seyhan T (2011) Split-thickness skin grafts. INTECH Open Access Publisher, London
19. Chua AWC, Khoo YC, Tan BK, Tan KC, Foo CL, Chong SJ (2016) Skin tissue engineering advances in severe burns: review and therapeutic applications. Burns Trauma 4:3
20. Kang Y, Ren L, Yang Y (2014) Engineering vascularized bone grafts by integrating a biomimetic periosteum and β-TCP scaffold. ACS Appl Mater Interfaces 6:9622–9633
21. Lesman A, Habib M, Caspi O, Gepstein A, Arbel G, Levenberg S, Gepstein L (2009) Transplantation of a tissue-engineered human vascularized cardiac muscle. Tissue Eng Part A 16:115–125
22. Mantovani A, Bussolino F, Dejana E (1992) Cytokine regulation of endothelial cell function. FASEB J 6:2591–2599
23. Mendes LF, Pirraco RP, Szymczyk W, Frias AM, Santos TC, Reis RL, Marques AP (2012) Perivascular-like cells contribute to the stability of the vascular network of osteogenic tissue formed from cell sheet-based constructs. PLoS One 7:e41051
24. Xue Y, Xing Z, Bolstad AI, Van Dyke TE, Mustafa K (2013) Co-culture of human bone marrow stromal cells with endothelial cells alters gene expression profiles. Int J Artif Organs 36:650
25. Li Q, Wang Z (2013) Influence of mesenchymal stem cells with endothelial progenitor cells in co-culture on osteogenesis and angiogenesis: an in vitro study. Arch Med Res 44:504–513
26. Chen L, Xing Q, Zhai Q, Tahtinen M, Zhou F, Chen L, Xu Y, Qi S, Zhao F (2017) Pre-vascularization enhances therapeutic effects of human mesenchymal stem cell sheets in full thickness skin wound repair. Theranostics 7:117
27. Portou M, Baker D, Abraham D, Tsui J (2015) The innate immune system, toll-like receptors and dermal wound healing: a review. Vasc Pharmacol 71:31–36
28. Sun BK, Siprashvili Z, Khavari PA (2014) Advances in skin grafting and treatment of cutaneous wounds. Science 346:941–945
29. Rose LF, Wu JC, Carlsson AH, Tucker DI, Leung KP, Chan RK (2015) Recipient wound bed characteristics affect scarring and skin graft contraction. Wound Repair Regen 23:287–296
30. Horch R, Bannasch H, Stark G (2001) Transplantation of cultured autologous keratinocytes in fibrin sealant biomatrix to resurface chronic wounds. Transplant Proc 33:642–644
31. Shokrollahi K, Azzopardi E, Boyce D, Dickson W, Laing J, Whitaker I (2013) Application of topical negative pressure (VAC) to split thickness skin grafts: a structured evidence-based review. Plast Reconstr Surg 132:154–155
32. Garbuzenko E, Nagler A, Pickholtz D, Gillery P, Reich R, Maquart FX, Levi-Schaffer F (2002) Human mast cells stimulate fibroblast proliferation, collagen synthesis and lattice contraction: a direct role for mast cells in skin fibrosis. Clin Exp Allergy 32:237–246
33. Martin P (1997) Wound healing—aiming for perfect skin regeneration. Science 276:75–81
34. Manske RC (2006) Postsurgical orthopedic sports rehabilitation: knee & shoulder. Elsevier Health Sciences, Philadelphia
35. Markeson D, Pleat JM, Sharpe JR, Harris AL, Seifalian AM, Watt SM (2015) Scarring, stem cells, scaffolds and skin repair. J Tissue Eng Regen Med 9:649–668
36. Zhang L, Xing Q, Qian Z, Tahtinen M, Zhang Z, Shearier E, Qi S, Zhao F (2016) Hypoxia created human mesenchymal stem cell sheet for prevascularized 3D tissue construction. Adv Healthc Mater 5:342–352
37. Amjad SB, Carachi R, Edward M (2007) Keratinocyte regulation of TGF-β and connective tissue growth factor expression: a role in suppression of scar tissue formation. Wound Repair Regen 15:748–755
38. Li B, Gao C, Diao JS, Wang DL, Chu FF, Li Y, Wang G, Guo SZ, Xia W (2016) Aberrant notch signalling contributes to hypertrophic scar formation by modulating the phenotype of keratinocytes. Exp Dermatol 25:137–142
39. Peura M, Siltanen A, Saarinen I, Soots A, Bizik J, Vuola J, Harjula A, Kankuri E (2010) Paracrine factors from fibroblast aggregates in a fibrin-matrix carrier enhance keratinocyte viability and migration. J Biomed Mater Res A 95:658–664
40. Neary R, Watson CJ, Baugh JA (2015) Epigenetics and the overhealing wound: the role of DNA methylation in fibrosis. Fibrogenesis Tissue Repair 8:18
41. MacFarlane DF (2006) Current techniques in skin grafting. Adv Dermatol 22:125–138
42. Ratner D (2003) Skin grafting. Semin Cutan Med Surg 22:295–305

Part VII

Quality of Life

Cultural Adaptation and Validation of the Freiburg Life Quality Assessment-Wound Module to Brazilian Portuguese

Elaine Aparecida Rocha Domingues, José Vitor da Silva, Maiume Roana Ferreira de Carvalho, Uiara Aline de Oliveira Kaizer, Tamy Ananda da Silva, and Thaís Mariane Soares

1 History of the Term Quality of Life

The quality of life began to be studied in the early 1950s, but it was linked to the work dimension, initially recognized as Quality of Work Life (QWL). The studies began in England and were carried out by Eric Trist and his collaborators at the Tavistock Institute, in which they intended to analyze the individual-work-organization relationship. During this research, a socio-technical approach to work organization was developed, based on the work satisfaction and concerning to it [1].

The term quality of life (QOL) was first mentioned in 1920 by Pigou in a book on economics and well-being, in which he linked the quality of life to government support offered to the underclasses and assessed its impact on the people's lives and finances [2].

Germano [3] emphasizes that until the 1970s, the interest in the theme "quality of life" was restricted to the human sciences, and it was studied by philosophers, social scientists, and politicians. From the 1970s, in the context of the epidemiological transition and with the growing interest in giving "voice" to patients, the term "quality of life" is now used in health, usually associated with the absence of disease and physical well-being.

As time goes, quality of life has become the focus of study and reflection increasingly, and since the 1980s, it began to be viewed in a multidimensional perspective, biological, psychological, economic, and cultural, since QOL depends on these factors and it is subjective [3].

The growing concern with issues related to the quality of life comes from the movement within the human and biological sciences, in the sense

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of valuing parameters broader than the control of symptoms, the reduction of mortality, or the increase of the life expectancy. Thus, quality of life is approached by many authors as a synonym of health and, by others, as a more comprehensive concept, in which health conditions would be one of the aspects to be considered [4].

