Needle-free jet injector-assisted drug delivery in dermatology

Towards effective and minimally invasive treatment of severe keloids

VAZULA BEKKERS

Needle-Free Jet Injector-Assisted Drug Delivery in Dermatology

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Needle-Free Jet Injector-Assisted Drug Delivery in Dermatology

Towards effective and minimally invasive treatment of severe keloids

Naaldvrije behandeling middels jet injectoren in de dermatologie

Naar effectieve en minimaal invasieve behandeling van ernstige keloïden

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Chapter 1

General introduction

Keloids

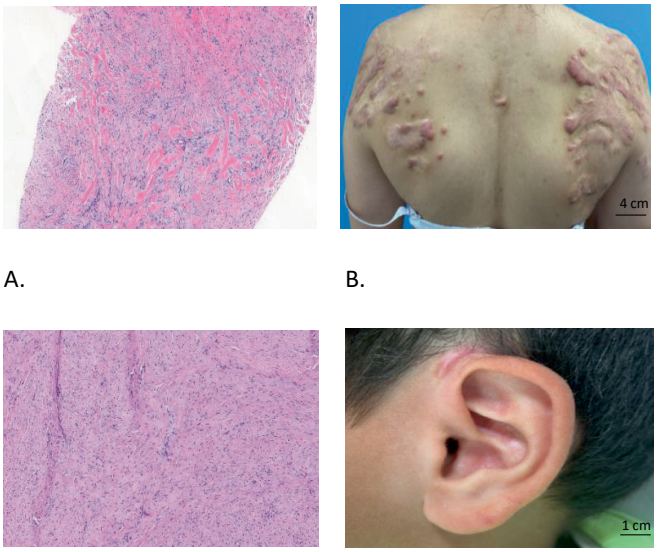
The word keloid is derived from the Greek word 'chele', meaning crab's claw because of its typical sideways growth into normal skin.[1] Keloids are fibroproliferative scars that cause reduced quality of life, due to pain, itch, social stigma and restriction of movement.[2] The mean Dermatology Life Quality Index (DLQI) in patients with keloids is 7.8 ± 5.1, meaning that keloids have a 'moderate effect' on patients quality of life, comparable to patients with mild to severe psoriasis vulgaris.[3]

Keloids can develop due to inflammation such as inflammatory acne and folliculitis or after (minor) trauma.[4] Keloid formation may be caused by a dysregulation in at least one of the four phases of wound healing: hemostasis, inflammation, proliferation and remodeling.[5,6] The inflammatory response in wound healing may play a crucial role in keloid formation. In keloids proinflammatory factors including interleukin-1α, -1β -6 and tissue growth factor-beta 1 (TGF-β1) are upregulated.[7] The upregulation of these proinflammatory factors suggests that the formation of keloids can be considered as an inflammatory disorder of the reticular dermis.[7] Moreover, recent studies have proposed that keloids possess features of an auto-immune disease, since anti-hnRNPA2B1 autoantibodies, Immunoglobulin A and M, and Complement components C1Q and C3 depositions were found in keloid tissue.[8,9]

Keloids vs. hypertrophic scars

Keloidal scars differ from hypertrophic scars because keloids will expand beyond the borders of the original wound while hypertrophic scars do not.[10] However, keloids also differ from hypertrophic scars in other aspects; keloids are histologically different, often cause more symptoms, and are more difficult to treat (Figure 1A-1D).[11,12] On histological examination, keloids show thicker collagen bundles, without a distinct pattern, while hypertrophic scars show relative thinner collagen bundles in a regular pattern.[13] The prevalence of keloids strongly differs among different ethnic populations. Keloids occur most frequently in the African, Asian and Hispanic population (prevalence up to 16%),

while the European population is least affected (prevalence of <0.1%).[14] However, hypertrophic scars develop much more frequently (prevalence of 32-72%).[15]



A. B.

C. D.

Figure 1.

A. Keloidal scar: dermis with increased cellularity, particularly fibrohistiocytic cells with intervening vessels and keloidal collagen.
B. A 34 year old female patient with multiple keloidal scars on the scapulae.
C. Hypertrophic scar: dermis containing intersecting bundles formed by proliferation of fibrohistiocytic cells with a small amount of collagen consistent with hypertrophic scar tissue.
D. A 12 year old male patient with a hypertrophic scar on the medial side of the left helix.

**All patients have provided written informed consent for publication of their photographs. The histological images (A, C) are provided by the pathology department of Erasmus MC without scale, and do not correspond to the clinical pictures (B, D).*

Patients with keloids: a heterogeneous population

The clinical presentation of keloidal scars highly varies among patients. Patients can have one small keloid that causes minimal symptoms, but patients can also be severely affected with >70 keloids spread over different anatomic locations causing extreme

physical symptoms, and is also associated with depression.[16] For this reason, keloids are a heterogenous group of fibroproliferative scars. Several genetic factors play a role in the development of keloids. A Japanese genome-wide association study (GWAS) was the first to reveal four single-nucleotide polymorphisms (SNPs) in three chromosomal regions: 1q41, 3q22.3-23 and 15q21.3.[17] Subsequently, another study found that these SNPs, specifically the rs8032158 SNP, may influence clinical keloid severity and may be a used as a biomarker for the prevention and treatment of keloidal scars.[18] Recently, also other genes such as TFCP2L1 have been identified by machine learning and RNA-sequence that may serve as potential biomarker for keloid development.[19] Other risk factors for keloid development include local factors (e.g. tension on scar and local infection), systemic factors (e.g. hypertension and rheumatism) and lifestyle factors (e.g. smoking).[20-22]

With regards to the phenotypes of keloids, a distinction has been made between 'mild' and 'severe' keloids.[9] Severe keloids are defined as multiple or large (>10 $cm^{2)}$) keloids.[23] The heterogeneity among keloids is often not considered in clinical studies and may explain differences in results between studies. Besides genetic factors, keloid severity and treatment response may be influenced by the location-, thickness-, and treatment history of keloids.[22,24] Therefore, the 'standard keloid' patient does not exist. However, especially in the severely affected patient group the burden of disease seems very high.

Keloid treatment

Several factors limit the risk of developing keloidal scars. Preventive measures include minimalizing tension on wounds (e.g. with z-plasty), infection prevention, hydration of wounds with silicon gels or - plasters, and of course minimalizing trauma to the skin as much as possible.[12,22,25] However, up to date, clinicians face difficulties in the treatment of keloidal scars. Several anti-inflammatory treatments have been used to improve keloidal scars, including intralesional treatment with corticosteroids, or chemotherapeutics e.g. bleomycin and 5-fluorouracil.[26] Yet, unfortunately no 'holy grail' to treat this heterogeneous group of fibroproliferative scars has been found. Especially severe keloids are challenging to treat in clinical practice, which can severely affect

patients' lives. Therefore, there is a high need for new efficacious, safe and minimal invasive treatment options for patients with severe and/or recalcitrant keloids.

Conventional hypodermic needles

Injection with conventional hypodermic needles is among the most commonly used procedures in clinical practice, but is hampered by high procedure-related pain and cannot be used in patients with (acquired) needle-phobia.[27] Previous literature shows that approximately 2 in 3 children and 1 in 4 adult patients fear needles.[28] The exact cause of needle-phobia is not fully understood, but traumatic experiences associated with needles during childhood (or during treatment) may set the stage for some patients.[7] Other disadvantages of conventional needle injections include a lack of standardization: volume, pressure and the depth of the injection varies and is highly operator dependent. Furthermore, the procedure related pain can significantly hamper the effectiveness of the treatment and some patients prematurely decide to discontinue treatment. In addition, conventional needle injections can technically be difficult to administer in keloids, because of the tough fibroproliferative scar tissue.[29]

Alternative dermal drug delivery techniques

Dermal drug delivery facilitates targeting of active substances to the appropriate skin layer(s) for the treatment of dermatological diseases.[30] However, the *stratum corneum*, the most superficial layer of the skin, plays a crucial role as the physical barrier against pathogens but also to the penetration of compounds into skin.[30] Drugs can only be delivered passively via the skin if it has an adequate lipophilicity and a low molecular mass preferably <500 Da.[31] Due to the physical barrier of the skin, medication containing larger drug molecules may not be absorbed via the skin at all, and therefore have no efficacy when applied topically.[32]

To overcome the main obstacle, the stratum corneum, drugs can be injected intradermally or intralesionally using conventional hypodermic needles. However, due to

the aforementioned limitations conventional needle injections are far from optimal. Therefore, several energy-based devices have been developed which can increase the skin bioavailability of the administered drug, including ablative (fractional) lasers, micro-needles, microdermabrasion, iontophoresis, electroporation, sonophoresis and needle-free jet injectors.[33,34]

Needle-free jet injectors

Needle-free jet injectors (NFI) are devices that are used for non-invasive drug delivery.[35] These devices operate by a high-velocity jet (between 100-200m/s) which punctures the skin and thereby enables delivery of therapeutics in the epidermis, dermis, subcutis or muscle.[36,37] A conventional NFI consists of 3 key components: (1) a nozzle, (2) an injection chamber which holds the drug, and (3) a pressure source that generates a high-velocity jet.[38] The pressure required to generate these high-velocity jets can be generated by a compressed gas such as CO2 or N2, or Lorentz- or piezoelectric actuators.[36]

The drug absorption and the injection-related pain of jet-injections is greatly influenced by the distribution pattern and penetration depth.[39] How a therapeutic is distributed is affected by several characters, including the *physical drug properties* (influenced by the density, viscosity and formulation of the fluid), *jet velocity* (influenced by pressure, filling volume, stand-off distance and nozzle diameter), and *skin characteristics* (influenced by elasticity, epidermis thickness, porosity, density and hardness).[36]

NFI can overcome several limitations that conventional hypodermic needles are faced with. They can minimize treatment-related pain, are free of risk for needle stick injuries and cross-contamination, and can be an alternative treatment option for patients experiencing needle phobia. Moreover, in a previous *ex-vivo* study with normal skin it was shown that NFI offers a more even distribution of fluids in the skin compared to hypodermic needles.[29]

Developing new NFI technologies

Up to date several types of jet injectors have become available and are increasingly used for intralesional treatment of various dermatological indications (Figure 2).[40] The first concept of a NFI was already developed in the 1860s. However, it then took several decades before the first spring loaded jet-injectors (SLI) was introduced in clinical practice in 1956.

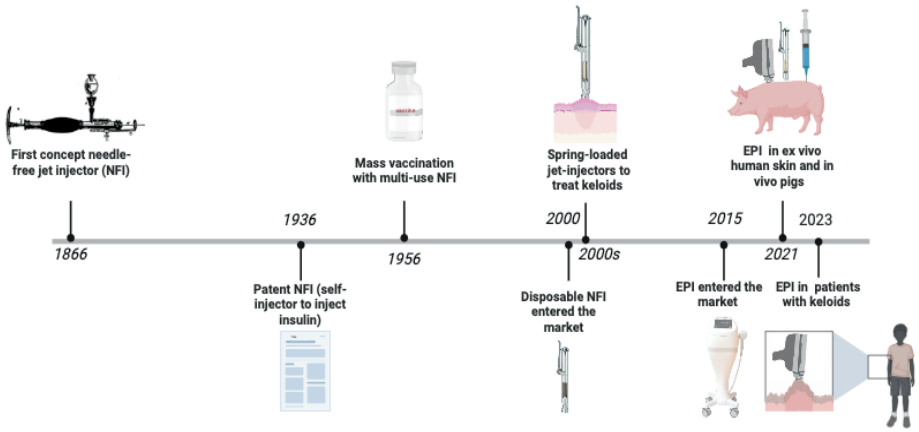


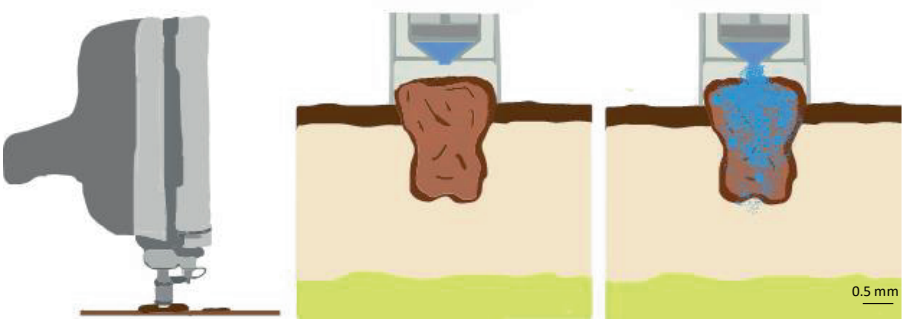
Figure 2.

Timeline from the first prototype of a needle-free jet injector developed in the 19th century to the clinical application of electronic pneumatic jet injectors to treat patients with keloids in the 21st century.

Various types of jet-injectors were subsequently used for mass vaccinations worldwide, to immunize people against e.g. smallpox and typhus (Figure 3).[41] Although the results were convincing as no smallpox epidemics were reported in 1990, in the same year a hepatitis B outbreak occurred which was related to contamination via the NFI.[42] This contamination was caused by splash-back of aerosols carrying hepatitis B viral particles when the same nozzle was used in multiple patients.[38]

Figure 3.
Since the 1950's, needle-free jet injectors have been employed globally to provide mass vaccination in both adults and children.

Consequently, innovative technological improvements such as the usage of disposable nozzles and the implementation of adjustable settings in NFI were made, that improved the safety and broadened the scope of their application.[36] Around 2015 electronically pneumatic jet injectors (EPIs), i.e. electronically controlled NFIs, were introduced (Figure 4).[27]



A. B. C.

Figure 4.

Illustration of an electronically-controlled pneumatic jet injector-assisted treatment with bleomycin and lidocaine in a keloid scar. (A) Before administering treatment, the electronically-controlled pneumatic jet injector (EPI) hand piece with the injector tip is placed perpendicularly on the keloid scar. (B) A cross-sectioned illustration of the injector tip and nozzle of an EPI-device. The liquid container within the EPI contains a solution with the combination of bleomycin and lidocaine (depicted in blue). (C) Illustration during injection. The EPI device generates a high-velocity jet stream that punctures the epidermis of the keloid, disperses the combination of bleomycin and lidocaine in the mid-deep dermis and creates visible skin papule or blanching.

Recently, also the first needle-free jet-injector that relies on laser energy, the Mirajet, was commercialized.[43] This EPI uses an Erbium YAG laser that generates vapor bubbles to generate pressure. These NFIs are yet being used to deliver small volumes in the superficial layers of the skin,[27] and therefore they are probably more appropriate for soft tissue rather than treatment of keloids or other rigid skin disorders. Currently, a smaller and more affordable NFI prototype is being developed for medical applications by the group of Prof. Fernandez Rivas from the University of Twente. These NFIs operate using lasers with microfluidic components. Up to date this prototype has been investigated in skin models such as porcine skin. Potentially, in the future these NFIs may be used as portable device in a home setting by patients for indications such as acne scars and alopecia areata.

The choice of jet injector may depend on the treatment indication, because the penetration depth and distribution patterns vary among jet injectors (Table 1). Other

factors that may play a role in the choice of NFI in clinical practice are costs and ease of use, for example SLIs are relatively easy to use, mobile and cheap. In the more innovative EPIs the volume and pressure can be adjusted, enabling more controlled and reliable drug delivery. The downside is that they are not portable, and relative expensive compared to the first generation SLIs. However, EPIs have shown to facilitate more standardized and consistent drug delivery compared to SLIs.

Clinical endpoints

In order for a treatment to be effective for keloids, it is important that the reticular dermis is reached.[44] A previous *ex vivo* study by Bik *et al.* has shown that the formation of a papule is an immediate skin response that is directly visible after NFI-assisted injections, and indicates successful dermal drug delivery in normal skin.[29] Therefore, it can be used as clinical endpoint when using NFI. However, the direct skin responses in keloids have not yet been studied, and may differ from normal skin because the fibrous tissue can be very thick and rigid.