According to the WHOQOL, in 1994, the term quality of life has many definitions because of its subjective nature, and there is no consensus about its meaning. There are several currents of thought that deal with quality of life and additional issues. Due to this wide divergence, most authors adopt the World Health Organization, which states quality of life as "the individual's perception of their position in life, according to the cultural context and value system and about its goals, expectations, standards and concerns" [5].

There is a lack of conceptual consensus on the quality of life among the authors, as they attribute the reason to the extreme complexity of the term and its use in several areas of study [2, 6]. The definition of quality of life in the specialized literature presents itself in different ways. Some define in a global way, emphasizing general satisfaction with life, or divided into sets, generating an approximation to the general concept. The approaches and the use of the indicators are intrinsically linked to the scientific and political interests of each study and research area, as well as the possibilities of operationalization and evaluation [2, 6].

Pereira [7], when conducting a bibliographic review, reaches the consensus that, depending on the area of interest, the concept is often enforced as a synonym for health, happiness, and personal satisfaction, living conditions, and lifestyle, among others. It states that indicators range from income to satisfaction with certain aspects of life. In the face of so many complexities, it becomes difficult to understand the theme quality of life, restricting, then, the operationalization in scientific analysis.

In 1994, the WHOQOL Group developed goals, expectations, standards, and concerns. Semantically, it compares to an ethical matter, from which we must analyze the individual perception of each one.

Etymologically distinguishing the term, quality derives from "qualis" (Latin) whose meaning is the specific manner of something, either considering itself or relating to another group, being able to assume both positive and negative characteristics. However, quality of life, most of the time, is characterized as something positive [8].

The quality of life is related to the subjective well-being, and it includes biological and psychological components, such as emotional well-being, abilities and disabilities awareness, adequate sleep and rest, vitality, and general satisfaction about one's life [9]. It is well known that there are countless definitions of the term quality of life, and there is still no definition that is widely accepted. It is very common to observe that most definitions are associated with several health factors, such as physical and mental well-being, but new literature has shown that quality of life can also be connected to other important elements such as friends, job, family, and everyday situations [9]. It is necessary to emphasize the relevance of some life aspects of people when it comes to an understanding the term quality of life. Kinds of literature emphasize that subjectivities should be considered, such as freedom, love, happiness, and satisfaction [9]. Nowadays, the most accepted concepts of quality of life try to handle with a multiplicity of dimensions discussed in the general or holistic approaches [7].

The main example that can be cited is the concept advocated by the World Health Organization (WHO), in which quality of life reflects the individuals' perception that their needs are being met or that opportunities to achieve happiness and self-fulfillment, regardless of their state of physical health or social and economic conditions, are being denied [10]. Although there is no consensual definition of quality of life, there is considerable agreement among researchers about some characteristics of the construct. According to it, there are three main features of the construct. They are shared by diverse opinion among the most different authors: subjectivity, multidimensionality, and bipolarity [10].

It is increasingly identified that the construct is subjective. It is not a matter of total subjectivity, since there are conditions called external to

the people who are present in the environment and in the working and living conditions that interfere with the analysis they make of their quality of life.

Concerning multidimensionality, it is a consensus among all researchers that quality of life includes at least three dimensions: physical, psychological, and social. When assessing the quality of life, other dimensions can be evidenced, for conceptual, pragmatic, and empirical reasons [11]. It can be deduced about bipolarity that the construct has positive and negative dimensions, which can be applied to several conditions such as the performance of social roles, mobility, autonomy, pain, fatigue, dependence, and others [11]. We can add two other features: complexity and mutability. The concept of quality of life (QOL) becomes complicated and challenging to evaluate because it is multidimensional, bipolar and subjective. On the other hand, the evaluation of this construct changes with time, person, place and cultural and social context. For the same person, changes according to his mood and health. These characteristics also increase the difficulty of evaluation [11].

2 Health-Related Quality of Life (HRQOL)

The interest in the QOL concept in the health area, stems in part, from the new paradigms that have influenced the policies and practices of the sector in the past decades. The determinants of the health-disease process are multifactorial and complex in nature. Thus, health and disease constitute processes understood as a continuum, related to economic, sociocultural aspects, to personal experience and lifestyles. According to this paradigm shift, QOL improvement has become one of the expected results of both healthcare practices and public policies for health promotion and disease prevention [12].

Quality of life in the health area can be identified in two concepts. Quality of life refers to a more generic concept, that is, it has a broader conception and it does not make any reference, different from the HRQOL that makes references to aggravations and dysfunction [2]. The concept of quality of life is very broad because it is linked to several approaches and problems. In the field of technical production, three key areas for analyzing quality of life stood out [2].

The first scope refers to the difference between material and immaterial aspects of quality of life. Material issues are those about basic human needs (housing conditions, water supply, and the health system, that is, the infrastructure aspect). Immaterial issues refer to the environment, cultural heritage, and well-being [2]. The second scope distinguishes individual and common aspects. Individual aspects are related to the economic, personal, and family condition, and the common ones are linked with basic and health services [2]. The third scope brings the difference among real aspects of quality of life that are easily apprehended through the definition of indicators of a quantitative nature and the subjective aspects that refer to subjective perception and the perception individuals have about quality of life [2].

It is possible to observe in the literature that, according to the authors mentioned above, the health approach is quite broad, and it is possible to find unique aspects in this approach that when interrelated gives meaning to the term quality of life in health. We can notice that there is a consensus to having quality of life; it is necessary that some elements be present: physical, emotional aspects, and relationships. All of them must be related to well-being.

To establish a more accurate analysis of these relationships, we used the World Health Organization (WHO) (2006) citation which defines that “Health is a large state of physical, mental and social well-being, not only lack of disease and infirmity.” Based on this definition, it is easy to understand that besides the preservation of physiological capacities, and the organism’s proper functioning, it is necessary that other factors are considered, such as the environment, social life, and relationships [12].

Several factors must be taken into account when relating quality of life and health; subjective and objective spheres need to be met, as well as the culture of the society. The subjective

spheres can be understood as personal actions, whereas the objective spheres can be understood as public programs linked to the population's condition of life improvements [13].

The individual action in their group is intrinsically linked to their state of health because they can define the perception of a positive or negative well-being. Human health state can constantly vary since it is extremely influenced by several factors. We can observe that, when a man is not satisfied with the environment he is in, he starts feeling uncomfortable because of that situation. As a consequence, he gradually feels unwell and even unhappy because he is in a place that takes his well-being out, and thus that position puts him off, jeopardizing one of the first indications defined by the WHO, which is that of mental and social well-being. Then the interference of the environment in the individual's life affects his state of health that, according to several studies, is related to the right quality [13].

Lifestyle can also significantly interfere with a person's state of health because life, when not based on the consciousness of right and wrong, is at risk of compromising and acting unthinkably. It is possible to give an example when we think about a person who abuses alcohol, the lifestyle of alcoholic changes drastically when he loses the notion of his attitudes, and the dependence of alcohol compromises not only health but also relationships with the people who surround him [13].

Human biology and the healthcare system organization are capable of interfering with the individual state of health and quality of life [13]. The relationship between health and quality of life needs to be followed by some elements. They can be divided in the following ways: functional domains, i.e., physical function, cognitive function, involvement in everyday life activities, and subjective health evaluation, and domains of well-being, i.e., body well-being, emotional well-being, self-care, and global perception of well-being [13].

Almeida [13] explains that a person's state of health can suffer changes due to the several characteristics that are part of it, as observed above, and because of these variants, we cannot associate the state of health/illness of a person only to a feature. To understand the health/illness relationship, it is necessary to consider both individual actions and public policies. Studies show that there is a close connection between quality of life and health and socioeconomic aspects. Some authors point that this relationship is focused on the access possibilities that the population has to receive healthcare and that the presence or lack of them directly influences their quality of life. It does not take much to conclude that a reduced healthcare population obviously cannot keep healthy and consequently suffers changes in the capacity to maintain their quality of life [13].

3 Quality of Life in Patients with Wounds

The wound is represented by the interruption of the corporeal tissue continuity, to a bigger or smaller extent, caused by any physical, chemical, and mechanical trauma or caused by a clinical affection, which activates the organic defense [14]. The healing of lesions involves complex anatomical and biological processes that, in a favorable clinical situation, can occur in acceptable time. However, when the lesions become chronic, the treatment can become quite complicated, causing an important impact on these patients' lives [15].

Wounds present classifications based on the characteristics of the lesion, such as etiology, the level of tissue involvement, and the time it lasts. As for the healing period, the wounds can be classified as acute, which are those that heal in less than 6 weeks, and chronic, which heal in a time superior to that mentioned [16–18].

Some types of wounds have significant social and economic impact, due to their enormous potential for chronicity and because they present high global prevalences, such as venous ulcers, pressure injury, injuries caused by diabetic complications, and burns [19–21]. Chronic ulcers are a serious and worldwide problem, responsible for significant morbidity and mortality rates, besides having a considerable economic impact [22].

The high rates of chronic wounds have particular relevance, given the Brazilian sociodemographic characteristics, as a longer life expectancy and less healthy, which contribute to the onset of

chronic diseases [23]. North American statistics indicate a prevalence of 14% of patients with wounds in the world population. Other studies suggest that the indexes may be higher, around 22.8% for the next few years [24].

The wound characteristics, such as the presence of exudate, its odor, pruritus, edema, and the extent of it, and the difficulty in performing basic activities such as bathing and walking are patient complaints that result in changes in lifestyle [25–27]. In the psychological domain, feelings such as fear, anger, depression, anxiety, disturbances, and social isolation are exaggerated in this population, especially in young people [28].

In a study where the authors evaluated the quality of life in 50 patients with venous ulcer who are being treated with compressive therapy by Unna boot, data collection was performed at the time of the study inclusion and was repeated at 4 months, 8 months, and 12 months after the first data collection using the Short Form 36-item (SF-36) questionnaire. During the inclusion of the patient in the study, the average scores of SF-36 were small [28], characterizing a fall in quality of life. After 12 months of Unna boot compressive therapy, the average score was 95.38, characterizing improvement in the quality of life of the analyzed patients ($p = 0.0001$). The authors concluded that patients with a venous ulcer at the beginning of data collection presented low quality of life, and after 8 months of treatment with the Unna boot, an improvement in the quality of life was observed.

The signs and symptoms presented by these individuals should not only be treated in the physical dimension but also emphasize the changes that the wound causes in their life. It is important to point out that tissue repair of a wound does not always mean an improvement in the individual's quality of life. That's possibly due to other comorbidities. That's why it is essential to bring up and care for these impacts caused by the wound, minimizing the biological, psychosocial, and emotional problems that directly affect their quality of life [29].

As soon as these patients demonstrate some dependence on managing activities like domiciliary, leisure, social, and family, they will have their autonomy impaired and automatically become dependent on their relatives and friends, and as a consequence, they have a decline in self-esteem and impairment of self-image and quality of life.

Diseases usually produce changes in the way patients live, especially in those who live with these conditions for a long period. The impact of chronic ulcers on patients' quality of life (QOL) has been the subject of studies since it involves research of many health professionals, known as health-related quality of life studies (HRQOL), and aims to identify critical aspects of the disease process in order to propose interventions that minimize suffering and its negative impact on patients' lives.

In the literature review, the HRQOL concept is dynamic and multidimensional, without consensus among the authors. It is associated with a state of emotional, mental, physical, social, and functional dimensions related to health and presents as synonymous with health perception or state of health. Therefore, there is no accurate measure of QOL; but it is necessary to evaluate the patients' well-being in these dimensions [27, 28]. HRQOL is also defined as the value given to life, weighted by the functional deteriorations, perceptions, and social conditions that are induced by the disease, complications, treatments, and the political and economic organization of the healthcare system. To measure such concepts, questionnaires were designed with the objective of evaluating QOL, which are classified as generic and specific instruments [29, 30].

In relation to the field of application, the measures can be classified as generic, if we use population-based questionnaires without specifying pathologies, being more appropriate to epidemiological studies, planning, and evaluation of the health system, related to the individuals' quality of daily life, subsequent to the experience of diseases, injuries, or medical interventions. Several instruments include indicators for subjective aspects of living with illnesses and injuries, such as feelings of shame and guilt, which have negative consequences on the perception of quality of life on the affected individuals and their families [31, 32].

Therefore, the availability of a particular instrument to evaluate the quality of life in patients with wounds will help with the integral care of the patient, and this instrument can be

used as an indicator of results on the performance of the health system [28].

4 Freiburg Life Quality Assessment-Wound (FLQA-wk)

The FLQA-wk, short version, was developed based on the Freiburg Questionnaire of Quality of Life in venous diseases, specific for venous ulcer and composed of 81 items [33]. For the development of FLQA-wk, 10 of the items were kept unchanged, 10 were modified, 61 questions were extracted, and there were 3 specific items of wounds [40]. The FLQA-wk instrument is composed of 24 questions, divided into 6 domains: physical symptoms (5 questions), daily life (5 questions), social life (3 questions), psychological well-being (4 questions), satisfaction (3 questions), and treatment (4 questions) [34].