Intralesional corticosteroid treatment

The most commonly used therapy for keloids is intralesional corticosteroid treatment using conventional needle injections, of which triamcinolone acetonide (10 to 40 mg per ml) is most frequently used.[45] It is easy to perform, widely available and relatively cheap.[46,47] Corticosteroids induce keloid regression through several mechanisms: suppression of inflammation by inhibition of cytokines, vasoconstriction which induces hypoxia, and inhibition of keratinocytes and fibroblasts proliferation due to its anti-mitotic effect.[48,49]

Overall, intralesional corticosteroids offer an effective treatment for keloids, and response rates vary from 50 to 100% after one year.[49] However, recurrence rates appear to be high, and range from 33%–50% after five years.[50] Moreover, intralesional corticosteroids appears to be less effective in severe keloids, and local adverse events such as fat atrophy,

hypopigmentation, and telangiectasia are frequently observed after treatment.[51] Although uncommon with intralesional corticiosteroid delivery, there is also a risk of systemic adverse events such as immunosuppression, adrenal insufficiency, and Cushing's syndrome, particularly with long-term use of high doses. Other intralesional treatment options are cryotherapy, and chemotherapeutics such as 5-Fluorouracil and bleomycin.[52] A meta-analysis showed that bleomycin treatment was associated with a lower risk of keloid recurrence compared to 5-Fluorouracil monotherapy or combination therapy with 5-Fluorouracil and triamcinolone.[53]

Intralesional bleomycin treatment

Bleomycin is an antibiotic with chemotherapeutic properties, derived from *Streptomyces verticillus* bacteria.[54] This antineoplastic agent inhibits collagen synthesis via a decrease in TGF-β and causes DNA damage and cell apoptosis.[55] Moreover, it inhibits endothelial cell migration and hereby also exhibits anti-angiogenic activities.[56]

Bleomycin has been used for intravenous treatment of head and neck tumors, and off-label as intradermal treatment for several dermatological indications, including recalcitrant warts, hemangioma and non-melanoma skin cancer.[57] Its topical use is limited due to the large molecular mass (1415 Da) and high hydrophilicity.[58] However, it has been used successfully as intradermal treatment for keloids using conventional needles. Treatment with intralesional bleomycin potentially may lead to low recurrence rates in keloids due to its antimitotic and anti-angiogenic effects.[59]

Clinical application of NFI-assisted treatment with bleomycin in keloids

Bleomycin can be degraded by the enzyme bleomycin hydrolase. Since there is a low expression of this enzyme in the lungs, high dosages of bleomycin can result in lung fibrosis.[60,61] In patients treated for dermatological indications, lung toxicity has not been reported, probably because of the use of low dosages (usually 2–4 U). However, potentially harmful aerosols can form when bleomycin is administered with NFIs.[62]

Therefore, it is important to use adequate safety measures such as protective gloves, goggles, and a smoke evacuator or a FFP-2 mask to effectively capture these aerosols.[62]

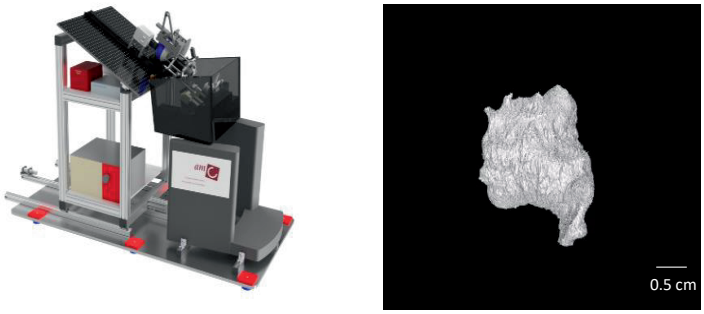
Moreover, intralesional bleomycin administration harbors the risk of mild to moderate adverse effects, including necrosis and ulceration. To minimize the risk of superficial necrosis, it is important to avoid too superficial drug delivery, and target the reticular dermis using a standardized and consistent drug delivery technique. EPI could potentially facilitate a standardized, effective and safe drug delivery of bleomycin in keloidal scars.

PRECLINICAL AND CLINICAL METHODOLOGY

In this thesis the clinical applicability of EPI-assisted jet injections in severe and recalcitrant keloids was studied using a variety of research techniques and designs. Prior to starting clinical research in patients with keloids, preclinical studies were performed using cutting-edge imaging techniques to provide a better understanding of the deposition and distribution of drugs in severe keloids after EPI-assisted drug delivery.

Previous work of Bik *et al.* has provided comprehensive information of how drugs are distributed in skin after EPI-assisted injection.[29,63] However, these experiments were limited to in vivo animal- and ex vivo human studies with normal skin. To gain more insights in the distribution of EPI-assisted jet injections in diseased tissue such as keloids, an ex vivo study that visualized distribution of EPI-assisted jet injections using the 3D-Fluorescent Imaging Cryomicrotome System (3D-FICS) in severe keloids was performed.

The 3D-FICS (Figure 5) is an innovative 3D imaging technique that enables visualization of fluorescent markers representing the biodistribution of fluorescent labeled-fluids. Various imaging techniques can be used to visualize the layers of the skin, including high frequency ultrasound, optical coherence tomography, and confocal microscopy.



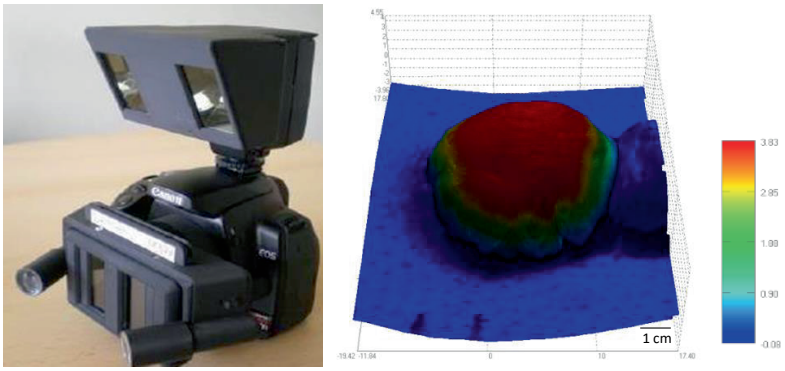
A. B.

Figure 5.

A. The 3D-Fluorescent Imaging Cryomicrotome System (3D-FICS), a custom-built innovative 3D imaging technique that enables visualization of fluorescent markers representing the biodistribution of fluorescent labeled-fluids.
B. A 3D image representing the biodistribution of fluorescent labeled triamcinolone acetonide administered in a keloid sample.

In contrast to older imaging techniques, our custom-built 3D-FICS can be used for high resolution segmentation of larger volumes, for example to visualize the biodistribution of fluorescence-labeled drugs in frozen tissue in 3D images. The 3D-FICS allows examination of deeper tissue layers, and can reconstruct volumes in organs with high spatial resolution (8-32 micrometer).[64] This novel 3D imaging technique has been used for the first time in cardiology to visualize monocytes in the coronary arteries.[65] In this thesis we describe the first time utilization of the 3D-FICS to explore the distribution of EPI- and needle assisted injections in ex vivo keloid- and normal skin samples.

In addition, a powerful 3D-camera to capture high resolution images was used in two studies in this thesis (Figure 6). Firstly, this technique was used to measure papule dimensions, directly after EPI-assisted jet injections in ex-vivo keloids. Secondly, a 3D-camera was used in the BLEOJET study, a randomized controlled split lesion clinical trial comparing the effects of bleomycin with normal saline. The 3D-camera facilitates the measurement of objective changes including volume, height, and roughness, before and after treatment of keloids.

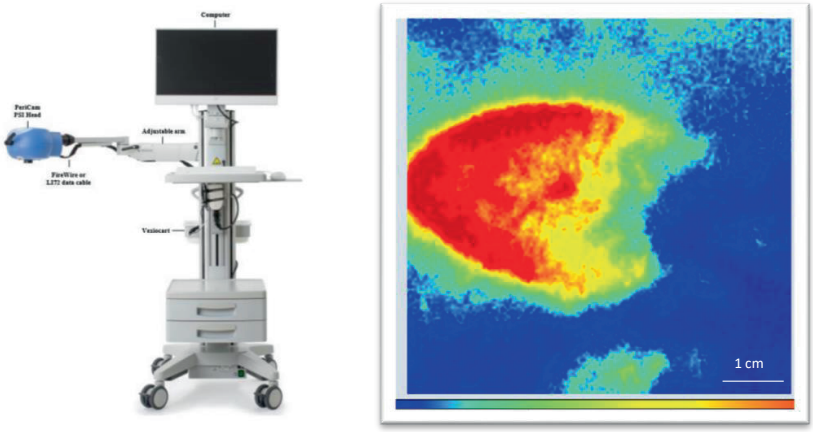


A. B.

Figure 6.

A. A stereophotogrammetric three-dimensional camera (LifeViz® Micro, Quantificare, Sophia Antipolis, France).
B. A three-dimensional reconstruction of a keloid lesion before EPI treatment, after image reconstruction with a heat map showing the height of the object which is used for the 3D analysis.

Furthermore, in the BLEOJET study, the Laser Speckle Contrast Imaging (LSCI) technique was used (Figure 7). This imaging technique is a highly sensitive technique to visualize the blood flow of the skin.[66] Erythrocytes act as a virtual contrast agent, outlining blood vessels and hereby enable the highly sensitive measurement of blood flow in the skin, which may correlate with the extent of dermal inflammation in keloids. In skin, erythrocytes are the main source of moving scatters. When coherent laser light interacts with skin tissue containing moving erythrocytes, it produces a speckle pattern because of the interference of scattered light waves. This pattern changes over time as erythrocytes move, with regions of slower flow exhibiting less variability in intensity compared to faster flowing areas.



A. B.

Figure 7.

A. The Laser Speckle Contrast Imaging (LSCI), a highly sensitive technique to visualize the blood flow of the skin.

B. Visualization of the blood flow of a keloid lesion before EPI treatment using the LSCI, with a heat map showing the intensity of the blood flow.

The final step in evaluating the clinical applicability of EPI-assisted jet injections in recalcitrant keloids was to examine the effectiveness, tolerability and patient satisfaction in a real-world setting. Tolerability, safety and patient satisfaction were assessed by daily photographs made by the patient using an innovative custom-made e-diary app and

routinely asking about injection-related pain and side effects. Two retrospective cohort studies that evaluated the effectiveness, tolerability and patient satisfaction in patients in a real-world setting were performed.

Since patients with recalcitrant keloids have been treated successfully and safe according to the BLEOJET study, hereafter adult patients from the outpatient clinic of Erasmus MC with recalcitrant keloids were offered treatment with a combination of bleomycin and lidocaine according to regular care. Lidocaine was added to the bleomycin in a real-world setting, because according to the data from the BLEOJET study, EPI-assisted injections with bleomycin monotherapy still resulted in relatively high injection-related pain, although assessed as less painful than needle-assisted injections. Patient reported outcome measures including the Patient and Observer Assessment Scale (POSAS) were used to assess effectiveness.

OBJECTIVES AND OUTLINE OF THIS THESIS

The aim of this thesis was to investigate the efficacy, safety and patient satisfaction of an innovative needle-free drug delivery device (Enerjet) to treat patients with keloidal scars. In order to study this, various research techniques and designs were used. First, the available evidence for needle-free jet injectors in dermatology was studied according to a systematic review approach. Hereafter, an ex vivo study was performed to visualize the distribution patterns of EPI-assisted jet injections in keloids. A randomized controlled trial was performed to study the efficacy and safety of EPI-assisted bleomycin in patient with recalcitrant keloids. Subsequently, two retrospective real-world studies using EPI-assisted drug delivery were performed. The first study aimed to explore the effectiveness, tolerability and patient satisfaction of EPI-assisted bleomycin and lidocaine in adult patients with recalcitrant keloids. The second study was performed to explore the effectiveness, tolerability and patient satisfaction of EPI-assisted triamcinolone in children with keloids or hypertrophic scars.

Chapter 2

The second chapter of this thesis describes the current evidence regarding the efficacy and safety of needle-free jet injectors to treat various dermatological indications. The available evidence for needle-free jet injectors in dermatology is summarized and critically appraised in a systematic review with risk of bias assessment.

Chapter 3

The third chapter describes the distribution of EPI-assisted jet injections in severe keloids by performing pre-clinical experiments. In this chapter we performed an *ex-vivo* study, in which EPI-assisted jet injections and conventional needle injections in normal skin samples and recalcitrant keloid samples were visualized using the 3D-FICS, an innovative 3D-imaging technique.

Chapter 4

The fourth chapter describes the clinical factors that may negatively influence the treatment response in keloids according to previous literature. In this chapter we

performed a systematic review, in which we describe the available evidence for clinically relevant keloid properties.

Chapter 5

The fifth chapter describes the clinical application of EPI-assisted bleomycin in *in vivo* severe and recalcitrant keloids. In this chapter we performed a double-blinded, randomized, placebo-controlled trial with split-lesion design, to investigate the efficacy and safety of EPI-assisted bleomycin compared to placebo in patients with severe keloids.

Chapter 6

The sixth chapter describes the findings of a retrospective cohort study that investigated the effectiveness, tolerability and patient satisfaction of EPI-assisted bleomycin combined with lidocaine in patients with recalcitrant keloids in a real-world setting.

Chapter 7

The seventh chapter describes treatment with the EPI in children, to explore a trauma-free, minimally invasive treatment option in children with keloids and hypertrophic scars. In this chapter we performed a retrospective cohort study in children, to study the effectiveness, tolerability and patient satisfaction of EPI-assisted triamcinolone in children with keloids or hypertrophic scars.

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Section I

Needle-free jet injectors in dermatology

Chapter 2

Efficacy and safety of needle-free jet injector-assisted intralesional treatments in dermatology—a systematic review

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ABSTRACT

Needle-free jet injectors are used for the intralesional treatment of various dermatological indications. However, a systematic review that evaluates the efficacy and safety of these treatments has not been published. The objectives of this study are to evaluate the efficacy and safety of needle-free jet injections for dermatological indications and to provide evidence-based treatment recommendations. An electronic literature search was conducted in April 2022. Two reviewers independently selected studies based on predefined criteria and performed a methodological quality assessment using the Cochrane Collaborations risk-of-bias 2.0 assessment tool and Newcastle-Ottawa Scale. Thirty-seven articles were included, involving 1911 participants. Dermatological indications included scars, alopecia areata, hyperhidrosis, nail diseases, non-melanoma skin cancer, common warts, local anesthesia, and aesthetic indications. Keloids and other types of scars (hypertrophic, atrophic, and burn scars) were investigated most frequently (n = 7). The included studies reported favorable efficacy and safety outcomes for intralesional jet injector-assisted treatment with triamcinolone acetonide/hexacetonide, 5-fluorouracil, bleomycin, or hyaluronic acid. Two high-quality studies showed good efficacy and tolerability of intralesional jet injections with a combination of 5-fluorouracil and triamcinolone acetonide in hypertrophic scars and with saline in boxcar and rolling acne scars. No serious adverse reactions and good tolerability were reported in the included studies. Overall, the methodological quality of the included studies was low. Limited evidence suggests that needle-free jet injector-assisted intralesional treatment is efficacious and safe for hypertrophic and atrophic acne scars. More well-powered RCTs investigating the efficacy and safety of jet injector treatment in dermatology are warranted to make further evidence-based recommendations.

INTRODUCTION

Intradermal drug delivery has many advantages over other routes of administration, especially high bioavailability in the skin.[1,2] Over the past decades, a variety of needle-free devices that enable intradermal drug delivery has been developed, including fractional ablative lasers, iontophoresis, sonophoresis, and various types of mechanical and energy-based jet injectors.[3-5] Jet injectors are commonly used for the intralesional treatment of several dermatological conditions such as keloids, hypertrophic scars, and recalcitrant viral warts.[6,7] Traditional mechanical jet injectors act with a fixed pressure predetermined by spring size.[8] Innovative electronically controlled pneumatic jet injectors are devices in which volume and pressure can be controlled by accelerated and compressed gas as pressure source, which dispense fluids into the skin.[7,9] Other types of jet injectors are controlled by Lorentz or piezoelectric actuators, lasers, and shockwaves to pressurize the injected drug.[10]

In contemporary healthcare, we are moving towards more patient-centered care. It is important to improve patient comfort and avoid physical or psychological harm as much as possible. According to a previous study, 63% of children and 24% of the adult population in the USA fear needles.[11] This is one of the reasons why jet injectors can be a viable alternative for conventional needles.Needle-free jet injectors can be an attractive alternative for hypodermic needles for patients experiencing needle phobia, minimize treatment-related pain, and are free of risk for needlestick injuries and cross-contamination. Additionally, jet injectors enable accurate and reproducible dermal delivery of liquid drugs and disperse the drug more evenly in the skin than conventional needle injections.[7, 9, 12, 13]

At present, there are a few overviews and narrative reviews describing the use of jet injector-assisted intralesional treatment for different dermatological indications.[7, 10, 12, 14] However, a systematic and critical review that evaluates the efficacy and safety of jet injector-assisted intralesional treatment in dermatology is lacking. In this review, we aimed to systematically review and evaluate the quality of clinical evidence for intralesional treatment of dermatological indications using needle-free jet injector systems and provide evidence-based recommendations for clinical practice.

MATERIALS AND METHODS

A literature search was conducted in April 2022 using Embase, MEDLINE ALL Ovid, Web of Science, and Cochrane Central Register of Controlled Trials databases, to identify relevant publications. This systematic review was registered in the PROSPERO (CRD42021258278) and followed the Preferred Reporting Items for the PRISMA 2020 checklist.[15]

Studies were included if they were human studies, written in English, published from inception to April 2022, randomized controlled trials (RCTs), controlled clinical trials (CCTs), prospective or retrospective cohort studies, and case series and included patients of all ages with dermatological indications eligible for intralesional treatment using needle-free jet injectors. Exclusion criteria included studies with fewer than 10 patients and intramuscular or subcutaneous drug delivery.

Selection of the articles, standardized data extraction, and methodological quality assessment of the included studies were performed independently by two authors (V.B. and J.V.H.). Articles were screened based on title and abstract. The primary outcome measure was efficacy, and the secondary outcome measure was safety. For data extraction, we converted pressure settings, total injection volume, and drug concentration to psi, ml, and mg/ml, respectively. If possible, efficacy measures were simplified to percentages in terms of clinical response compared to baseline. Methodological quality was assessed using the Cochrane Collaborations risk-of-bias 2.0 tool (ROB 2.0) for RCTs and CCTs, and the Newcastle–Ottawa Scale (NOS) for cohort studies and case series.[16–19] Final selection of the articles was based on screening of full texts. Discrepancies between reviewers were discussed and resolved by consensus and involved a third author (L.B.) if necessary. Illustrations of the methodological quality assessments were created using Robvis.[17]

RESULTS

Our literature search identified 1326 records. Duplicates were removed. Based on title and abstract, 985 articles were screened. Full texts of 71 articles were assessed for eligibility of which 37 studies were selected with a total of 1911 participants (Fig. 1). The included studies comprised 6 RCTs, 6 CCTs, 16 prospective cohorts, 5 retrospective cohorts, and 4 case series. The studies investigated needle-free jet injector-assisted intralesional treatments for atrophic and hypertrophic scars, keloids, alopecia areata, hyperhidrosis, nail diseases (psoriasis, lichen planus, and idiopathic onycholysis), non-melanoma skin cancer (basal cell carcinoma (BCC), squamous cell carcinoma (SCC), Bowen's disease, and Paget's disease), common warts, granuloma annulare, lichen simplex chronicus, psoriasis, seborrheic dermatitis, aesthetic indications (wrinkles, rejuvenation, rhytides, facelift), and local anesthesia.

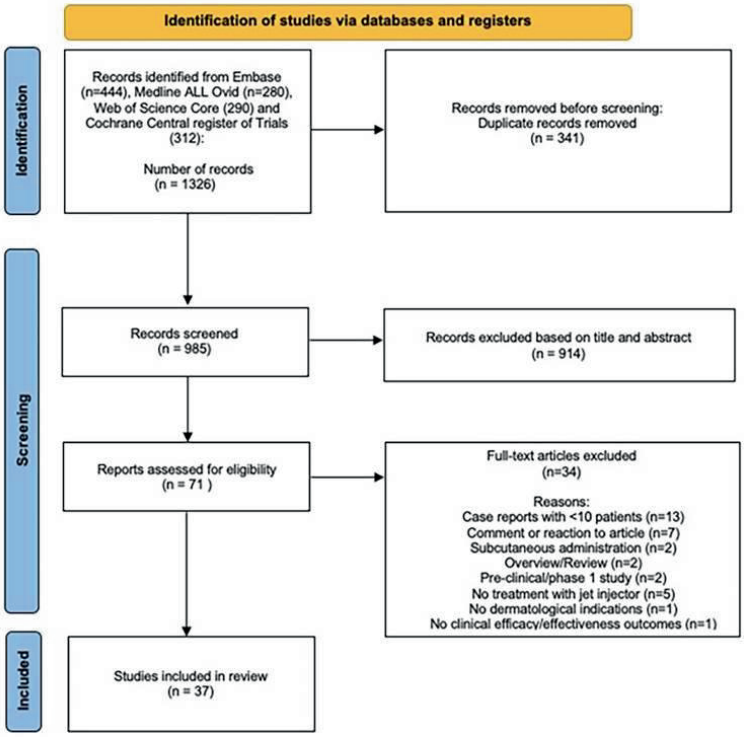


Figure 1.

Study flow diagram of exclusion process resulting in 37 included studies

Scars and keloids

Seven studies, investigated jet injections to treat various scar types (Table 1).[20–28] Compared to baseline, spring-loaded jet injections with triamcinolone acetonide (TCA) and silicone sheets showed significant scar thickness reduction in hypertrophic scars, while silicone sheets alone did not (3–5 treatments; $p < 0.05$; $p > 0.05$).[21] Moreover, pneumatic jet injector-assisted treatment with a mixture of hyaluronic acid and hypertonic glucose led to a reduction in mean scar volume of 0.4 mm3 compared to the untreated side in atrophic facial acne scars (single treatment; $p < 0.05$).[23] Spring-loaded jet injections with bleomycin in keloids and hypertrophic scars led to reduced pain and pruritus with respectively 88% and 89% (2–6 treatments; no comparative intervention; no statistical analyses reported).[26] Furthermore, pneumatic jet injections with 5-fluorouracil (5-FU) diluted in corticosteroids (TCA or methylprednisolone acetate) and lidocaine led to a significant reduction of pain and pruritus in patients with keloids, with respectively 69% and 79% compared to baseline (7 treatments; no comparative intervention; $p < 0.01$; $p < 0.05$).[27] Pneumatic jet injections of hypertonic glucose resulted in a mean Global Aesthetic Improvement Scale (GAIS) of 2.3 ± 0.8 in atrophic scars, striae, and wrinkles compared to baseline (1–5 treatments; no comparative intervention; no statistical analyses).[24] In comparison, jet injections with non-crosslinked and crosslinked hyaluronic acid injections in acne and hypertrophic scars resulted in overall GAIS of 1.9 and 1.8 respectively (mean 2.5 treatments; no statistical analyses).[25] Jet injections (unknown injector type) with triamcinolone hexacetonide resulted in “good,” “acceptable,” and “negative” results in respectively 68.2%, 15.9%, and 15.9% of children with burn scars (1–4 series, no comparative intervention; no statistical analyses).[28]

Local anesthesia

Three studies investigated local anesthesia administered by a spring-loaded jet injector before suturing or performing dermatological surgery (Table 2).[48–50] Jet injections with mepivacaine chloride resulted in “no pain” in 79.6% of the lesions during surgery (no comparative intervention; no statistical analyses).[50] Lidocaine administered with a jet injector compared to injections with a hypodermic needle resulted in a mean anesthesia-related Visual Analogue Scale (VAS) score of 1.1 vs. 4.4 respectively ($p < 0.0001$), while

suturing-related pain was not significantly different ($p > 0.05$).[48] Lidocaine administered with a jet injector vs. needle injections resulted in “no pain” during suturing in respectively 94% vs. 83% of the children.[49]

Aesthetics

Six studies investigated intralesional pneumatic jet injections in the face or neck for aesthetic purposes (Table 2).[51–56] Jet injections with hypertonic glucose compared to isotonic glucose improved GAIS with a mean score of respectively 2.5 ± 0.7 vs. 3.1 ± 0.9 (3 treatments; $p = 0.005$).[51] To compare, jet injections with non-crosslinked hyaluronic acid resulted in “improved” and “much improved” GAIS in 42.9% and 57.1% of the patients respectively (5 treatments; no comparative intervention; no statistical analyses).[54] Crosslinked hyaluronic acid using jet injections reduced mean Fitzpatrick–Goldman Wrinkle Classification with 21.2% and 27.6%, respectively in the neck and face (1–4 treatments; no comparative intervention; $p < 0.05$; $p < 0.05$).[56] Hyaluronic acid with jet injections or multi-needle injections and placebo with jet injections or multi-needle injections reduced Wrinkle Severity Rating Scale compared to baseline with 1.0 ± 0.6 vs. 1.5 ± 0.6 vs. 0.5 ± 0.8 vs. 0.5 ± 0.6, respectively (3 treatments; $p < 0.05$; $p < 0.01$; $p > 0.05$; $p > 0.05$).[52] Jet injections with hyaluronic acid reduced Mean Lemperle Rating Score with one point in all areas (2.5 treatments; no comparative intervention; no statistical analyses).[53] Jet injections with hypertonic glucose showed “slight” or “notable” improvement in 91% of the patients (1 treatment; no comparative intervention; no statistical analyses).[55]

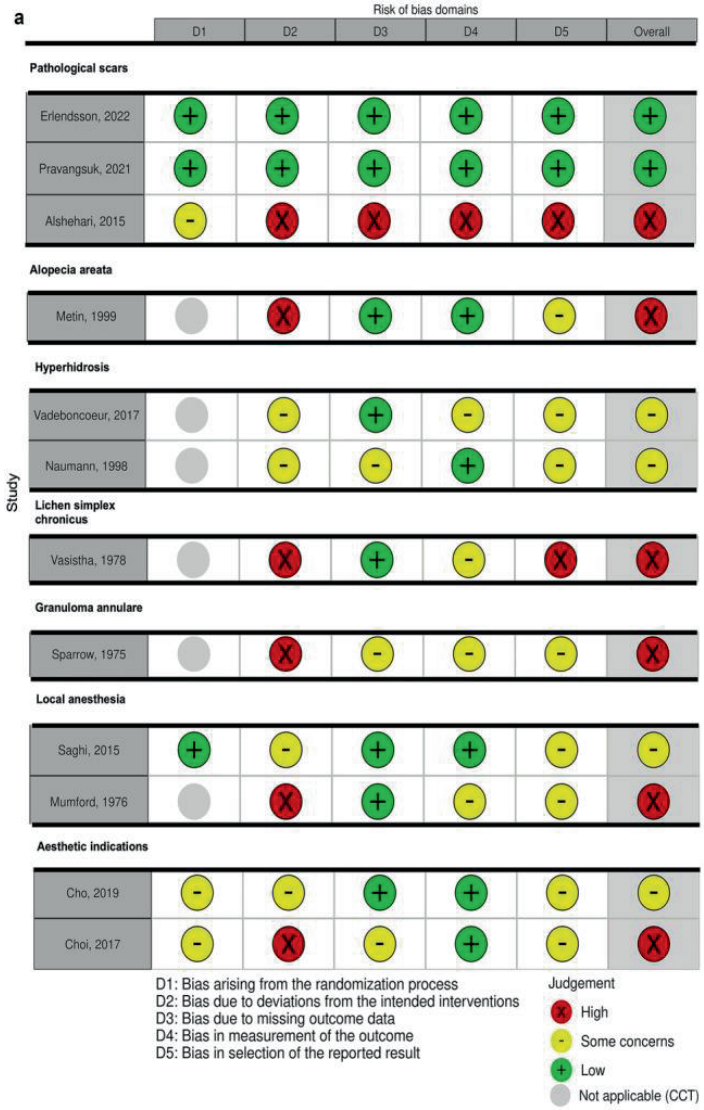
Adverse reactions

The majority of the adverse reactions were mild and the most common were local erythema, pain, hypo- and hyperpigmentation, bruising, hematoma, atrophy, swelling, and itching (Tables (Tables 1 and and2). No serious adverse events were reported. However, two studies that investigated bleomycin or interferon alfa-n2 delivered with a spring-loaded jet injector for palmar and plantar warts reported severe events including cellulitis, lymphangitis, and large hematomas, which needed surgical drainage and debridement.[42, 43] Also, TCA administered by a spring-loaded jet injector for the

treatment of alopecia areata resulted in bleeding from the arteria temporalis in one patient, which was controlled by firm pressure.[32]

Methodological quality assessment

Overall risk of bias assessed with Cochrane's ROB 2.0 tool was "high" in six RCTs and CCTs, "some concerns" in four studies, and "low" in two studies (Fig. 2a). Methodological quality was particularly poor due to deviations from the intended intervention and selection bias (Fig. 2b).



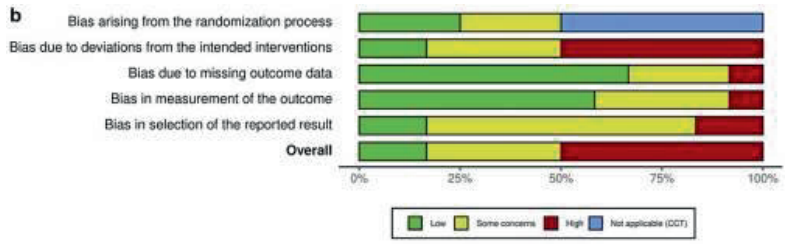
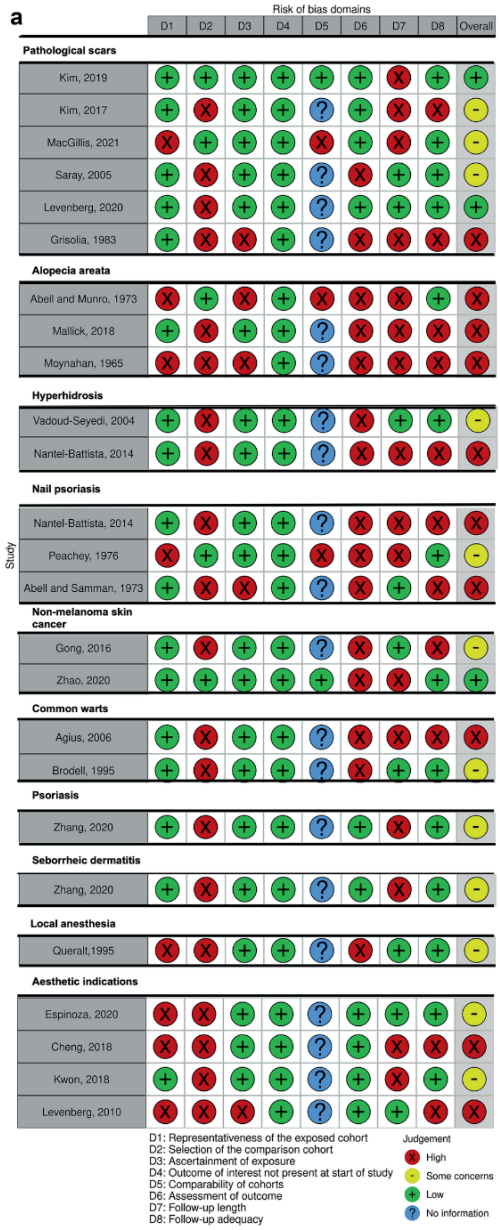


Figure 2.

A. Risk of bias in the included (non) randomized controlled trials was categorized as high, low or some concerns according to the Cochrane risk-of-bias 2.0 assessment tool. Overall, risk of bias was high because of poor methodological quality, particularly in domain 2 and 5.

B. Methodological quality of the (non) randomized controlled trials according to the Cochrane Collaborations risk-of-bias 2.0 tool assessment

According to the Newcastle–Ottawa Scale, overall risk of bias in the included cohorts and case series was “high” in eleven, “some concerns” in another eleven, and “low” in three studies (Fig. 3a).[16] Methodological quality was particularly poor due to lack of comparative cohorts, lack of blinding, and short follow-up time (Fig. 3b).



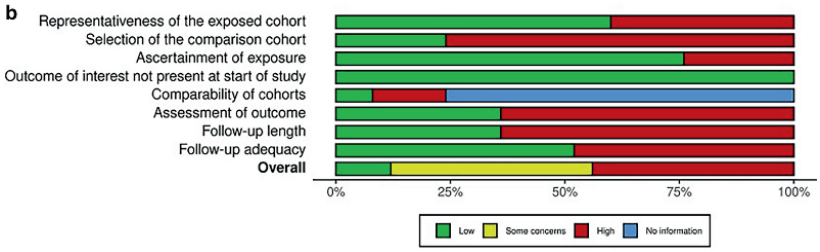


Figure 3.

A. Risk of bias in the included cohort studies and case series was categorized as high, low, some concerns or not applicable according to the Newcastle–Ottawa Scale. Overall, risk of bias was high because of poor methodological quality, particularly in domains 2, 6, and 7.
B. Methodological quality of the included cohort studies and case series according to the Newcastle–Ottawa Scale

DISCUSSION

In this systematic review, we summarized and critically appraised the current evidence on the efficacy and safety of jet injector-assisted intralesional treatments for dermatological indications. We selected 37 studies including 12 (randomized) controlled trials. The majority of studies had a “high risk of bias” or “some concerns” and only five studies (investigating acne scars, hypertrophic scars, keloids, and non-melanoma skin cancer) had “low risk of bias”. Furthermore, 19 of 37 studies lacked statistical analysis for the reported outcomes.

Due to large heterogeneity among studies with respect to a.o. study design, indication, type of jet injector, therapeutics, and outcome measures, a meta-analysis could not be performed.

Significant favorable effectiveness was reported in 13 of 15 studies, in which statistical analyses were reported. These studies investigated intralesional jet injections in scars, hyperhidrosis, nail psoriasis, non-melanoma skin cancer, seborrheic dermatitis, local anesthesia, and aesthetic indications. Most studies investigated keloids and other types of scars (hypertrophic, atrophic, and burn scars) and showed good efficacy and high tolerability.[21–26] Additionally, our review shows that despite differences in viscosity, several fluids have been successfully administered with jet injectors.