The domain "physical symptoms" refers to the physical well-being of the patient, which can be influenced by the condition of the wound. These are specific issues: pain, discharge, pruritus, and bad smell. The "daily life" scale refers to how the wounded individual manages his daily life, while "social life" mentions the patient's relationships with other people. Psychological well-being lists the feelings that the wounded patient can present. The "treatment" domain refers to how the patient feels about the treatment offered by the health team, and the "satisfaction" scale includes how satisfied the individual feels about their health, treatment, and wound conditions.

The answers of the "daily life," "social life," "treatment," and "satisfaction" scales range from never (1 point), few times (2 points), moderately (3 points), quite a few (4 points), and a lot (5 points). For the domains of "psychological well-being" and "physical symptoms," we have the following answers: never (1 point), rarely (2 points), sometimes (3 points), often (4 points), or always (5 points).

The domain "treatment" has a question that evaluates the time spent by the individual to take care of his wound; the answers are no time (1 point), less than 10 min (2 points), 1–30 min (3 points), 30–60 min (4 points), and more than 60 min (5 points).

The FLQA-wk can be filled by the patients themselves, but, if it is necessary, it can be conducted by the researcher through an interview. To calculate the score, it is necessary that 75% of the items are answered and that at least five of the six scales are complete. It evaluates the quality of life of people with chronic wounds in the last week. The scales are calculated by the average of each answer, after recoding the "satisfaction" scale. The total score is computed from the average values of each domain.

The questionnaire also has three visual analog scales, graded from 0 (very bad) to 10 (very good). The individual evaluates his/her quality of life, general health, and wound conditions in the last week. This scale helps in the control of values of the domains, that is, the values are compared with the score of the whole instrument. The higher the value of the score, the bigger the interference with quality of life. The score varies from 1 (better quality of life) to 5 (worse quality of life).

The validation occurred in three distinct studies with individuals with acute and chronic wounds. The first study is a multicenter, uncontrolled research conducted with 175 patients. The research consisted of the evaluation of the quality of life of patients with acute and chronic wounds treated with vacuum therapy. Patients answered the questionnaire, and physicians filled an instrument on wound conditions, both before and after therapy [34]. The tests for validation in this research were the internal consistency, the convergent validity through the correlation of the FLQA-wk items with a generic instrument of quality of life, and the items of the visual analog scale. The ability to detect changes in the quality of life according to the patient's clinical alterations was performed by comparing the FLQA-wk with these variables: wound area and conditions, generic quality-of-life instruments, and global evaluation of quality of life of the visual analog scale.

A cross-sectional observational study of 384 patients with lower limb ulcers undergoing different treatments also evaluated the psychometric properties of FLQA-wk. Patients and physicians completed the questionnaires only in a moment.

The tests performed were internal consistency, convergent validity through the correlation of the FLQA-wk with visual analog scale items, and pain intensity (ranges from 0 to 10) [34].

And finally, a multicenter, randomized research verified the efficiency and safety of keratinocyte transplantation along with compressive therapy in 198 venous ulcer patients. The patients answered the questionnaires on 0 (time 1), on the 28th (time 2), and 56th (time 3) day of therapy. At times 1 and 2, all subjects received the treatment; from time 2 to 3, the patients were randomized. The tests performed were the internal consistency, test-retest, and sensitivity through the correlation of the FLQA-wk items with the success score in the treatment, scored from 1 (very successful) to 5 (no success), and FLQA-wk before and after treatment [34].

The questionnaire presented proper internal consistency in the three studies, with values ≥0.85. The test-retest and the validity were satisfactory [34].

5 Technique

5.1 Cultural Adaptation Procedures

The translation and cultural adaptation process of the FLQA-wk were carried out according to the methodological rules recommended by internationally recognized publications [35–39]. The following steps were carried out: translation, synthesis of translations, back translation, committee of experts, pretest, and focus group. It is pointed out that the author of the questionnaire was previously consulted and the authorization was provided to be adapted and validated for the Portuguese language of Brazil.

5.2 Translation of the Instrument into Portuguese

For the accomplishment of this stage, the first translation of the FLQA-wk instrument for the Portuguese language of Brazil was carried out by two independent, qualified, and bilingual translators, whose native language is Portuguese. Two independent translations were conducted, one of the translators was a nurse with knowledge in the area of wounds, and the other one was a language teacher, who was not from the area and did not know the purpose of the work [35, 37] resulting in two versions: Translation 1 (T1) and Translation 2 (T2).

5.3 Translation Synthesis

Following the translation stage, the two versions (T1 and T2) were synthesized. The analysis of the divergences of both versions was carried out by the two translators, with the researcher who reached a consensus, producing a different translation, synthesis version (SV).

5.4 Back Translation

The synthesis version (SV) of the instrument obtained by consensus was again translated into the original language of the instrument by two other translators who did not participate in the first stage of the process. This procedure checked the validity to ensure that the translated final version is in agreement with the original version of the instrument.

The back translation was carried out by two bilingual translators who had the same mother tongue as the adapted questionnaire and had knowledge of Portuguese language. Both were not from the health area and didn't know about the concepts and objectives of the research, performing the translations independently.

The questionnaire was also sent by e-mail to the authors to verify the adequacy of their original content. It is worth mentioning that the authors requested the instrument in the Portuguese language version so that one of their translators could perform a back translation. At the end of this stage, the versions back translation 1 (BT1) and back translation 2 (BT2) were produced.

Thus, the probability of imperfections decreased, having the quality guarantee of the cultural adaptation of the instrument chosen for this research [35, 39].

5.5 Committee of Experts

The committee of experts components are crucial to achieve transcultural equivalence of the translated instrument and should be made up of bilingual people and specialists from the knowledge area of the instrument, composed of health professionals, language teacher, and methodology specialist [35, 37]. This committee should be multidisciplinary and informed about the objectives and concepts of the study.

At first, the experts received an invitation letter to participate as a member of the committee of experts. After they had accepted, the specialists received all versions of the instrument: translations into Portuguese (T1 and T2), synthesis (SV) and back translations (BT1 and BT2), and a questionnaire for evaluation, which was developed specifically for this stage.

Judges reviewed and compared all translated versions modifying the format of the instrument and changing or deleting the inappropriate items for a final translation. The committee's work aims to determine the semantic, linguistic, cultural, and conceptual equivalence between the original questionnaire and the Portuguese version, to guarantee the understanding and cultural equivalence of the final version [35, 38].

Semantic equivalence is related to the meaning of words, considering the vocabulary and grammar, and the idiomatic equivalence considers the use of idioms and colloquial expressions referring to both languages. In cultural equivalence, the events mentioned in the items of the original questionnaire must be according to the Brazilian cultural experience. Conceptual equivalence is the pertinence appreciation of the concepts and the events experienced by the subjects that are part of Brazilian culture [35].

To facilitate evaluation by the committee, the questionnaire was split into items, that is, each question answered one item. The title was the first item; the general statement was the second item, and so on. The full questionnaire consisted of 42 items.

The committee of experts was formed by:

Judge 1: Nurse, university professor with experience in the care area in wound treatment
Judge 2: Language teacher
Judge 3: Nurse, Ph.D. in nursing, university professor with experience in research methodology
Judge 4: Nurse, Ph.D. in nursing, with experience in studies about quality of life and older adults
Judge 5: Nurse, master in nursing, university professor, with experience in the area of care and studies related to wounds and therapeutic communication
Judge 6: Nurse, enterostomy therapist, university professor, with experience in the care area and studies related to wounds, incontinence, and stoma
Judge 7: Nurse, master of nursing, university professor with experience in studies on quality of life
Judge 8: Nurse, master in nursing, university professor with experience in research methodology

After that, the content of the instrument was validated. The content validity verifies the degree of extension which the subject of interest is contemplated in the dimensions of the instrument [40]. This type of validity refers to a careful analysis of an instrument with the objective of verifying if the items and subitems proposed have a representative within the instrument [41, 42].

Independently, the judges carried out the analysis of the items, verifying their clarity, pertinence, and comprehensiveness, besides evaluating the overall appearance of the instrument. The content validity was assessed by calculating the content validity index (CVI). This test evaluates the level of concordance among judges on certain aspects of the adapted questionnaire and its items [42].

The judges scored the items with values from 1 to 4: 1, not equivalent; 2, it is impossible to evaluate equivalence without reviewing the item;

3, equivalent, but it needs minor changes; and 4, absolutely equivalent.

The score was calculated by summing the items that were highlighted with "3" and "4" dividing the value by the number of judges. The items with grades "1" and "2" were reviewed. For the research, the level of agreement was set equal to or higher than 0.8 [42, 43].

Thus, after the experts' assessment, we had a first translated version (VT1) of the instrument, which was used for the pretest.

5.6 Retest

The final stage of the adaptation process consisted of the pretest when the questionnaire was applied to a sample of subjects with wounds that were not part of the final sample of the study. For this phase, the authors advocated a total of 30–40 people [35, 37].

Patients who agreed to participate in the pretest received guidance on the informed consent form and explanation of the purpose of the research. The purpose of this stage was to evaluate participants' understanding and acceptance of the content of the translated version, verifying in practice how it will be managed. At the end of each completed questionnaire, the participant expressed his/her difficulties in understanding the questions of the questionnaire.

5.7 Focus Group

Due to the difficulties of understanding presented by the interviewees during the pretest related to the items of the questionnaire, it was decided to carry out a focus group with the purpose of adjusting the questionnaire to the highlighted population, for comprehensibility.

The focus group is a qualitative method that aims to obtain data from the discussion focused on specific points. In order to do this, there must be an interaction between the researcher and the research participants. The number of participants may vary from 6 to 10, and the members of the focal group should have similar characteristics and be related to the item to be studied [44]. In this study specifically, the focus group was carried out to assist in the adaptation of a quantitative questionnaire which needed to be improved to facilitate the understanding of the study population. The steps for this group consist of the assembly, conduction of the group, and, finally, the data analysis. Recruitment was carried out by invitation to individuals performing dressing on a skin lesion unit.

5.8 Evaluation of Measurement Properties

The psychometric properties of the FLQA-wk questionnaire were evaluated through reliability and convergent validity.

5.9 Reliability Assessment

The reliability, also called accuracy and reliability of the instrument [40, 45], refers to the "degree of consistency or precision with which the instrument measures the attribute it proposes to measure" [40]. The reliability of the questionnaire was evaluated using two methods: homogeneity (internal consistency) and stability (test-retest).

Internal consistency or homogeneity analyzes whether all items in an instrument measure the same characteristic [46, 47] or the degree of interrelationship between items [45]. It was verified by calculating Cronbach's alpha coefficient for the total score (Cronbach, 1951), and Cronbach's alpha higher than or equal to 0.70 was considered interesting [48, 49].

Stability refers to the consistency of the measurements' repetitions. The use of this method requires that the factor to be measured remains the same as applied to a sample of subjects at two different times, comparing the results obtained [41].

Stability was evaluated through the test-retest, calculating the intraclass correlation coefficient (ICC). The FLQA-wk questionnaire was carried out in 71 subjects under the same conditions, within a 7-day interval.

6 Validity Assessment of FLQA-Wk

Validity is an important measure property to evaluate the quality of an instrument [41], and it refers to the degree to which the data of an instrument actually measures what it is proposed to [39, 40, 46].

The convergence construct validity consists of comparing how one dimension of a new instrument correlates with dimensions of another instrument that measures the same concept, and it is applied concomitantly [39, 41, 50]. The convergent validity was carried out through the correlation of the four domains of Ferrans and Powers Quality of Life Index-Wound Version (health and functioning, psychological and spiritual, family and socioeconomic) with the domains of the FLQA-wk questionnaire. The domains Ferrans and Powers Quality of Life Index-Wound Version (FPQLI-WV) instruments were chosen, taking into account the similarity of the content of these domains with the FLQA-wk questionnaire, as shown in Table 1.

Validity was also evaluated by correlating the total score of the FLQA-wk questionnaire with the patient's score on the visual analog scale (VAS) of quality of life. This scale complements the FLQA-wk instrument and scores the individual's quality of life from 0 (very poor) to 10 (very good) (Table 2).

7 Discussion

The evaluation of the quality of life in health, mainly in the treatment of wounds, has been gaining more space in the clinic, therapeutics, and research services, providing the necessary assistance to the patient by the professionals [51]. Therefore, the application of instruments can be considered an objective measure that assists in the evaluation of the patient's well-being, contributing to quality intervention [52].