None of the included studies compared the use of spring-loaded vs. pneumatic jet injectors. In studies published before 2000, only spring-loaded jet injectors were used because pneumatic jet injectors were not yet introduced. Importantly, spring-loaded jet injectors were associated with a number of severe adverse reactions, including fluctuating cortisol levels and arteria temporalis damage in alopecia areata treated with TCA. Cellulitis, large hematomas, and lymphangitis occurred in patients with warts treated with spring-loaded devices and bleomycin or interferon alfa-n3.[30, 32, 42, 43] In contrast, no severe adverse reactions were reported in studies that investigated pneumatic jet injectors. Possibly, this could be related to the tunable settings for pneumatic jet injectors enabling safer and more effective treatment settings based on clinical endpoints, which are not available for spring-loaded injectors.[57]

Only five of the included studies compared patient-reported pain between needle-free jet injectors and conventional needle injections.[33, 34, 41, 48, 49] Jet injections with lidocaine caused significantly less injection-related pain, and less procedure-related pain with 5-ALA and PDT treatment in non-melanoma skin cancer compared to needle injections with 5-ALA and PDT.[34, 41, 48] Jet injections with botulinum toxin for palmar and axillar hyperhidrosis and with xylocaine for local anesthesia in children were better tolerated than conventional needle injections; however, no statistical analyses were performed.[34, 49] On the other hand, two studies investigating local anesthesia with lidocaine and palmar hyperhidrosis with onabotulinumtoxinA reported no significant difference in procedure-related pain between jet injections and conventional needle injections.[33, 48]

Risk of bias assessment resulted in two high-quality RCTs. The results of these studies suggest that jet injections with 5-FU and TCA and jet injections with saline in atrophic acne scars (boxcar and rolling) are efficacious, safe, and well-tolerated.[20, 22] Also, favorable efficacy and safety were found in cohort studies with low risk of bias for intralesional jet injections with 5-FU combined with corticosteroids in keloids and with hyaluronic acid in atrophic acne scars.

To our knowledge, this is the first systematic review that evaluated the efficacy and safety of intralesional treatment with jet injectors for dermatological indications. The strengths of this study include the use of a comprehensive database search, reporting of outcome measures as efficacy and adverse reactions, addressing jet injector settings, critical methodological quality assessment, and inclusion of all study designs with no limitation to publication date. Limitations of this systematic review include a majority of studies in cohorts or case series, noncomparative studies, poor methodological quality of the included studies, and missing of important clinical data such as skin type.

At our tertiary outpatient clinic, patients with keloids, hypertrophic scars, and recalcitrant warts are commonly treated with spring-loaded or pneumatic injectors to administer TCA, bleomycin or a mixture of both.

Moreover, we believe there is a significant clinical benefit of jet injector treatment in children (e.g., for keloids and hypertrophic scars), because in our experience they tolerate

the jet injections much better and cause less anxiety than conventional hypodermic needle injections.

Importantly, we strongly recommend the use of protective safety measures such as smoke evacuators and face masks due to the potential formation of harmful aerosols, especially when antineoplastic drugs such as bleomycin or 5-FU are administered.[9] Moreover, caution should be taken when using spring-loaded jet injectors in anatomical areas around large vessels, nerves, and bone, because potential damage can be inflicted with this type of fixed-setting jet injectors.[32]

Contemporary deficiencies of modern jet injectors include drug spill (residual fluid on the skin surface and formation of potentially harmful airborne small-droplet aerosols). Also, gas-compressed energy-based jet injectors create a relatively loud noise during the injection phase which may lead to anxiety in some patients.[6, 12, 58, 59] Therefore, opportunities for improvement of the needle-free injection technology in the future will lie in optimizing the injection efficiency, creating less noisy (smaller) devices, and the development of new technology to reduce the production or capture potentially harmful aerosols. Moreover, with respect to future research, good quality RCTs investigating the efficacy and safety of jet injectors in dermatology are highly needed to conduct a meta-analysis and produce stronger evidence that can be used to provide solid evidence-based recommendations for the use of jet injectors in clinical practice.

In conclusion, this systematic review presents an overview and methodological quality assessment of clinical data on the efficacy and safety of intralesional jet injection treatments for dermatological indications. Limited good quality data suggest that intralesional jet injection treatments with 5-FU and TCA in hypertrophic scars and with saline in atrophic acne scars are efficacious and well-tolerated.[20,22] In addition, some evidence suggests that jet injector treatment might be less painful for patients than conventional needle injections for certain indications. More high-quality randomized controlled trials are needed to provide future evidence-based recommendations for clinical practice.

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We thank Wichor M. Bramer, biomedical information specialist at Erasmus MC Rotterdam, for his contribution to the electronic database search.

2. Efficacy and safety of needle free jet injector assisted intralesional treatments in dermatology—a systematic review.

First author, year of publi-cation	Dermatological indication	Study design	No. of patients (+ lesions)	Skin-type	Type jet injector (brand) + (pressure)	Pressure in study	Fluid
Erlendsson, 2022	Hypertrophic scars	RCT	20 (?)	I-V	Pneumatic (Enerjet 2.0) (A)	30.4 – 52.2 psi	A: 5-FU + TCA
Alshehari, 2015	Hypertrophic scars	RCT	30 (?)	NR	Spring-loaded (Dermojet) (F)	1,420 psi	A: TCA + silicone sheet
Pravangsuk, 2021	Atrophic acne scars (boxcar and rolling)	RCT	18 (108)	III-IV	Pneumatic (Innojector) (A)	Unclear (level 2-3)	A: SAL
Kim, 2019	Atrophic acne scars (boxcar, rolling and icepick)	Prospective cohort	10 (13)	II-IV	A: Pneumatic (Airjet) (A)	Unclear (50% of total)	A: HA in hypertonic glucose
Kim, 2017	Atrophic scars (post acne, carbuncle, furuncle), striae and wrinkles	Prospective cohort	13 (13)	III-IV	Pneumatic (SheMax) (A)	52.6-67.3 psi	Hypertonic glucose
MacGillis, 2021	Scars, skin rejuvenation, striae	Retro-spectve cohort	115 (325)	NR	Pneumatic (Enerjet /Airgent) (A)	NR	Crosslinkec HA in SAL
Saray, 2005	Keloids and hypertrophic scars	Prospective cohort	14 (15)	II-IV	Spring-loaded (MadaJet XL) (F)	1800 psi	Bleomycin in SAL
Levenberg, 2020	Keloids	Retro-spectve cohort	20 (38)	NR	Pneumatic (Enerjet 2.0) (A)	43.5-82.3 psi	5-FU in MA/TCA + lidocaine 2%
Grisolia, 1983	Burn scars	CS	44 (?)	NR	NR	NR	TH in SAL
Metin, 1999	Alopecia areata	CCT	35 (?)	NR	NR	NR	A: BDSP or SAL

Total volume per lesion each treatment	Concentration	Total no treatments and interval	Comparison	Results per patient + significance (results per lesion + significance)	Follow-up time	Adverse reactions
0.32–0.70 ml	5-FU: 50 mg/ml, TCA: 10 or 40 mg/ml	1	B: No treatment	NR (Total VSS decreased in 55% and 25%, resp. in A and B, with median reduction of -1 in VSS score (0 in control; p= 0.09))	1 month	Severe: none Minor: punctate defects, hyperpigmentation
Single or multiple doses of 0.1 ml	40 mg/ml	3-5, 3 weeks	B: Silicone sheet alone	Scar thickness reduced in A and B, p<0.05; p>.05. (NR)	6 months	Severe: none Minor: pain
Unclear (first two treatments shots of 0.15 ml, third treatment shot of 0.1 ml)	9 mg/ml	3, 4 weeks	B: Needle sub-cision	NR (Mean scar volume reduced with 11.7% and 12.0% compared to baseline, resp. in A and B, p<0.001; p<0.001. No statistical difference between treatments)	1 month	Severe: none Minor: bruises, scale, hyperpigmentation, hematoma, edema, erythema and subcutaneous emphysema
NR (0.085 ml injection)	HA: 1 mg/mL, glucose: 200 mg/ml	1	B: No treatment	Mean scar volume reduced with ca. 0.4 mm3 and 0.0 mm3, resp. in A and B (p<.05). (NR)	2 months	Severe: none. Minor: swelling, spot bleeding
NR (0.08-0.1 ml per injection)	200 mg/ml	1-5, 3 weeks	None	Mean GAIS 1 month after final treatment 2.3 ± 0.8. NS. (NR)	2 months	Severe: none. Minor: spot bleeding, crusting, PIH
NR (0.05- 0.75 ml per injection)	2.5 mg/ mL	Mean 2.85, 12 weeks	Non-crosslinked HA	Overall GAIS score 1.78 and 1.6 resp. NCL-HA and CL-HA. NS. (NR)	> 3 months	Severe: none. Minor: bruises, temporary local edema
< 3.5 ml	1.5 IU/ml	2-6, 4 weeks	None	NR. (Mean scar height, pliability, erythema, pain- and pruritus score reduced resp. 3.20 mm, 2.64 mm, 2.13 mm, 88%, 89%, p < .001; p <.001; p <.001; p =.01. No recurrences)	16-24 months	Severe: none. Minor: hyperpigmentation and skin atrophy
0.5 – 10 ml	5-FU: 50 mg/ml, MA/TCA: 40 mg/mL	7, 2 weeks	None	NR. (Total VSS score decreased with 53% in all components, p <.05. Overall POSAS patient score decreased in all components from 39.54 ± 5.31 to 19.63 ± 6.30, P < .05. Pain and pruritus lessened resp. 69% and 79%, p < .05. No recurrence)	12 months	Severe: none. Minor: superficial ulceration
NR (mass < 5 mg)	2 mg/ml	1-4 series, 1-3 weeks	None	'Good' in 68.2%, 'acceptable' in 15.9% and 'negative' in 15.9%. NS. (NR)	NR	Severe: none. Minor: telangiectasia, increased hair growth, subcutaneous atrophy, ulcer
NR	NR	4, 3 weeks	B: Cyclosporine A or SAL	NR. (Regrowth in 88.2% and 66.6%, resp. in BDSP and Cyclosporine A. Regrowth in 11.7% and 16.6% in resp. A and B with SAL. NS.)	NR	NR

2. Efficacy and safety of needle free jet injector assisted intralesional treatments in dermatology—a systematic review.

First author, year of publi-cation	Dermatological indication	Study design	No. of patients (+ lesions)	Skin-type	Type jet injector (brand) + (pressure)	Pressure in study	Fluid
Abell, 1973	Alopecia areata	Prospective cohort	84 (111)	NR	Spring-loaded (Port-O-Jet) (F)	NR	TCA
Mallick, 2018	Alopecia areata	CS	100 (?)	NR	Spring-loaded (Dermojet) (F)	1,420 psi	TCA
Moynahan, 1965	Alopecia areata	CS	60 (60)	NR	Spring-loaded (Porton needleless injector) (F)	NR	TCA
Vadebon-coeur, 2017	Palmar hyperhidrosis	CCT	20 (40)	NR	Pneumatic (Med-Jet) (A)	140-150 psi	A: direct OnabotA in SAL
Naumann, 1999	Palmar and axillar Hyperhidrosis	CCT	20 (40)	NR	A: Spring-loaded (Dermojet) (F)	1,420 psi	A: BTX-A in SAL
Kim, 2020	Axillar and palmoplantar hyperhidrosis	Prospective cohort	20 (?)	NR	SheMax (A)	29.7 psi	BoNTA in SAL and lidocaine 2%
Vadoud-Seyedi, 2004	Plantar hyperhidrosis	Prospective cohort	10 (20)	NR	Spring-loaded (Dermojet) (F)	1,420 psi	BTX-A in SAL
Nantel-Battista, 2014	Nail psoriasis	Prospective cohort	16 (16)	NR	Pneumatic (Med-Jet) (A)	130-170 psi	TCA
Peachey, 1976	Nail psoriasis	Prospective cohort	37(37)	NR	Spring-loaded (Port-O-Jet) (F)	NR	A: TCA

Total volume per lesion each treatment	Concentration	Total no treatments and interval	Comparison	Results per patient + significance (results per lesion + significance)	Follow-up time	Adverse reactions
Mean: 2.8 ml	5 mg/ml	3, 1-2 weeks	None	Regrowth in 86% and 62%, resp.after 6 and 12 weeks. NS. (NR)	3 months	Severe: fluctuating cortisol Minor: hemorrhage, atrophy
NR (0.1 ml per injection)	10mg/ml	3-4, 4 weeks	None	Regrowth in 75%. Stratification for age, gender, duration, fam. history and size all >.05 (NR)	3 weeks	NR
< 6.0 ml	5 mg/ml	≤. 3, 1 week	None	Regrowth in 49% and 43% of adults and children, resp. NS. (NR)	NR	Severe: a. temporalis damage Minor: bleeding
5 ml	20 U/ml	1	B: NPT + lidocaine, CNI + OnabotA	HDSS score reduced with 1.6 and 1.25 resp. in A and B after 1 month, p = .031. Reduction at 3 and 6 months not statistically significant. (NR)	6 months	Severe: none. Minor: weakness, vasovagal symptoms, ecchymosis
BTX-A: 50 MU, SAL: 5ml	20 U/ml	1	B: CNI + BTX-A in SAL	Sweat production of 77.8 +/-8.4 and 72.2 +/-10.1 mg/ml at baseline, and 53.1 +/- 7.8 and 18.1 +/-3.3 mg/ml post treatment resp. in A and B, p<.05; p<.0001. (NR)	3- 4 weeks	Severe: none Minor: hematoma, transient paresthesia
4.8-6.4 ml	6.25 U/ml	1	None	NR (Median HDSS reduction from 3 to 1 and from 4 to 1, resp. axillar and palmoplantar, p <.001; p < .001)	1 month	Severe: none Minor: subcorneal blisters
UBTX-A: 50 U, SAL: 5ml	NR	1	None	After 5 months 70% was free of symptoms. NS. (NR)	8 months	Severe: none. Minor: localized hematoma
Ca 0.07 ml	8 mg/mL	4, 4 ± 1 weeks	None	Mean baseline NAPSI score was 6.5, mean final NAPSI score was 2.8, p = .0007. (NR)	12 months	Severe: none. Minor: spot bleeding
0.1-0.3 ml	5 mg/ml	A: 3, 4-6 weeks	B: TCA 3-9 treatments, inter-vals 2-4 weeks. B: TH, 3-4 treatments, intervals 5-7 weeks	Study ended premature due poor results. Improvement in 90% and 26% in resp. nail-matrix and nail-bed and/or hyponychial. NS. (NR)	1 month	Severe: none. Minor: pain and atrophy

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First author, year of publi-cation	Dermatological indication	Study design	No. of patients (+ lesions)	Skin-type	Type jet injector (brand) + (pressure)	Pressure in study	Fluid
Abell, 1973	Nail dystrophy	Prospective cohort	100 (693)	NR	Spring-loaded (Port-O-Jet) (F)	NR	TCA
Gong, 2016	Non-melanoma skin cancer	Prospective cohort	54 (54)	NR	Spring-loaded (INJEX) (F)**	3000 psi	5-ALA
Zhao, 2020	Non-melanoma skin cancer	Retro-spectve cohort	381 (381)	NR	A: Pneumatic (Airjet) (A)**	NR	A: 5-ALA
Agius, 2006	Plantar warts	Prospective cohort	47 (138)	NR	Spring-loaded (Dermojet) (F)	1,420 psi	Bleomycin
Brodell, 1995	Palmar/plantar warts	CS	22 (>49)	NR	Spring-loaded (Dermojet) (F)	1,420 psi	Interferon alfa-n3

Total volume per lesion each treatment	Concentration	Total no treatments and interval	Comparison	Results per patient + significance (results per lesion + significance)	Follow-up time	Adverse reactions
0.1-0.4 ml	5 mg/ml	At least 3, 2-10 weeks	None	Matrix improvement in matrix psoriasis, combined psoriasis, lichen planus in resp. 84%, 95% and 73%. Onycholysis Improvement in combined psoriasis, psoriatic- and idiopathic onycholysis in resp. 70%, 50% and 47%. Overall, 42% relapsed. NS. (NR)	24 months	Severe: none. Minor: hemorrhage, atrophy, penetration of the nail plate
0.4 ml	200 mg/ml	6, 2 weeks	None	CR of 81% and PR of 13%. Recurrence rate of 9 % at follow-up. NS. (NR)	12 months	Severe: none. Minor: swelling, rash, hyperpigmentation
0.5 ml	200 mg/ml	6, 1-2 weeks	B: CNI + 5-ALA in SAL C: BPT + 5-ALA in SAL	CR 77%, 65%, 66% resp. in NPT, CNI and BPT. Recurrence rate of 4% at follow-up, p =.012. (NR)	6 months	Severe: none. Minor: swelling, rash, burning, itching, hyperpigmentation, headache, chills, puffy eyelids
Mean of 1–3 ml	1 U/ml	5, 5 weeks	None	NR. (CR was 51.5%, 60.1%, 73.9%, 77.5% and 77.5% after resp. first, second, third, fourth and fifth treatment. NS)	5 weeks	Severe: cellulitis, large hematomas (surgical drainage and debridement) Minor: pain, hematoma
0.1 ml	NR	Mean: 15, 0.5 weeks	None	CR in 73% at 8 weeks, rest at least some improvement. Recurrence in 14%. NS. (NR)	9.5 ± 1.5 months	Severe events: lymphangitis Minor: mild discomfort

Table 1: Characteristics and summary of results of included studies using needle-free jet injectors in scars and keloids, alopecia areata, hyperhidrosis, nail diseases, non-melanoma skin cancer and warts