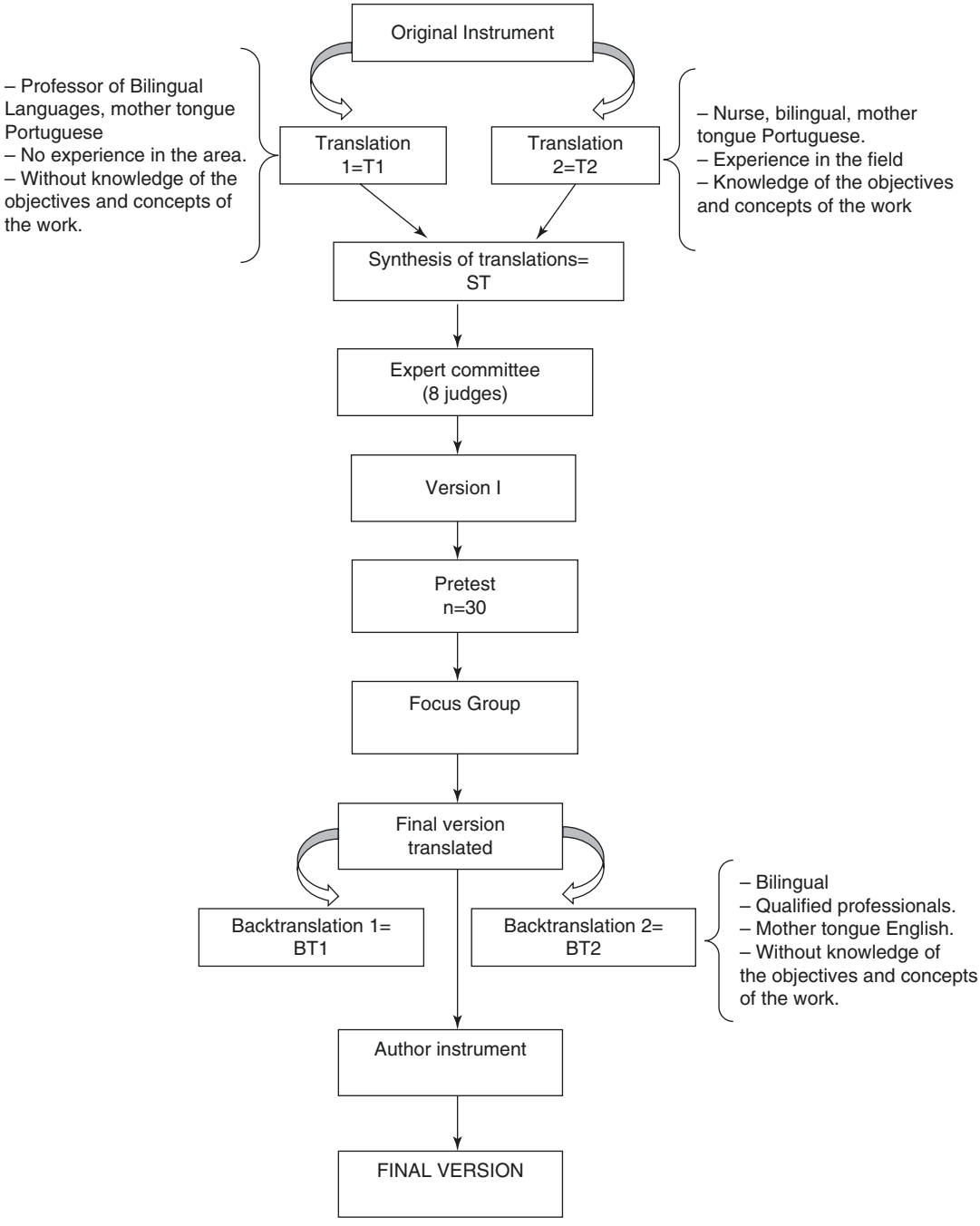
In this context, the cultural adaptation of any instrument is effective but complex and with different recommended methods in the literature [38]. In this research, this process followed the steps of translation, translation synthesis, back translation, committee of experts evaluation, pretest, and focus group [35].

The content validity was carried out with the help of a committee of judges that proved that the questionnaire contains relevant questions, and small changes were made in some items to facilitate the understanding of the highlighted population.

Table 1 Correlations of the FPQLI-WV domains indicated for the achievement of the convergent validity of the Freiburg Life Quality Assessment-Wound questionnaire

Questionnaire	Domains		Freiburg life quality assessment-wound (FLQA-wk)—adapted
Ferrans and powers quality of life index-wound version	Health and functioning	X	Physical symptoms
	Health and functioning	X	Daily life
	Health and functioning	X	Treatment
	Health and functioning	X	Social life
	Psychological/spiritual	X	Psychological well-being
	Psychological/spiritual	X	Satisfaction
	Socioeconomic	X	Satisfaction
	Family	X	Psychological well-being
	Health and functioning	X	Total score
	Total score	X	Total score
	Total score	X	Psychological well-being
	Total score	X	Satisfaction

Table 2 Flowchart of the transcultural translation process



The committee suggested small changes in items, such as synonym substitutions, sentence inversions, and some spelling mistakes following semantic, cultural, conceptual, and idiomatic equivalences. Considering the agreement rate >0.80, the committee of experts suggested small changes in some questions, which were followed. After these changes, Version I was created, which was used in the pretest.

The pretest included 30 subjects with chronic wounds of different etiologies. The individuals' average age was 63.0 years old (standard deviation = 12.8), represented by 79.3% female, 41.4%

married, and 69.0% with incomplete primary education. The venous ulcer presented a higher frequency with 58.6%. The average wound duration was 42 months (standard deviation = 63.7) and with an average number of 1.5 wounds.

Patients with different levels of schooling were selected to allow evaluation of the questionnaire in different degrees of difficulty. Five patients attended the meeting, the average age was 65.4 years, and the educational attainment levels of the participants were two illiterates, two with incomplete elementary education, and one with complete higher education.

The instrument was applied by interview, and 5–10 min was spent for the application. At the end of the interview, participants were asked to express their opinions on the questionnaire verbally. Patients reported that some words made it difficult to understand the questions. Due to these difficulties presented by the participants in the comprehension of some questions, which could produce false results in the analysis of the questionnaire, it was decided to carry out a focus group, to adapt the instrument to the specific population.

The focus group was started explaining the purpose of the meeting and the importance of each participation. The questions of the instrument were read separately and in an easy way to facilitate the follow-up by all, enabling them to understand what was intended with the focus group.

Conducting the focus group were one moderator and two observers. The moderator was responsible for reading the questionnaire and discussion, while the observers analyzed expressions during the discussion. The whole session was recorded with the permission of the attendees. The discussion lasted 1 h and 10 min, and the questionnaire was analyzed and discussed twice. During the reading of the instrument to each question, the participant was asked about the comprehension of the content. According to the reports, associated with the nonverbal expressions that indicated doubt, the item was discussed and they requested to vote for possible changes. After this step, the final translated version (FTV) was elaborated – which was sent to back translation to evaluate the discrepancies between the FTV and the original instrument.

The FVT was sent to one of the authors for consideration. Small changes were requested in the Portuguese questionnaire so that the cultural adaptation of the Freiburg Life Quality Assessment-Wound (FLQA-wk) questionnaire, abbreviated version, reflected the content of the original version. After the changes in the questionnaire, the final version (FV) was created.

Regarding the medical properties, the reliability of the FLQA-wk was evaluated through the internal consistency, represented by Cronbach's alpha, and the stability, through the test-retest, using the intraclass correlation coefficient (ICC).