? = unclear; ** = addition to photodynamic therapy; a = adjustable pressure; 5-ala = 5-aminolevulinic acid; bdsp = betamethasone dipropionate sodium-phosphate; bont-a = botulinum neurotoxin-a; btx-a = botulinum toxin type a; cct = clinical controlled trial; cni= conventional needle injection; cs= case series; f = fixed pressure; fu = follow-up; 5-fu = 5 – fluorouracil; gais = global aesthetic improvement scale; ha = hyaluronic acid; hdss = hyperhidrosis disease severity scale; napsi = nail psoriasis severity index; no = numbers; npt = needle free jet injection nr = not reported; ns = no significance reported; oi= overall improvement; onabotA = onabotulinumtoxina; pih = post inflammatory hyperpigmentation; posas = patient and observer scar assessment scale; rct = randomized controlled trial; sal= normal saline; tca = triamcinolone acetonide; th = triamcinolone hexacetonide; vss = vancouver scar scale;

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First author, year of publication	Dermatological indication	Study design	No. of patients (+ lesions)	Skin-type	Type jet injector (brand) + (pressure)	Pressure in study	Fluid
Sparrow, 1975	Granuloma Annulare	CCT	45 (58)	NR	Spring-loaded (Port-O-Jet) (F)	NR	TCA
Vasistha, 1978	Lichen simplex chronicus	CCT	30 (?)	NR	Spring-loaded (Dermojet) (F)	1,420 psi	A: TCA
Bleeker, 1974	Psoriasis	Prospective cohort	18 (?)	NR	A: Spring-loaded (Port-O-Jet) (F)	NR	TCA
Zhang, 2020	Seborrheic dermatitis	Retrospective cohort	72 (72)	NR	NR	NR	1: Vitamin B6, 2: glycyrrhizin, 3: metronidazol, 4: HA
Saghi, 2015	Local anesthesia	RCT	53 (?)	NR	Pneumatic (NR) (NR)	NR	Lidocaine
Mumford, 1976	Local anesthesia	CCT	82 (NR)	NR	Spring-loaded (Syrijet) (A)	2000 or 2600 psi	Xylocaine
Queralt, 1995	Local anesthesia	Prospective cohort	168 (206)	NR	Spring-loaded (MadaJet) XL (F)	1800 psi	Mepivacain chloride
Cho, 2019	Aesthetic (facelifts)	RCT	10 (20)	III-IV	Pneumatic (SheMax) (A)	52.6 psi	A: Hypertonic glucose

Total volume per lesion each treatment	Concentration	Total no treatments and interval	Comparison	Results per patient + significance (results per lesion + significance)	Follow-up time	Adverse reactions
0.1-0.3 ml	5 mg/ml	Mean: 2-4, 2-8 weeks	SAL	63.6% cleared more with TCA, 36.4% same response with SAL and TCA (CR in 68% and 44% in resp. TCA and SAL. NS)	2-24 months	Severe: none. Minor: erythema, atrophy
0.1 ml	10mg/ml	8, 1 week	B: NPT + distilled water	'Excellent' in 66% in A and 46% in B, p= .80. (NR)	1 month	Severe: none. Minor: hypo- and depigmentation, aggravation of new patches
Skin < 5 ml Nails: 0.2-0.6 ml	5mg/ml	1	B: CNI + TCA	13.3% 'better' results with NPT, 6.7% 'better' results with CNI and 80% 'equal' results. NS. (NR)	Unclear	NR
1: 4 ml, 2: 20 ml, 3: 8 ml, 4: 6 ml	1: 50 mg/ml, 2: 2 mg/ml, 3: 5mg/ml, 4: 0.5 mg/ml	3, 2 weeks	None	Mean IGA 6.79 ± 1.20, 6.28 ± 0.98 and 5.58 ± 0.93 resp. baseline, 4 and 6 weeks. Erythema and hydration improved (p<.001; p<.05). Roughness of the skin and lipid level not significant. (NR)	2 weeks	Severe: none. Minor: itching
1 ml	10mg/ml	1	B: CNI + lidocaine 10 mg/ml	VAS injection: 1.1±1 and 4.4±1.4, resp. in A and B, p<.0001. No difference in suture pain, p>.05. (NR)	No FU	NR
Unclear	20 mg/ml	1	CNI + lidocaine 20 mg/ml	No pain in 94% and 83%, resp. NPT and CNI. Children preferred NPT unanimous. NS. (NR)	No FU	Severe: none. Minor: oozing
Ca 1.3 ml	10 mg/ml	1	None	NR. (CR in 79,61%, in others minimal discomfort. NS)	None	Severe: none. Minor: edema
8 ml	200 mg/ml	3, 4 weeks	B: Isotonic glucose 50 mg/ml	NR (Mean overall GAIS in A and B resp. 2.5 ± 0.707 and 3.1 ± 0.876, P = .005)	3 months	Severe: none. Minor: bleeding, redness, worsening

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First author, year of publication	Dermatological indication	Study design	No. of patients (+ lesions)	Skin-type	Type jet injector (brand) + (pressure)	Pressure in study	Fluid
Choi, 2017	Aesthetic (wrinkles)	RCT	24 (24)	III-V	A: Pneumatic (Innojector) (A)	? (level 5)	HA
Espinoza, 2020	Aesthetic (wrinkles)	Retrospective cohort	34 (34)	NR	Pneumatic (AirGent 2.0) (A)	NR	HA
Cheng, 2018	Aesthetic (skin rejuvenation)	Prospective cohort	28 (28)	III-V	Pneumatic (JetPeel-3V) (A)	103 psi	Non cross-linked HA
Kwon, 2018	Aesthetic (facelift/skin rejuvenation)	Prospective cohort	22 (22)	III-IV	Pneumatic (Ultra Beau-jetT) (A)	18.1 -72.5 psi	Hypertonic glucose
Levenberg, 2010	Aesthetic (skin rejuvenation)	Prospective cohort	34 (69)	I–IV	Pneumatic (AirGent) (A)	NR	Crosslinked HA

Total volume per lesion each treatment	Concentration	Total no treatments and interval	Comparison	Results per patient + significance (results per lesion + significance)	Follow-up time	Adverse reactions
1.05 ml	NR	3, 2 weeks	B: MNI + HA C: NPT + placebo D: MNI + placebo	WSRS reduction of 1.00 +/- 0.63 and 1.50 +/- 0.55, resp. A and B in week 16, p<.05; p<.01. Reduction in C and D not significant. (NR)	3 months	Severe: none. Minor: pain
NR (0.09 ml per injection)	NR	Mean: 2.5-3, 12 weeks	None	Mean Lemperle Rating Score decreased 1 degree in all treated areas. NS. (NR)	6 months	Severe: none. Minor: bruises, swelling, erythema, scabs
5 ml	NR	5, 1 week	None	'Improved' and 'much improved' GAIS score rated by patients and dermatologists in week 5 in resp. 42.86% and 57.14%. NS. (NR)	3 months	Severe: none. Minor: none
0.4-6.0 ml	200 mg/ml	1	None	Improvement in 91% post-treatment. NS. (NR)	3 months	Severe: none. Minor: erythema, blebs
NR (mass 2 mg)	NR	1-4, 3-4 weeks	None	80% was (very) satisfied. (Long term wrinkles reduced 27.6% and 21.2%, resp. face and neck, p< .05; p<.05. Long term OI in dorsal hands was good, p<.05)	1-18 months	Severe: none. Minor: bleeding, erythema, edema, tenderness, PIH

Table 2 Characteristics and summary of results of included studies using needle-free jet injectors in other dermatological indications (granuloma annulare, psoriasis, seborrheic dermatitis, local anesthesia and aesthetics)

? = unclear; a = adjustable pressure; bpt = plum-blossom needle injection; cct = clinical controlled trial; cni= conventional needle injection; cr; complete response; cs = case series; f = fixed pressure; fu= follow-up; ha = hyaluronic acid; gais = global aesthetic improvement scale; iga = investors global assessment; mni = multi needle injection;; npt = needle-free jet injection; no = numbers; nr = not reported; ns = no significance reported; pih = post inflammatory hyperpigmentation; pr = partial response; rct = randomized controlled trial; sal = normal saline; tca =triamcinolone acetonide; vas = visual analogue scale; wsrs = wrinkle severity rating scal

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Section II

Biodistribution using different injection techniques in severe keloids and properties of severe keloids

Chapter 3

Biodistribution of needle-injections and needle-free jet-injections visualized by a 3D- Fluorescent Imaging Cryomicrotome System

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ABSTRACT

Introduction Intralesional corticosteroid injections are a first-line treatment for keloids; yet clinical treatment results are highly variable and often suboptimal. Variation in triamcinolone acetonide (TCA) biodistribution may be an important reason for the variable effects of TCA treatment in keloids. In this exploratory study we investigated the biodistribution of TCA in keloids and normal skin using different drug delivery techniques.
Methods Fluorescent-labeled TCA suspension was administered into keloids and normal skin with a hypodermic needle and an electronic pneumatic jet injector. TCA biodistribution was represented by the fluorescent TCA volume and 3D biodistribution shape of TCA, using a 3D-Fluorescence-Imaging Cryomicrotome System.
Results Twenty-one keloid and nine normal skin samples were analyzed. With needle injections, the mean fluorescent TCA volumes were 990 µl ± 479 in keloids and 872 µl ± 227 in normal skin. With the jet injector, the mean fluorescent TCA volumes were 401 µl ± 252 in keloids and 249 µl ± 67 in normal skin. 3D biodistribution shapes of TCA were highly variable in keloids and normal skin.
Conclusion TCA biodistribution in keloids is highly variable for both needle and jet injection. This may partly explain the variable treatment effects of intralesional TCA in keloids. Future research is needed to confirm this preliminary finding and to optimize drug delivery in keloids.

INTRODUCTION

Keloids are fibroproliferative scars caused by chronic inflammation in the reticular dermis. Keloids may cause pain, pruritus, movement restriction, and cosmetic concerns.[1,2] They can be challenging to treat. Intralesional corticosteroid administration (ICA) by needle injection is traditionally considered a first-line treatment for keloids, with triamcinolone acetonide (TCA) being used most frequently.[3,4] Nevertheless, clinical results of this treatment are highly variable and often suboptimal.[5,6]

Treatment efficacy can be influenced by various factors, such as the duration, size, anatomic location, genetic predisposition and treatment history of keloids.[7] Additionally, treatment efficacy is influenced by drug biodistribution, which may depend on characteristics of the tissue that is injected.[8,9] Drug biodistribution may also depend on the drug delivery technique. Conventional needle injection using hypodermic needles has been used predominantly for ICA in the past few decades. Yet, a wide variation in this injection technique exists in current clinical practice.[4] Alternatively, different types of jet injectors can be used for ICA. Electronic pneumatic jet injectors, referred to hereinafter as 'jet injectors', are needle-free injectors that use pressured gasses (e.g. air or CO2) to create a high velocity jet stream of liquid drugs that penetrates the skin without using a needle.[10] The drug volume and gas pressure can be adjusted to match the dose and depth of drug administration to the specific clinical requirements.

To date, the biodistribution of TCA administered with different drug delivery techniques has not been investigated in different tissues. Variation in TCA biodistribution may be a major reason for the variable treatment effects of TCA in keloids reported in clinical trials and observed in clinical practice. The aim of this exploratory study is to assess the biodistribution of TCA in ex vivo keloids and normal skin with different drug delivery techniques.

MATERIALS AND METHODS

Collection procedures were in compliance with article 7:467 BW of the Dutch law. Formal approval for this exploratory ex vivo study from the Medical Ethics Board Committee (METC) was not required, because the tissue samples only involved anonymously collected material that was discarded following routine elective surgery.

Study design

In this exploratory study, the TCA biodistribution using different drug delivery techniques in ex vivo keloids and normal skin was investigated. TCA 40 mg/mL suspension (Kenacort, Bristol-Myers Squibb, New York City, New York, U.S.) was labeled with a fluorescent dye (Texas Red 10 µg/mL; 3000 MW, Invitrogen). TCA biodistribution was represented by the fluorescent TCA volume and 3D biodistribution shape of TCA, using a 3D-Fluorescence-Imaging Cryomicrotome System (3D-FICS).

The drug delivery techniques were (1a) needle injection in the superficial, mid, and deep layer of the keloid; (1b) perforation technique, i.e. making multiple cross-sectional passes with a thick needle prior to injection in the mid-layer of the keloid; and (2) jet injection using pressures of 4, 5 and 6 Bar. For jet injections, the residual TCA volume on the keloid and skin was determined by wiping off the fluid on the surface of the keloid and skin using a gauze and measuring the weight increase of the gauze. Then, this residual weight was converted to residual volume using a conversion rate of 1.0496 (1 mL TCA = 0.9527 g, based on own measurements). Papule formation after each jet injection was directly captured using a 3D-camera (LifeViz Micro 600D, Quantificare, Sophia Antipolis, France).

Study samples

The selection of keloids was performed by two plastic surgeons (FN and OL) and one dermatologist (EP) experienced in keloid treatment. Keloids were included if a specimen of at least 1.5 x 1.5 cm could be harvested; regardless of the anatomic location, duration, etiology, and pretreatment. Keloids were excluded if (1) the differentiation between keloid and hypertrophic scar could not be made clinically, (2) the sample had been

preserved in any preservative fluid, or (3) the patient had objected to the use of discarded material for scientific purposes.

The keloids were obtained from patients who underwent elective keloid excision and adjuvant radiotherapy (Department of Plastic Surgery Amsterdam UMC, Department of Dermatology Erasmus Medical Centre, The Netherlands). Normal skin was obtained from patients who underwent abdominoplasty (Department of Plastic Surgery, Jan van Goyen Medical Center, The Netherlands). After removing excessive subcutaneous fat, the keloids and normal skin tissues were stored at -80 ^{o}C or at -20 ^{o}C, the latter for a maximum of 6 months.

Experiments

Prior to the experiments, the normal skin and keloid samples were thawed to room temperature, fixed under mild tension, kept moist with wet gauzes, and marked with 1.5 x 1.5 cm zones. All experiments were conducted in triplicate, by a dermatologist experienced in ICA in keloids (AW).

For the experiments with 'conventional' needle, a 25-gauge needle and 1 mL syringe were used to administer 100 µL fluorescent-labeled TCA 40 mg/mL per sample. For the experiments with the jet injector, a needle-free jet injector (Enerjet 2.0, Perfaction, Rehovot, Israel) was used to administer 100 µL (device range: 50-130 µL) fluorescent-labeled TCA 40 mg/mL with pressures of 4, 5 and 6 bar (device range: 2-6 bar) per sample. All jet-assisted injections were administered perpendicularly in the center of the sample.

Image acquisition

The biodistribution of fluorescent-labeled TCA suspension was visualized using the 3D-FICS (Figure 1). Firstly, all samples were embedded in a 3% carboxymethylcellulose with black ink to reduce background signaling. All samples were sectioned vertically into slices of 48 µm thickness. After each section, images were taken from the remaining bulk with a camera with an in-plane resolution of 13.66 x 13.66 µm. Prior to the experiments, wavelengths and exposure times were optimized using test samples. Eventually, a 595 nm excitation and 620 nm emission wavelength with an exposure time of 500 ms were used

to visualize the fluorescent-labeled TCA suspension. A reflection image at 549 nm was used to reconstruct the tissue borders.

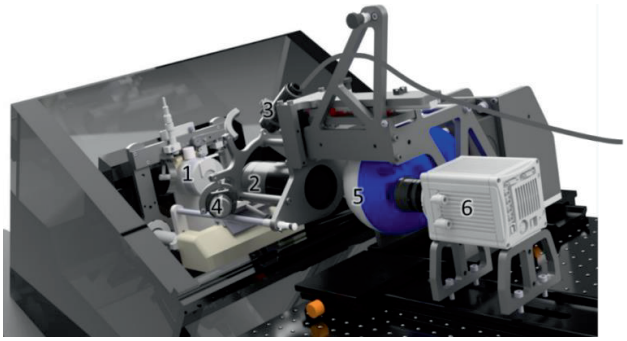


Figure 1.

3D FICS. 1: Sample holder, 2: Lens (Olympus SZX16 stereo microscope system), 3: Tunable supercontinuum laser, 4: UV led illumination, 5: Tunable filter wheel, 6: Camera

Image analysis

The 2D images were first cropped using LabVIEW (National Instruments, Austin, Texas, USA) and the resulting stack of images was resampled to a volume image with a resolution of (x,y,z) = 27.4 x 27.4 x 35.0 µm, which was sufficient to observe and quantify the fluorescent-labeled TCA. The 3D model of the fluorescent distribution (Figure 2) was obtained by image segmentation using custom software (Dobbe, 2019). During segmentation, all voxels in the fluorescent region above a pragmatically chosen intensity threshold (2000) were included, while excluding voxels representing autofluorescent tissue as much as possible. The volume of the segmented fluorescent regions represents the fluorescent TCA volume. This is not equivalent to the actual injected TCA volume due to the point spread function of the imaging system and the chosen arbitrary intensity threshold. However, the measured fluorescent TCA volume enables comparison of the arbitrary fluorescent TCA volumes between samples.