The adapted instrument (FLQA-wk), containing 24 items, presented an excellent internal consistency since it obtained a total Cronbach's alpha of 0.86. In relation to the domains, the Cronbach's alpha was physical symptoms, 0.63; daily life, 0.83; social life, 0.53; psychological well-being, 0.70; treatment, 0.68; and satisfaction, 0.86.

Concerning stability evaluation, a high value of ICC (0.93) is contacted, which is considered excellent, since the closer to 1, the better the stability of the instrument [46]. The adapted questionnaire showed temporal stability when applied in different moments for the same participants in similar conditions.

The convergent validity was evaluated by carrying out the FLQA-wk correlation with the Ferrans and Powers Quality of Life Index-Wound Version, as well as the quality of life score of the visual analog scale. The analysis was carried out using Spearman's correlation coefficient.

Correlations appear as negative or inverse relationships since the FLQA-wk score indicates that the higher the score, the worse the quality of life, whereas in the FPQLI-WV, the score is the opposite: the higher the score, the better the quality of life.

The correlation of FLQA-wk with the FPQLI-WV domains. The treatment domains (−0.32), daily life (−031), physical symptoms (−0.27), social life (−0.24), and the total score (−0.41) of the FLQA-wk presented satisfactory to moderate correlation and significant with the health and functioning domain of FPQLI-WV.

The psychological well-being domain of FLQA-wk had a moderate and significant correlation with the psychological/spiritual domains (−0.44), family (−0.38), and the total score (−0.40) of FPQLI-WV.

The domain satisfaction showed a moderate and significant correlation with the psychological/

spiritual domains (−0.48), socioeconomic (−0.46), and total score (−0.37) of the IQVFP-VF. The total score (0.36) of the FLQA-wk had a moderate correlation with the total FPQLI-WV score.

Concerning the visual analog scale, the FLQA-wk total score had a moderate and significant correlation with the total quality of life score (−0.38).

Conclusions

The cultural adaptation of the Freiburg Life Quality Assessment-Wound was performed according to the recommended international methodology, resulting in a real version. In the application, the instrument proved to be easy to understand and apply.

The questionnaire was found to be reliable, with a Cronbach's alpha (0.87) and satisfactory temporal stability (0.93). Concerning the validity, the adapted FLQA-wk questionnaire presented a correlation of satisfactory magnitude to moderate magnitude and significant (−0.24 to −0.48; $p < 0.0001$) with the domains of the IQVFP-VF questionnaire.

Therefore, FLQA-wk presented enough healing properties when applied in patients with chronic wounds in the Brazilian population.

References

1. Rodrigues MVC (1991) Qualidade de Vida no Trabalho: evolução e análise no nível gerencial. UNIFOR, Fortaleza
2. Belasco AGS, Sesso RCC (2006) Qualidade de vida: princípios, focos de estudo e intervenções. In: Diniz DP, Schor N (eds) Qualidade de vida. Manole, São Paulo, pp 1–10
3. Germano CM, Bonato D, Maion VH, Avó LR, Melo DG, Fontanella BJ (2016) Possíveis novos determinantes da qualidade de vida de pacientes com câncer de tireóide tratado: um estudo qualitativo. Cien Saude Colet 21(8):2451–2462
4. Fleck MP, Louzada S, Xavier M, Chachamovich E, Vieira G, Santos L, Pinzon V (1999) Aplicação da versão em português do instrumento de avaliação de qualidade de vida da Organização Mundial da Saúde (6WHOQOL-100). Rev Saúde Pública 33(2): 198–205
5. Whoqol Group (1994) The development of the World Health Organization quality of life assessment instrument (the WHOQOL). In: Orley J, Kuyken W (eds) Quality of life assessment: international perspectives. Springer, Heidelberg, pp 41–60
6. Farquhar M (1995) Definitions of quality of life: a taxonomy. J Adv Nurs 22(3):502–508
7. Pereira EF, Teixeira CS, Santos A (2012) Qualidade de vida: abordagens, conceitos e avaliação. Rev Bras Educ Fís Esporte 26(2):241–250
8. Santin S (2002) Cultura corporal e qualidade de vida, vol 27. Kinesis, Santa Maria, pp 116–186
9. Aguglia A, Salvi V, Maina G, Rossetto I, Aguglia E (2011) Fibromyalgia syndrome and depressive symptoms: comorbidity and clinical correlates. J Affect Disord 128(3):262–266
10. Nutbeam D (1998) Promoción de la salud: glossário. World Health Organization, Genebra
11. Paschoal SM (2016) Qualidade de vida na velhice. In: de Freitas EV, Py L (eds) Tratado de Geriatria e Gerontologia, 4th edn. Guanabara Koogan, Rio de Janeiro, pp 79–86
12. Schuttinga JA (1995) Quality of life from a federal regulatory perspective. In: Dimsdale JE, Baum A (eds) Quality of life in behavioral medicine research. Lawrence Erlbaum Associates, Hillsdale, NJ, pp 31–42
13. Almeida MAB, Gutierrez, GL, Marques R (2012) Qualidade de vida: definições e conceitos. In: Almeida MAB, Gutierrez GL, Marques R, organizadores. Qualidade de vida: definição, conceitos e interfaces com outras áreas de pesquisa. Escola de Artes, Ciências e Humanidades, Universidade de São Paulo, São Paulo, pp 13–50
14. Oliveira ACDS, Silva CI, Ramos RS (2016) Úlceras venosas, Arteriais e Mistas. In: Gamba MA, Petri V, MTF C (eds) Feridas: Prevenção, causas e tratamento. Guanabara Koogan, Rio de Janeiro, p 261
15. Yamada BFA, de Gouveia SantosIISantos VLC (2009) Construção e Validação do Índice de Qualidade de Vida de Ferrans & Powers - Versão Feridas. Rev Esc Enferm USP 43:1105–1113
16. Werdin F, Tennenhaus M, Schaller HE, Rennekampff HO (2009) Evidence-based management strategies for treatment of chronic wounds. Eplasty 9:e19
17. Dealey C (2008) Cuidando de feridas. Atheneu, São Paulo
18. de Brito KKG, de Sousa MJ, de Sousa ATO, de Ardújo Menses LB (2013) Dos Santos Oliveira SH, ães Oliveira Soares MJG. Feridas crônicas: abordagem da enfermagem na produção científica da pós-graduação. Rev Enferm 7(2):414–442
19. de Souza Smaniotto PH, Ferreira MC, Isaac C, Galli R (2012) Sistematização de curativos para o tratamento clínico das feridas. Rev Bras Cir Plást 27(4):623–626
20. Hopkins RB, Burke N, Harlock J, Jegathisawaran J, Goeree R (2015) Economic burden of illness associated with diabetic foot ulcers in Canada. BMC Health Serv Res 15(13):1–9
21. Padula WV, Makic MB, Wald HL, Campbell JD, Nair KV, Mishra MK, Valuck RJ (2015) Hospital-acquired pressure ulcers at academic medical centers in the United States, 2008-2012: tracking changes since the