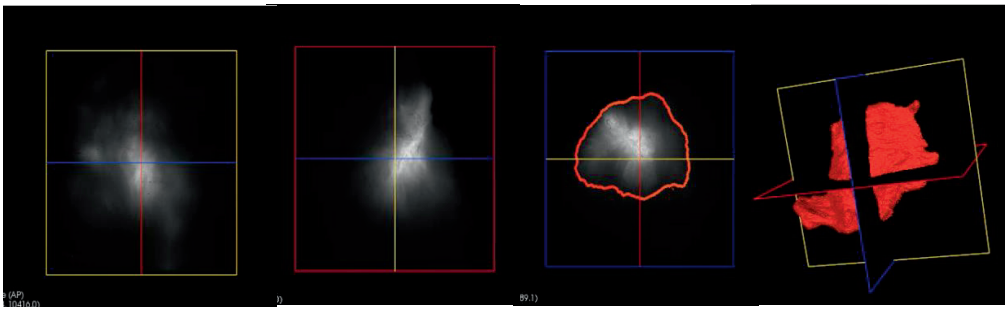


Figure 2.

Fluorescent TCA biodistribution in keloid sample in axial, coronal and sagittal axis respectively; segmented fluorescent region representing the fluorescent TCA volume and the 3D biodistribution shape of TCA in a keloid sample.

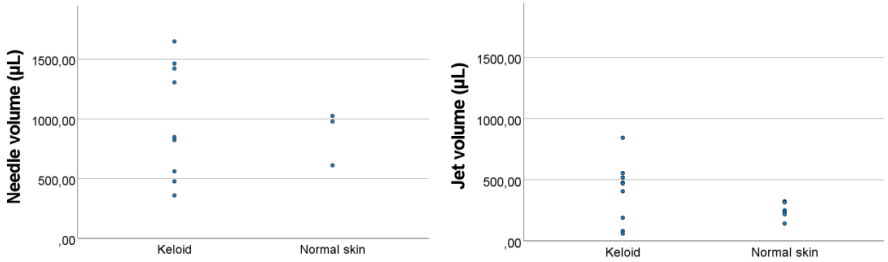
RESULTS

A total of 30 samples (21 keloid, 9 normal skin) were analysed for the biodistribution of TCA injected with a hypodermic needle and jet injector. The keloids were obtained from six patients who underwent elective keloid excision and adjuvant radiotherapy, and were located on the abdomen, chest, mandibula or shoulder. Normal skin was obtained from three patients who underwent abdominoplasty.

Fluorescent TCA volume

A large variation in fluorescent TCA volumes was observed in keloids (Figure 3a, 3b). With the perforation technique, the mean fluorescent TCA volumes were similar (975 µl ± 284) compared to the 'conventional' needle injections (990 µl ± 479). Considerable operator injection force was needed for the needle injections and changing the needle position was sometimes necessary to inject the predefined volume of 100 µl.

With the jet injector, only a single attempt was made to inject the TCA. With the jet injector, the fluorescent TCA volume in keloids (401 µl ± 219) and normal skin (249 µl ± 59) seems to be smaller compared to needle injections (Table 1, 2).



A. **B.**

Figure 3.

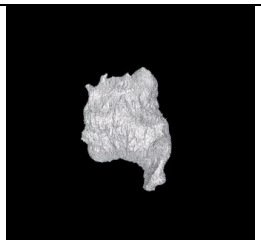
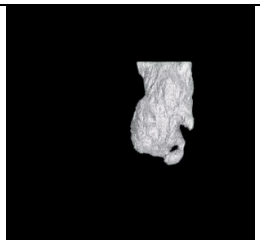
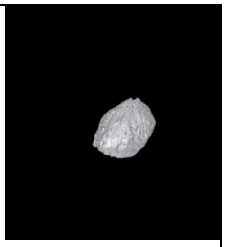
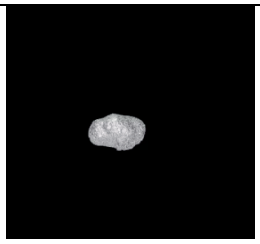
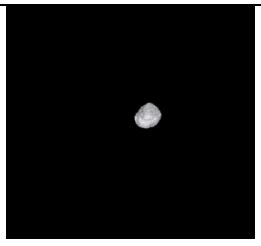
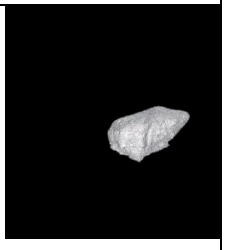
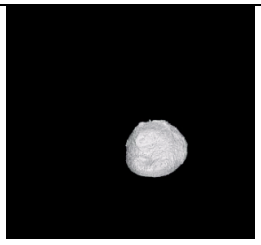
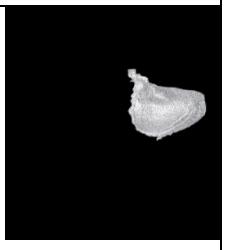
Fluorescent TCA volumes

A. Fluorescent TCA volumes in keloid and normal skin after needle injections.

B. Fluorescent TCA volumes in keloid and normal skin after jet injections.

3D biodistribution shape

The 3D biodistribution shape of TCA in keloids and normal skin using needle and jet injectors was highly variable (Figure 4a and 4b).

1	 Keloid, 4 Bar		2	 Keloid, 5 Bar		3	 Keloid, 6 Bar	
4	 Keloid, 4 Bar		5	 Keloid, 5 Bar		6	 Keloid, 6 Bar	
7	 Keloid, 4 Bar		8	 Keloid, 5 Bar		9	 Keloid, 6 Bar	
10	 Normal skin, 4 Bar		11	 Normal skin, 6 Bar		12	 Normal skin, 4 Bar	

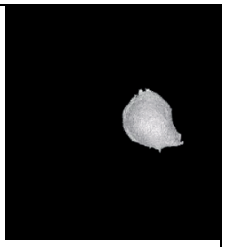
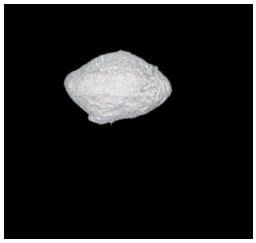
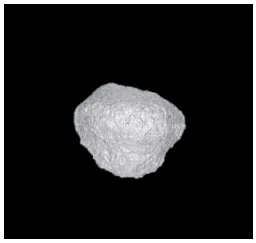
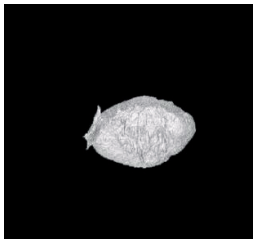
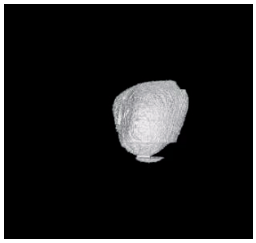
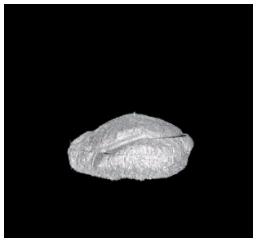
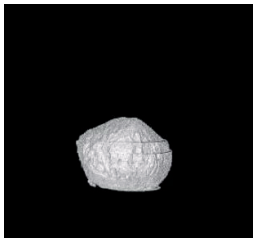
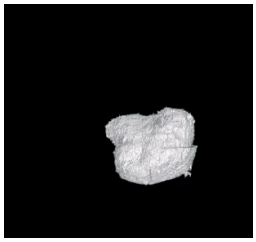
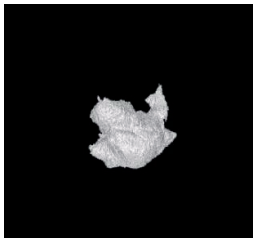
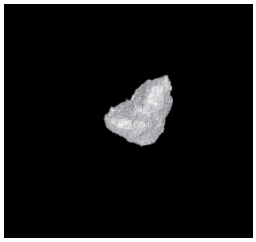
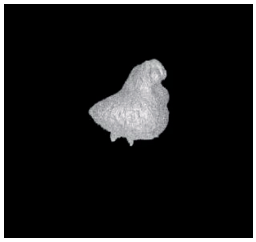
13			14			15	
	Normal skin, 6 Bar			Normal skin, 4 Bar			Normal skin, 6 Bar

Figure 4A.

3D images of segmented fluorescent regions representing the TCA volume using a jet injector in keloid samples (1 to 9), and normal skin (10 to 15)

16	 Keloid, superficial	17	 Keloid, superficial	18	 Keloid, superficial
19	 Keloid, mid	20	 Keloid, mid	21	 Keloid, mid
22	 Keloid, deep	23	 Keloid, deep	24	 Keloid, deep
25	 Keloid, perforation	26	 Keloid, perforation	27	 Keloid, perforation

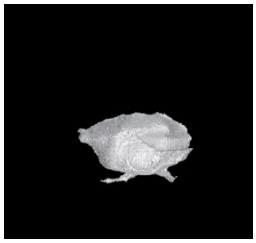
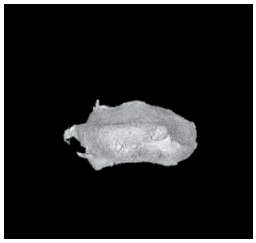
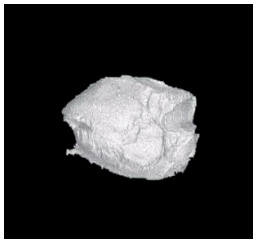
28		29		30	
	Normal skin		Normal skin		Normal skin

Figure 4B.

3D images of segmented fluorescent regions representing the TCA volume using needles in keloid samples (16 to 27), and normal skin (28 to 30)

Clinical endpoints jet injections

The residual TCA volume on the skin surface after jet injections was high, especially in keloids (Table 2). Using higher pressure of 6 Bar seems to result in larger fluorescent TCA volumes in keloids, compared to lower pressures (Table 2). Contradictory, higher pressure also resulted in larger residual volumes in our experiments. Papule formation was observed in 66% (6/9) of the keloid samples, while this was observed in all (6/6) normal skin samples. No clear relation was observed between papule formation in keloids and mean fluorescent TCA volume.

DISCUSSION

To date, studies about the treatment of keloids have focused on the various drugs for intralesional administration while the challenges and limitations of optimal biodistribution in keloids have been neglected. In this exploratory study, the 3D biodistribution of TCA using different drug delivery techniques in ex vivo keloids and normal skin was assessed using the 3D-FICS.

Large heterogeneity in TCA volumes was observed in keloids. This may be the result of the large variation in mechanical properties such as rigidity and viscoelasticity among different keloids and even within the same keloid. This variation in mechanical properties may depend on the anatomic location, prior treatment and genetic predisposition (1, 3, 22). Moreover, with jet injectors the fluorescent TCA volume seems to be smaller compared to needle injections. This is in line with the large residual TCA volume remaining on the skin surface of keloids (71.9 µl ± 14.3) after jet injection. Pressures generated by the jet injector may not be sufficient for penetrating the recalcitrant solid keloids in this ex vivo setting. Notably, the considerable operator injection force as applied in the experiments with needle injection could only be possible in clinical practice if prior local anesthesia has been applied, as it could be very painful otherwise. And for jet injections, repeating the injection would preferably be performed in clinical practice if a high amount of residual fluid is observed directly after jet injection. For needle injections, blanching is an endpoint of infiltration in clinical setting. However, this could not be used as a reference in these experiments using ex vivo samples without blood perfusion.

Compared to lower pressures, a higher pressure of 6 Bar seems to result in larger fluorescent TCA volumes in keloids. It should be emphasized that fluorescent TCA volumes are not equivalent to actual delivered TCA volumes. We assume that even though 6 Bar may result in larger fluorescent TCA distribution compared to lower pressures, the actual delivered TCA dose was lower, as reflected by the larger residual volumes. The reason for the latter is unclear; and the effect of different pressure levels on the biodistribution in keloids needs further investigation.

Considering the 3D biodistribution shape of TCA, we noticed substantial heterogeneity. These variable patterns of 3D TCA shapes contribute to the observation of the large heterogeneity in TCA biodistribution and ultimately clinical response.

There are several strengths to this study. To the best of our knowledge, there are no similar studies assessing the 3D biodistribution of TCA in keloids. Moreover, several drug delivery methods were studied, including needle injection, perforation technique, and jet injection. Furthermore, an innovative 3D imaging technique was used. Various imaging techniques may be used for assessing layers of the skin, including confocal microscopy, optical coherence tomography and high frequency ultrasound. In contrast to these imaging techniques, the custom-built 3D-FICS can be used for high resolution segmentation of large 3D volumes [11]. This novel imaging technique has previously been used in other medical specialties including cardiology to visualize the perfusion distribution within the heart and in neurology for imaging fluid distribution in brain structures [12, 13].

However, there are also several limitations to this study. Firstly, the sample size was limited, because keloids are excised infrequently. Secondly, differentiation in keloid characteristics such as anatomical location, tissue density and prior treatment was not performed due to the small sample size. Moreover, the included keloids were selected for excision and adjuvant brachytherapy, and differ from the usually smaller, thinner and less rigid keloids in clinical practice. Additionally, we visualized the fluorescent marker that was labeled to the TCA suspension, being a proxy for the TCA suspension. Although the fluorescent TCA volumes are not equivalent to the actual injected TCA volumes, they enabled comparison of the fluorescent biodistribution between samples. Furthermore, the observed fluorescent TCA volume and 3D biodistribution shape could be affected by the optical properties of tissue types, which may be different for normal and keloid tissue. Finally, inherent to the exploratory design of the study, in-vivo conditions such as blood flow, skin turgor, and hydration of the skin could not be taken into consideration.

Despite the above-mentioned limitations, the preliminary findings of this exploratory study are important for the clinical practice. Improving knowledge of drug biodistribution in keloids is not only important to enhance efficacy and safety for TCA, but also for other intralesionally administered drugs such as 5-fluorouracil and bleomycin. This exploratory study provides a framework for future studies on drug distribution. Future research may focus on improving biodistribution in keloids, either by changing the device for injection, injection technique of the physician (e.g. level of injection, perforation technique) or mechanical tissue properties (e.g. by hyperthermia, cryotherapy, radiotherapy and lasers). Interestingly, previous research in tumors demonstrated better biodistribution of certain tumor drugs when applied with a multiside hole needle (22 holes at the side of the needle) instead of the conventional 'end hole needle' [8]. Other future research challenges to better understand drug biodistribution are the measurement of absolute TCA volumes, concentrations and dimensions. A quantitative technique such as ELISA could be used to measure the drug concentrations in different skin levels.

In conclusion, our experiments indicate that TCA biodistribution in keloids is highly variable for both needle and jet injection. This may partly explain the variable treatment effects of intralesional TCA in keloids. Moreover, with jet injectors the fluorescent TCA volume seems to be smaller compared to needle injection in keloids. However, more experiments are needed to confirm the findings of this exploratory study.

	Level of needle injection	Mean TCA volume (μL)
Normal skin (n = 3)	Middermal[1]	872 ± 227
Keloid (n = 3)	Superficial[1]	1069 ± 516
	Mid[1]	745 ± 159
	Deep[1]	1158 ± 697
	Perforation[2]	975 ± 284

Table 1.

Mean fluorescent TCA volumes in mid-dermis of normal skin; and superficial, mid, and deep layers of keloid after needle injection.

[1] Standard technique, i.e. using one injection for TCA administration.

[2] 'Perforation technique', i.e. making multiple cross-sectional passes with a thick needle prior to injection in the mid-layer of the keloid.

Specified data is reported in Supplement 1.

	Jet pressure (Bar)	Mean TCA volume (μl)	Mean residual volume (%)
Normal skin (n = 3)	4	210 ± 59	28 ± 24
	6	287 ± 59	27 ± 8
Keloid (n = 3)	4	329 ± 447	59 ± 12
	5	384 ± 184	80 ± 12
	6	489 ± 27	89 ± 20

Table 2.

Mean fluorescent TCA volumes and residual volumes in normal skin and keloid samples after jet injection.

Specified data is reported in Supplement 2.

SUPPLEMENTARY FILES

Tissue (location)	Location of injection	TCA volume (µL)
Keloid (Abdomen)	S	477,6
Keloid (Back)	S	1422,1
Keloid (Presternal)	S	1306,3
Keloid (Abdomen)	M	562,1
Keloid (Back)	M	823,6
Keloid (Presternal)	M	849,4
Keloid (Presternal)	M*	648,7
Keloid (Presternal)	M*	1109,9
Keloid (Presternal)	M*	1166,8
Keloid (Abdomen)	D	359,8
Keloid (Back)	D	1463,6
Keloid (Presternal)	D	1649,1
Normal skin	M	612,0
Normal skin	M	978,6
Normal skin	M	1026,0

Supplement 1.

Needle injections: location of injection and TCA volumes.

*Perforation technique', i.e. making multiple cross-sectional passes prior to injection in the mid-layer of the keloid through one of the passes.

Tissue (location)	Pressure (Bar)	TCA volume (µL)	Residual volume (%)
Keloid (Presternal)	4	844,6	55.5
Keloid (Mandibula 1)	4	81,8	72.3
Keloid (Mandibula 2)	4	60,8	48.9
Keloid (Presternal)	5	555,1	69.3
Keloid (Mandibula 1)	5	406.7	93.8
Keloid (Mandibula 2)	5	189.9	78.2
Keloid (Presternal)	6	477,2	112.3
Keloid (Mandibula 1)	6	519.2	74.9
Keloid (Mandibula 2)	6	470.3	82.1
Normal skin	4	142,9	55.4
Normal skin	4	250,3	11.8
Normal skin	4	238,0	17.5
Normal skin	6	324,7	18.5
Normal skin	6	317,1	27.9
Normal skin	6	219,1	34.6

Supplement 2.

Injected TCA volumes and percentage 'residual volume' using a jet injector.

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Chapter 4

Effects of keloid properties on treatment efficacy

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ABSTRACT

Background: The efficacy of keloid treatment in randomized studies is highly variable. However, no systematic review has been performed to evaluate the effect of different keloid properties on treatment efficacy.

Objective: to identify clinically relevant keloid properties, that may influence treatment efficacy.

Methods & Materials: An electronic database search was conducted. Two reviewers independently selected randomized controlled trials (RCTs) and performed a methodological quality assessment using the Cochrane risk-of-bias 2.0 tool.

Results: 1520 studies were screened, and 16 RCTs, involving 1113 patients, were included. We found lower efficacy in older keloids (n=3), keloids located on the chest, extremities, pinna, and shoulder (n=3), larger keloids (n=2), lower baseline VSS score (n=1), and keloids with history of recurrence (n=1). Overall, the majority of studies had a high risk of bias.

Conclusion: Only a minority of studies specifically addressed keloid properties, which makes comparisons between studies challenging. Our results suggest that keloid location, - duration prior to treatment, -size, -history of recurrence, and -severity are clinically relevant keloid properties that affect treatment efficacy. Further studies are crucial to corroborate our findings, establish a clinically relevant keloid classification, and ultimately develop an evidence-based treatment algorithm that takes these properties into account.

INTRODUCTION

Keloid, derived from 'cheloides', the Greek word for 'crab's claw,' is a fibroproliferative scar that expands beyond the initial border of injury and rarely shows spontaneous regression. These pathological scars can cause severe pain, pruritus, and functional- or aesthetic complaints, which can decrease patients' quality of life.[1]

The reported clinical efficacy of keloid treatments is highly variable, and may strongly depend on keloid- and patient characteristics. However, reaching consensus on a standardized keloid classification system based on the most relevant clinical properties remains challenging. Ideally, this classification should be based on high level evidence that shows the impact of specific properties on treatment efficacy. This could be a crucial step towards developing evidence-based guidelines for selecting the most efficacious treatment for individual keloid patients.

However, to date, no systematic review has been performed to evaluate the evidence regarding the impact of different keloid properties on treatment efficacy. This systematic review aimed to assess the impact of the various keloid properties on treatment efficacy.

METHODS

A comprehensive electronic literature search was performed by a Biomedical Informatics Specialist in Cochrane Central Register of Controlled Trial, Embase, Google Scholar, Medline ALL and Web of Science Core Collection (Supplementary files). This systematic review was registered in PROSPERO (CRD42023451685) and the PRISMA 2020 checklist was followed (Supplementary files).

Duplicates were removed, and titles and abstracts were screened for eligibility independently by two reviewers (V.B.; P.B). Hereafter, full-text articles were assessed for eligibility. Randomized controlled trials (RCTs) were included if the full-text was published in English from inception to August 2023, and if they assessed the efficacy of any keloid treatment in patients of all ages, with at least one keloid property analyzed. Studies were excluded if they did not provide separate analyses for keloids when hypertrophic scars were also included.

Standardized data extraction, and methodological quality assessment of the included studies were performed independently by V.B and P.B. Discrepancies between reviewers were discussed and resolved by consensus and if necessary, discussed with A.W. The collected data included the (1) analyzed keloid properties, (2) treatment efficacy, (3) primary outcome measure, (4) total no. of keloids and patients, and (5) keloid therapies used. Methodological quality was assessed using the Cochrane risk-of-bias 2.0 tool (ROB 2.0), and figures of the methodological quality assessment were created with Robvis.[2]

RESULTS

Our literature search identified 1520 studies, of which 16 studies with a total of 1113 patients were included for data assessment (Fig. 1, Table 1).

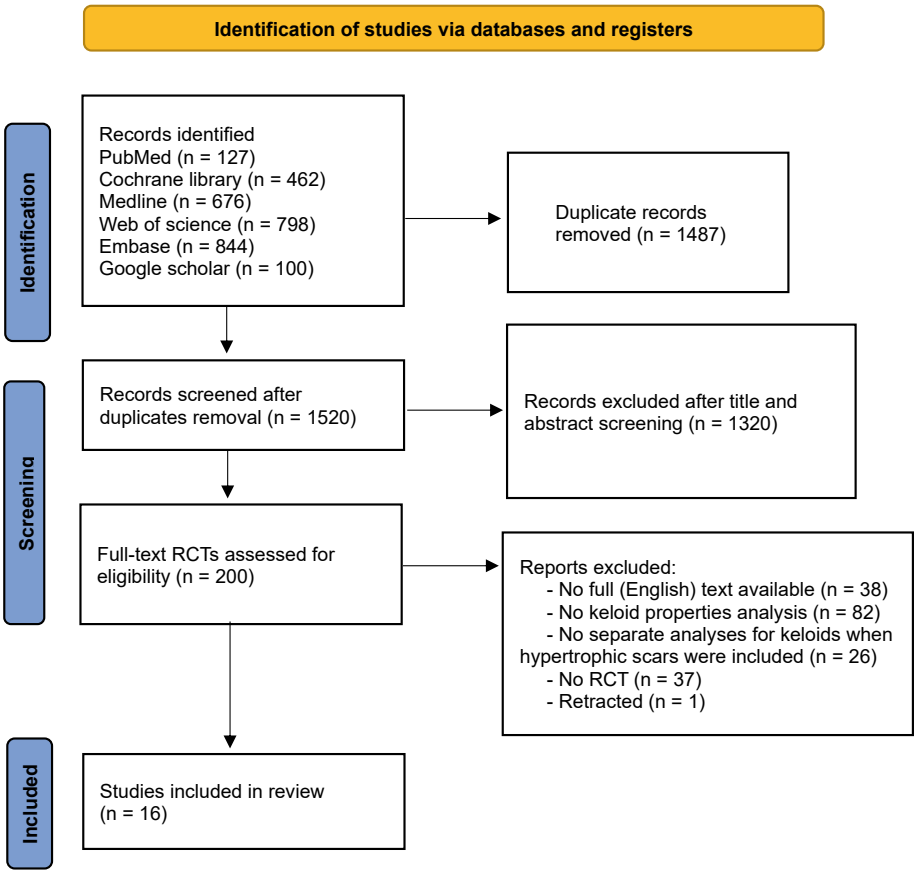

Fig. 1. Study flow diagram resulting in 16 included studies.

Keloid duration prior to treatment

Twelve studies involving 811 patients investigated keloid duration prior to treatment.[3-14] Three studies reported higher efficacy in younger keloids compared to longer existing keloids.[7,9,12] Rani et al. reported higher efficacy in younger keloids (<2 years), however, without performing sub-analysis per treatment group (intralesional triamcinolone acetonide (il TCA); il 5-FU; il TCA + cryotherapy; surgical excision + topical imiquimod).[12] The two other studies did not report a specific cut-off point for duration, but reported significantly higher efficacy in younger keloids treated with respectively Nd:YAG laser- or cryotherapy.[7,9] The remaining studies found no significant correlation.[3,4,6,8,10,11,13,14]

Keloid location

Seven studies involving 440 patients investigated keloid location.[7, 9, 12, 13, 15-17] One study found significantly higher 'cure rates' for keloids treated with il TCA that were located on the cheek, forehead, submandibular area and lip compared to the pinna, while no sub-analysis was reported for keloids treated with excision and radiotherapy.[15] Belie et al. reported higher efficacy for keloids located on the trunk compared to the extremities after treatment with il TCA or il verapamil.[16] Rani et al. reported higher efficacy for keloids located on the earlobes, face and back, compared to the chest and shoulders, without a sub-analysis per treatment group (il TCA; il 5-FU; il TCA + cryotherapy; surgical excision + topical imiquimod).[12] The other studies found no significant correlation.[9,14,15,17]

Keloid size

Three studies involving 195 patients investigated keloid size.[3,5,8] Two studies reported higher efficacy in smaller keloids (<1 cm^2 and <5 cm^3) compared to larger keloids, after respectively contact cryosurgery or il 5- fluorouracil.[3,5] The other study found no significant difference between smaller and larger keloids.[8]

History of recurrence

One study involving 26 patients investigated treatment history.[18] After intralesional cryotherapy a volume reduction of 40% versus 1% was observed in respectively naïve versus recurrent (previous corticosteroid injections or excision) keloids.

Baseline VSS core

One study involving 60 patients investigated baseline Vancouver Scar Scale (VSS).[8] Higher efficacy was reported in keloids with higher baseline VSS scores compared to keloids with lower baseline VSS scores. However, no specific cut-off point for VSS and sub-analysis per treatment group (il botulinum toxin type-A, il platelet rich plasma, and il TCA) were mentioned.

Other keloid properties

Fitzpatrick skin type (n=2; 324 patients),[4,6] baseline Patient & Observer Scar Assessment Scale (POSAS)-score (n=1; 164 patients),[6] ethnicity (n=1; 39 patients),[17] etiology (n=1; 50 patients),[7] keloid thickness (n=1; 90 patients),[11,14] and number of lesions (n=1; 44 patients),[13] did not show a significant correlation with treatment efficacy.

Risk of bias assessment

The overall risk of bias was rated as “high” in eight studies, “some concerns” in seven studies, and “low” in one study (Fig. 2). Methodological quality was particularly poor due to bias arising from the randomization process, deviations from the intended intervention and selective reporting.

4. Effects of keloid properties on treatment efficacy

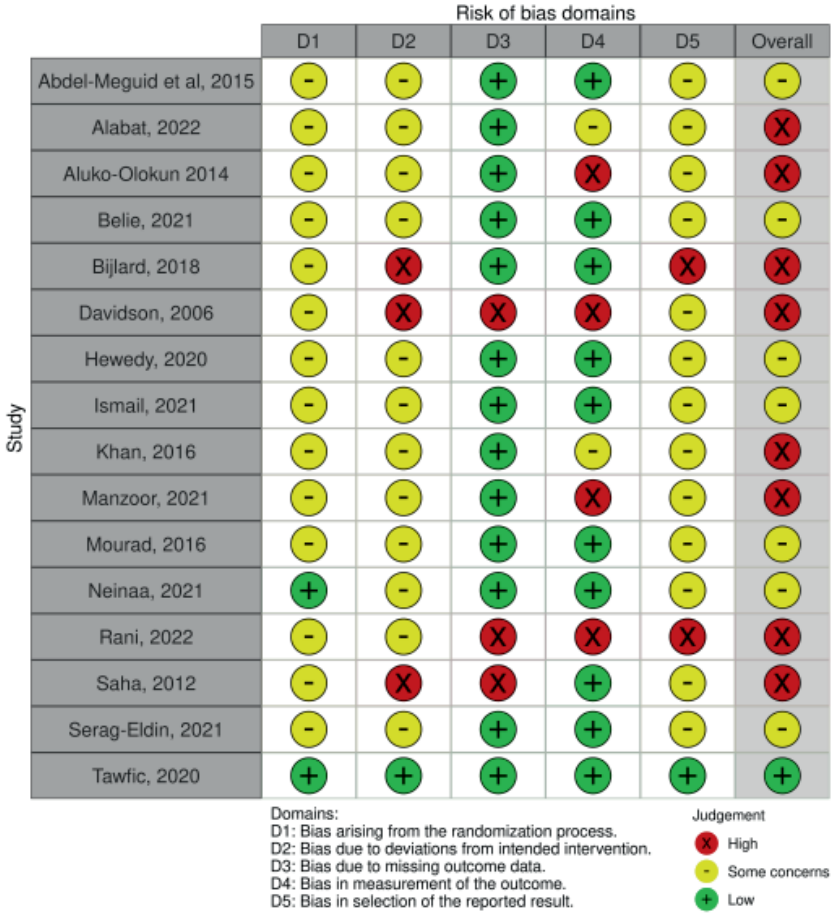

Fig. 2. Risk of bias of the included RCTs. Half of the articles (50%) were judged as having 'high risk of bias'. One article (6%) was assessed as having 'low risk of bias', and the remaining articles (44%) were judged as having 'some concerns.

DISCUSSION

This systematic review aimed to identify clinically relevant keloid properties that may impact treatment efficacy. In total, only 16 RCTs performed a separate analysis for specific keloid properties. In these 16 studies, keloid duration prior to treatment and location were the most frequently analyzed properties followed by size, Fitzpatrick skin type, baseline POSAS score, baseline VSS score, keloid thickness, ethnicity, etiology, number of keloids, and history of recurrence.

Our findings suggest a lower treatment efficacy in keloids with a longer duration prior to treatment, location on the chest, extremities, pinna, or shoulder, a larger size, history of recurrence, and a lower baseline VSS score of keloids. However, we cannot exclude clinical relevance of the other keloid properties because the number, sample size, and quality of the studies is insufficient to draw firm conclusions. Hence, more research is needed with a focus on these keloid properties.

Some keloid properties were found to be relevant for specific treatments only, suggesting that the influence of keloid properties depends on the treatment used. In line with this finding, some experts proposed treatment algorithms addressing specific keloid properties.[19,20] Although size and number of keloids were mentioned in these algorithms as important properties to take into account, other potentially clinically relevant properties such as duration prior to treatment and location of keloids were not mentioned. Moreover, these algorithms were not based on a systematic review.

Importantly, a diversity of outcome instruments and scales were used in the included studies. For instance, some studies used the Swada and Sone,[7] POSAS,[4,6] or VSS scale[8,9] to evaluate keloid treatment outcomes, while others used reduction in keloid size[3,5,18] as the primary outcome. This variation in outcome measures makes it challenging to compare results between studies, decreasing the value of these studies and contributing to waste in research. Therefore, it is imperative that a consensus-based Core Outcome Set will be implemented in future research and reporting in this field.

The strengths of this systematic review include the use of a comprehensive database search, inclusion of RCTs with no limitation of publication date, and a critical methodological quality assessment using the ROB 2.0. Limitations of this review include the low number of eligible studies and the heterogeneity of outcome measures and scales which precludes a meta-analysis. Moreover, the study populations were generally small which makes it difficult to detect differences in efficacy between keloid properties. In conclusion, only a minority of studies performed sub-analyses for specific keloid properties and even fewer studies found clinically relevant keloid properties. Our results suggest that keloid duration prior to treatment, -location, -size, -history of recurrence, and -severity influence treatment efficacy. Nonetheless, more high quality head-to-head RCTs using validated outcome measures should report on the potentially relevant keloid properties. These further investigations are crucial to corroborate our findings, establish a clinically relevant keloid classification, and ultimately develop an evidence-based treatment algorithm for clinical practice that takes these properties into account.

4. Effects of keloid properties on treatment efficacy

Study	Keloid properties	Treatment groups	Results	Outcome measure	Relevant study characteristics (n = no. keloids)
Abdel-Meguid et al., 2015	Duration, size	A: IL cryosurgery; B: contact cryosurgery	- Duration: NS. - Size: smaller keloids had a better response to contact cryosurgery, while size of the keloids did not significantly affect the response to intralesional cryosurgery.	Keloid height	**Duration** : ≤ 2 years (n=48), > 2years (n=18). **Size**: small <1 cm2 (n=41), medium 1-5 cm2 (n=21), large >5 cm2 (n=4). **Total no. keloid patients:** 33.
\Albalat et al., 2022	Duration, skin type	A: IL TCA; B: IL verapamil; C: IL 5-FU; D: IL PRP	- Fitzpatrick skin type and duration: NS.	POSAS score	**Mean duration (months)**: 4 (n=40), 5 (n=40), 4 (n=40), 5 (n=40). **Fitzpatrick skin type**: Type 3 (n=109), type 4 (n=45), type 5 (n=6). **Total no. keloid patients:** 160.
Aluko-Olokun et al., 2014	Location	A: IL TCA; B: excision + radiotherapy	- Location: higher cure rate on cheek, forehead, submandibular and lip with TCA compared to the pinna. NS between different locations in excision + radiotherapy group.	Keloid height and recurrence	**Location (TCA group):** Pinna (n=13), cheek (n=16), forehead (n=7), submandibular (n=9), lip (n=11). **Location (excision + radiotherapy group):** Pinna (n=12), cheek (n=13), forehead (n=7), submandibular (n=12), lip (n=9). **Total no. keloid patients:** 107.
Belie et al., 2021	Location	A: IL verapamil; B: IL TCA monotherapy	- Location: significant decrease in pain and pruritus in keloids located on the head, neck and trunk with TCA, at resp. the 2nd and 3rd visit, whereas the response in the VTG showed a significant reduction in symptoms in both regions at the 4th visit.	VAS for pain and pruritis	**Pain:** Head/neck (n=17), Trunk (n=19), Upper limb (n=6), lower limb (n=1). **Pruritis**: Head/neck (n=19), Trunk (n=20), Upper limb (n=4), lower limb (n=8).