CMS nonpayment policy. Jt Comm J Qual Patient Saf 41(6):257–263
22. Castro SLS, Ferreira NMLA, Roque M, Souza MBB (2012) Vivendo uma situação difícil: a compreensão da experiência da pessoa com úlcera venosa em membro inferior. Rev Estima 10(1):12–19
23. Neves JS, Azevedo RS, Soares SM (2014) Atuação multiprofissional na construção de grupo operativo envolvendo pacientes com lesão de membros inferiores. Rev Norte Mineira Enferm 3(1):86–95
24. Evangelista DG, Magalhães ERM, Moretão DIC, Stival MM, Lima LR (2012) Impacto das feridas crônicas na qualidade de vida de usuários da estratégia de saúde da família. Rev Enferm Centro Oeste Mineiro 2(2):254–263
25. Fagervik-Morton H, Price P (2009) Chronic ulcers and everyday living: Patients' perspective in the United Kingdom. Wounds 21(12):318–323
26. Salomé GM, Pellegrino DMS, Blanes L, Ferreira LM (2011) Self-esteem in patients with diabetes mellitus and foot ulcers. J Tissue Viability 20(3):100–106
27. Salomé GM, Ferreira LM (2012) Quality of life in patients with venous ulcers treated with Unna's boot compressive therapy. Rev Bras Cir Plást 27(3):466–471
28. Dias TYAF, Costa IKF, Salvetti MG, Mendes CKTT, Torres GV (2013) Influência da assistência e características clínica na qualidade de vida de portadores de úlcera venosa. Acta Paul Enferm 26(6):529–534
29. Santo PFE, Almeida SA, Salomé GM (2013) Avaliação do nível de depressão em indivíduos com feridas crônicas. Rev Bras Cir Plást 28(4):665–668
30. Salomé GM, Blanes L, Ferreira LM (2014) The impact of skin grafting on the quality of life and self-esteem of patients with venous leg ulcers. World J Surg 38:233–240
31. Santos LSF, Camacho ACLF, Oliveira BGRB, Nogueira GA, Joaquim FL (2015) Influência da úlcera venosa na qualidade de vida dos pacientes: revisão integrativa. Rev enferm UFPE 9(3):7710–7722
32. Augusto FS (2015) Tradução, adaptação cultural e validação do Cardiff Wound Impact Schedule para a língua portuguesa do Brasil. Dissertação (Mestrado)-Universidade Federal de São Paulo – Programa de Pós-Graduação em Cirurgia Translacional, São Paulo
33. Augustin M, Dieterle W, Zschocke I, Brill C, Trefzer D, Peschen M et al (1997) Development and validation of a disease specific questionnaire on the quality of life of patients with chronic venous insufficiency. Vasa 26(4):291–301
34. Augustin M, Herberger K, Rustenbach SJ, Zschocke I, Blome C (2010) Quality of life evaluation in wounds: validation of the Freiburg life quality assessment-wound module, a disease-specific instrument. Int Wound J 7(6):493–501
35. Beaton D, Bombardier C, Guillemin F, Ferraz MB (2007) Recommendations for the cross-cultural adaptation of the DASH & QuickDASH outcome measure [online]. Institute for Work and Health. http://www.dash.iwh.on.ca/sites/dash/files/downloads/cross_cultural_adaptation_2007.pdf Accessed 8 Apr 2017
36. Guillemin F (1995) Cross-cultural adaptation and validation of health status measures. Scand J Rheumatol 24(2):61–63
37. Beaton DE, Bombardier C, Guillemin F, Ferraz MB (2000) Guidelines for the process of cross-cultural adaptation of self-report measures. Spine 25(24):3186–3191
38. Alexandre NMC, Guirardello EB (2002) Adaptación cultural de instrumentos utilizados en salud ocupacional. Rev Panam Salud Publica 11(2):109–111
39. Mokkink LB, Terwee CB, Patrick DL Alonso J, Stratford PW, Knol DL, Bouter LM, de Vet HCW (2012) COSMIN checklist manual. http://www.cosmin.nl/the-cosmin-checklist_8_5.html. Accessed 8 Apr 2017
40. Mcdowell I, Newell C (1996) Measuring health. A guide to rating scales and questionnaires, 2nd edn. Oxford University Press, New York
41. Polit DF, Beck CT (2011) Fundamentos de Pesquisa em enfermagem: avaliação de evidências para as práticas da enfermagem, 7th edn. Artmed, Porto Alegre
42. Alexandre NMC, Coluci MZO (2011) Validade de conteúdo nos processos de construção e adaptação de instrumentos de medida. Ciênc Saúde Coletiva 16(7):3061–3068
43. Grant JS, Davis LL (1997) Focus on quantitative methods: selection and use of content experts for instrument development. Res Nurs Health 20(3): 269–274
44. Lervolino SA, Pelicioni MCF (2001) A utilização do grupo focal como metodologia qualitativa na promoção da saúde. Rev Esc Enf USP 35(2):115–121
45. Lohr KN, Aaronson NK, Alonso J, Burnam MA, Patrick DL, Perrin EB et al (1996) Evaluating quality-of-life and health status instruments: development of scientific review criteria. Clin Ther 18(5):979–992
46. Lobiondo-Wood EHJP (2001) Avaliação crítica e utilização. Guanabara- Koogan, Rio de Janeiro
47. Terwee CB, Bot SD, de Boer MR, Van Der Windt DA, Knol DL, Dekker J, Bouter LM, de Vet HC (2007) Quality criteria were proposed for measurement properties of health status questionnaires. J Clin Epidemiol 60(1):34–42
48. DeVon HA, Block ME, Moyle-Wright P, Ernst DM, Hayden SJ, Lazzara DJ, Savoy SM, Kostas-Polston E (2007) A psychometric toolbox for testing validity and reliability. J Nurs Scholarsh 39(2): 155–164
49. Houser J (2008) Precision, reliability, and validity: essential elements of measurement in nursing research. J Spec Pediatr Nurs 13(4):297–299
50. Fayers PM, Machin D (2000) Quality of life: assessment, analysis and interpretation. John Wiley and Sons Ltd, Chichester
51. Jankovic S, Vukicevic J, Djordjevic S, Jankovic J, Marinkovic J, Basra MK (2013) The Cardiff Acne Disability Index (CADI): linguistic and cultural validation in Serbian. Qual Life Res 22(1):161–166
52. Anand SC, Dean C, Nettleton R, Praburaj DV (2003) Health-related quality of life tools for venous-ulcerated patients. Br J Nurs 12(1):48