4. Effects of keloid properties on treatment efficacy

					Total no. keloid patients: 78.
Bijlard et al., 2018	History of recurrence	A: IL cryotherapy + excision + IL TCA B: IL cryotherapy + excision + brachytherapy	- History of recurrence: IL cryotherapy resulted in 40% reduction in scar volume in treatment naïve keloids, compared to 1% reduction in recalcitrant keloids.	Keloid volume	**Primary keloid:** excision with TCA (n=5), IL cryotherapy (n=5). **Recalcitrant keloid:** excision with brachytherapy (n=7), IL cryotherapy (n=9). **Total no. keloid patients:** 26.
Davison et al., 2006	Ethnicity, location	(Postoperative) A: IL interferon alpha-2b; B: IL TCA	- Ethnicity and location: NS.	No. recurrences	**Ethnicity**: African American (n=21), Caucasian (n=13), Hispanic (n=4), Asian (n=1). **Location**: ear (n=10), face/scalp (n=8), chest (n=7), extremity (n=6), abdomen (n=4), neck (n=4). **Total no. keloid patients:** 34.
Hewedy et al., 2022 (10)	Duration	A: IL TCA + PRP; B: IL TCA	- Duration: NS.	VSS score	**Mean duration (months)**: 15.8 (n=20); 16.5. (n=20). **Total no. keloid patients:** 40.
Ismail et al., 2021	Duration, size	A: IL BTX-A; B: IL 5-FU	- Size: NS in groups receiving BTX-A. However, small, and medium lesions in the group receiving IL 5-FU showed a significantly better response than larger lesions. - Duration: NS.	Keloid height	**Duration**: ≤ 2 years (n=43), > 2 years (n=26). **Size**: Small <1 cm3 (n=43), medium 1-5 cm3 (n=20), large >5 cm3 (n=6). **Total no. keloid patients:** 50.
Khan et al., 2019	Baseline POSAS, duration skin type	A: IL bleomycin; B: IL TCA	- Fitzpatrick skin type, keloid duration and baseline POSAS score: NS.	POSAS score	**Mean duration (months)**: 4 (n=164). **Skin type**: type 2 (n=31), type 3 (n=63), type 4 (n=54), type 5 (n=16). **Mean POSAS Baseline:** 90 (n=82), 91 (n=82). **Total no. keloid patients:** 164.
Manzoor et al., 2022	Duration	A: IL 5-FU; B: IL TCA alone, C: IL TCA + 5-FU	-Duration: NS.	VSS score	**Mean duration (months)**: 5.03 (n=30), 6.30 (n=30), 5.27 (n=30).

					Total no. keloid patients: 90.
Mourad et al., 2016	Duration, etiology, location	A: IL cryotherapy; B: cryospray	- Duration: negative correlation between keloid duration and treatment efficacy. - Etiology and location: NS.	Swada and Sone scoring	**Mean duration (months):** NR **Etiology**: acne (n=16), burn (n=18), surgery (n=7), trauma (n=8), vaccine (n=2). **Location**: ear (n=10), face/scalp (n=8), chest (n=7), extremities (n=6), abdomen (n=4), and neck (n=4). **Total no. keloid patients:** 50.
Neinaa et al., 2021	Baseline VSS, duration, size	A: IL BTX-A; B: IL PRP C: IL TCA	- Duration and size: NS. - Baseline VSS: Higher baseline VSS scores were significantly correlated to better treatment outcomes in all studied groups (BTX-A and PRP were the most efficacious treatments).	VSS score	**Mean duration (months)**: 5.2 (n=20), 8.4 (n=20), 7.4 (n=20). **Mean baseline VSS:** 9.4 (n=20), 9.7 (n=20), 8.8 (n=20). **Mean size (cm2)**: 7.6 (n=20), 8.4 n=20), 7.4 (n=20). **Total no. keloid patients:** 60.
Rani et al., 2022	Duration, location	A: IL TCA, B: IL 5-FU, C: cryotherapy + IL TCA, D: surgical excision + topical 5% imiquimod	- Duration: negative correlation between keloid duration and treatment efficacy. Lesions of < 2 years showed better efficacy than lesions of >2 years ($p < 0.05$). - Location: 'excellent response' on earlobes, face and back. Poor efficacy on chest and shoulder ($p<0.05$).	Unclear	**Duration**: <1 year (n=42), 1-2 year (n=16), >2 year (n=22). **Location:** chest (n=38), earlobes(n=16), shoulders (n=11), face (n=1). **Total no. keloid patients:** 80.
Saha et al., 2012	Duration, location, no. of lesions	A: IL 5-FU; B: IL TCA	-Duration, location and number of lesions: NS.	Keloid volume	**Duration**: : ≤ 2 years (n=22), > 2years (n=22). **No of lesions** (range): 1-6 (n=NR). **Locations**: arms (n=NR), back (n=NR), chest (n=NR). **Total no. keloid patients:** 44.

4. Effects of keloid properties on treatment efficacy

Serag-Eldin et al, 2021	Duration, thickness	A: IL TCA, B: IL pentoxifylline, C: IL TCA + IL pentoxifylline	Duration and thickness: NS.	VSS score	**Mean duration(months):** 7.0 (n=10), 7.2 (n=10), 12.4 (n=10). **Mean thickness** (cm): 2.9(n=10), 4.4(n=10), 3.0 (n=10). **Total no. keloid patients:** 30.
Tawfic et al., 2020	Duration, location	A: Fractional CO2; B: Nd:YAG laser; C: CO2 + Nd:YAG lasers	- Duration: significant negative correlation between keloid duration and treatment efficacy with Nd:YAG laser (NS for fractional CO2 laser or a combination of fractional CO2 laser followed by Nd:YAG laser). - Location: NS.	VSS score	**Mean duration (years)**: 8.84 (n=30). **Location:** upper extremities (n=13), lower extremities (n=5), trunk (9), lower extremities + trunk (n=3). **Total no. keloid patients:** 30.

Table 1.

Results of the included studies

Abbreviations: BTX-A, Botulinum toxin type A; 5-FU, 5-flurouracil; duration, duration prior to treatment; IL, intralesional; No, number; NR, Not reported; NS, no significant correlation with treatment efficacy; POSAS, Patient and Observer Scar Scale; PRP, platelet rich plasma. TCA, triamcinolone acetonide; VAS, Visual Analogue Scale; VRS, Verbal Rating Scale; VSS, Vancouver Scar Scale; VTG, Verapamil treatment group

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Section III

Efficacy and safety of intralesional bleomycin treatment in keloids

Chapter 5

Needle-free electronically-controlled jet injector treatment with bleomycin is efficacious and well-tolerated in patients with severe keloids: results of a randomized, double-blind, placebo-controlled trial

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5. Needle-free electronically-controlled jet injector treatment with bleomycin is efficacious and well-tolerated in patients with severe keloids: results of a randomized, double-blind, placebo-controlled trial.

ABSTRACT

Background Severe keloids are difficult to treat. Corticosteroid injections with needles are painful and associated with frequent recurrences. Therefore, more effective, safe and patient friendly alternative treatments are urgently needed.

Objectives To assess the efficacy, tolerability, and patient satisfaction of intralesional bleomycin treatment using a needle-free electronic pneumatic jet injector (EPI) in severe keloids.

Methods Patients with severe keloids were included in this double-blind, randomized placebo-controlled trial with split-lesion design. Three EPI treatments with bleomycin or saline, were administered every four weeks in respectively the intervention and control side. Outcome measures were change in scar volume assessed by 3D-imaging, Patient and Observer Scar Assessment Scale (POSAS), skin perfusion with laser speckle contrast imaging (LSCI), spilled volume, procedure related pain, adverse events, and patient satisfaction.

Results Fourteen patients (9 female, 5 men) were included. The estimated mean keloid volume was significantly reduced with 20% after EPI-assisted bleomycin, compared to a slight increase of 3% in the control side ($p<0.01$). The estimated mean POSAS patient and observer scores decreased with respectively 26% and 28% ($p = 0.02$; $p = 0.03$). LSCI showed no significant change in perfusion. EPI treatment was preferred over previous needle injections in 85% of patients. The estimated mean spilled volume after EPI was around 50%, and NRS pain scores were moderate. Adverse events included bruising, hyperpigmentation, and transient superficial necrosis.

Conclusion Three EPI-assisted bleomycin treatments are efficacious and well-tolerated in severe keloids. Moreover, EPI treatment was preferred by most patients and may serve as a patient-friendly alternative treatment.

INTRODUCTION

Keloids are abnormally healing scars, which are associated with a substantially reduced quality of life due to pain, itching, and restriction of movement.[1,2] Keloids are most common in Fitzpatrick skins types 4-6 and are more prevalent in the African and Asian populations (prevalence of 5-10%), while Fitzpatrick skins types 1-3 are less frequently affected (prevalence of < 0.1%).[3] Recent studies suggest that a dysregulated transforming growth factor beta 1 (TGF-β1) pathway contributes to keloid formation by inducing neovascularization and the formation of abnormal fibrosis.[4] Neoangiogenesis and an increased activation and proliferation of fibroblasts, lead to increased collagen deposits, which plays an important role in keloid formation.[5]

Severely affected keloid patients are defined as having a single keloid exceeding a surface area of 10 cm^2 and/or multiple keloids.[6] Various factors, including Fitzpatrick skin type, anatomical location- and lesion duration may play a role in developing severe keloids.[7-9] Also, external factors such as low income and severe manipulation of keloids have been associated with the development of more severe keloids.[10]

The first-line treatment for keloids consists of conventional intralesional needle injections with corticosteroids.[11] Other treatment options include cryotherapy, intralesional 5-fluorouracil (5-FU) injections and (non-)ablative laser treatments.[12,13] However, drug delivery techniques as conventional needle-injections and laser treatment can be painful. Moreover, corticosteroids and 5-FU often lead to adverse effects and treatment failure.[14] Therefore, alternative treatment options are urgently needed.

Bleomycin, an antineoplastic agent, is a second-line option for intralesional keloid treatment. Its mechanism of action comprises delaying the cell cycle in the G2-phase, inhibition of DNA and RNA synthesis, apoptosis of fibroblasts, and suppression of collagen production.[16,17] Moreover, bleomycin induces endothelial cell damage by inhibiting cytokines including TGF-β1, resulting in a reduction of the perfusion of keloids.[18]

Intralesional administration of bleomycin with conventional needles has several disadvantages: it is not a patient-friendly treatment in patients with severe keloids because multiple painful injections are usually needed to achieve significant clinical improvement, and it cannot be used in patients with needle phobia.[19] As an alternative for needle injections, less painful intralesional delivery methods such as needle-free electronic pneumatic assisted injection (EPI) were developed.[20] A few retrospective studies showed that intralesional EPI-assisted triamcinolone acetonide (TCA) was effective, minimally painful and resulted in high treatment satisfaction in patients with keloids and hypertrophic scars.[21,22] However, since intralesional TCA is associated with frequent recurrences, it is often less effective in severe keloids.[23] Therefore, in this double-blind, randomized, vehicle-controlled, split-lesion trial, we investigated whether intralesional bleomycin delivered with an EPI is a patient-friendly delivery method with better treatment responses than placebo, in severe keloids.

METHODS

Study design

BLEOJET (NCT04582305) is a double-blind, randomized, placebo-controlled trial, with split-lesion design to evaluate the efficacy and tolerability of bleomycin compared to placebo, in keloids using an EPI. The Medical Ethics Review Committee (METC) of Erasmus MC in Rotterdam approved the study in May 2021 (NL74548.078.20). The study was conducted between March 2022 and December 2022 at the Dermatology department of Erasmus University Medical Center, Rotterdam, the Netherlands.

Patients

Inclusion criteria were age ≥ 18 years, at least one keloid of ≥ 4 cm in length, or two separate keloids of ≥ 2 cm with a minimum > 1 cm apart in the same anatomical regional, and willingness to fill in questionnaires and take photos using an e-diary application. A maximum of two large (≥ 4 cm) keloids were included to be treated in the trial.
Exclusion criteria were hypersensitivity to any component of the test materials, pregnancy or breast-feeding women, previous bleomycin treatment of the keloid within the last 12 weeks prior to screening, non-response to previous bleomycin treatments of the keloid, and any medical or psychiatric condition which would preclude the participant from adhering to the protocol or completing the study per protocol.

Randomization

The participants, treating physicians, and other investigators were blinded for the allocation of treatment. One larger keloid (≥ 4 cm) that was divided into two comparable halves, or two comparable smaller keloids (< 4 cm) were included. Each lesion half was randomly assigned to three consecutive treatments with bleomycin, or three consecutive placebo (physiological saline) treatments. Allocation and sequence were randomized by a validated computer system (SAS version 9.4 M6, SAS Institute, Cary, NC, USA) in blocks of four by a study-independent statistician. The randomization list was administrated and stored in a locked office at our hospital pharmacy. Blinding was concealed by an

unblinded pharmacist that prepared identical syringes, with either bleomycin or physiological saline. Blinding was ensured until data was locked.

Intervention

For each lesion, a transparent sheet was consistently used, indicating "lesion 1" and "lesion 2." To prevent a carry-over effect, an exclusion zone of 1 cm was respected between the lesions (Figure S1). A physician blinded to treatment administered three treatments every 28 days. Each treatment consisted of intralesional bleomycin in one lesion and physiological saline in the other lesion, using an EPI (Enerjet 2.0; Sinclair Pharma, Rehovot, Israel). This device contained a 10 ml syringe and a 200 µm nozzle. Approximately every square centimeter of the included keloid lesion received one intralesional injection. Each injection volume was 100 µl, delivered with a starting pressure of 3 bar and (device range 50–150µl; 2-6 bar). Pressure ranged from 3 – 5 bar depending on the scar characteristics, and was increased with 10% if the clinical endpoint (papule or blanching) was not observed after injection. In each keloid lesion 1 USP/ml of bleomycin (Bleomedac, Pharmanovia Benelux, Breda, The Netherlands) was delivered, while in the control lesion NaCl 0.9% was delivered. The syringes with bleomycin could not be distinguished from the syringes with NaCl. A maximum dose of 2 USP bleomycin was administered per treatment.

Primary and secondary outcomes

The primary outcome was change in keloid volume. Secondary outcomes included change in height, Patient and Observer Scar Assessment Scale (POSAS), change in perfusion, spilled volume during treatment, procedure related pain scores, adverse events and patient satisfaction. All outcome measures were assessed at all three treatment visits and at follow-up (week 12, four weeks after the third treatment).

Outcome assessments

The outcome assessments used are as follows.

(i) Change in volume (in mm3) and height (in mm) of keloid tissue measured by a three-dimensional (3D) camera (LifeViz Micro; Quantificare, Sophia Antipolis, France) at baseline compared with follow-up.

(ii) Change in POSAS at baseline compared with follow-up.

(iii) Change in skin perfusion measured by laser speckle contrast imaging (LSCI; Perimed PeriCam LSCI; Perimed AB, Järfälla, Sweden) at baseline compared with follow-up.

(iv) Average spilled volume assessed by weighing a filtration paper before and after each EPI-assisted injection. Postinjection weight was determined by weighing the filtration paper after dabbing it at the injection site.

(v) Procedure-related pain score measured with an 11-point numerical rating scale (NRS pain) directly after every EPI-assisted treatment.

(vi) Incidence and type of adverse events, assessed every 4 weeks by the treating physician, and by the patient, who was instructed to take photographs daily and report adverse events with an e-diary app.

(vii) Treatment satisfaction measured with the five-point Likert scale, evaluated by the patient at follow-up.

Statistical analysis

Sample size calculation was based on prior studies that investigated bleomycin treatment in keloids. We employed a 2-sided paired t-test with $\alpha = 0.05$ to detect a significant difference of at least 35% in volume reduction between treatments. To reach a statistical power of 90% and account for a corresponding coefficient variance of the difference of 40%, a sample size of 11 patients was determined to be necessary. Anticipating a dropout rate of 25%, 14 patients were needed to demonstrate a treatment effect. Descriptive statistics and tables are presented as least squares mean (LSM) with confidence intervals (CI). A mixed effects model with a random subject factor and pre-value as covariate was

used to compare bleomycin- and placebo treatment. Statistical analysis was performed using SAS for windows V9.4 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Demographics

Fourteen patients with at least one keloid were included (age range 18–48 years, 9 females; Fitzpatrick skin type I–VI, Table 1 and Table S1). In four patients, two smaller separated keloid lesions were assigned to treatment with bleomycin or placebo. In the remaining ten patients, one large (> 4 cm diameter) keloid was divided in two smaller lesions which were assigned to treatment with bleomycin or placebo. Anatomical locations included the abdomen (n = 1), neck (n = 1), upper extremity (n = 2), chest (n = 4), and shoulder (n = 6). All patients completed the three consecutive treatments. However, one patient was lost to follow-up and missed the follow-up visit.

Scar volume by 3D imaging

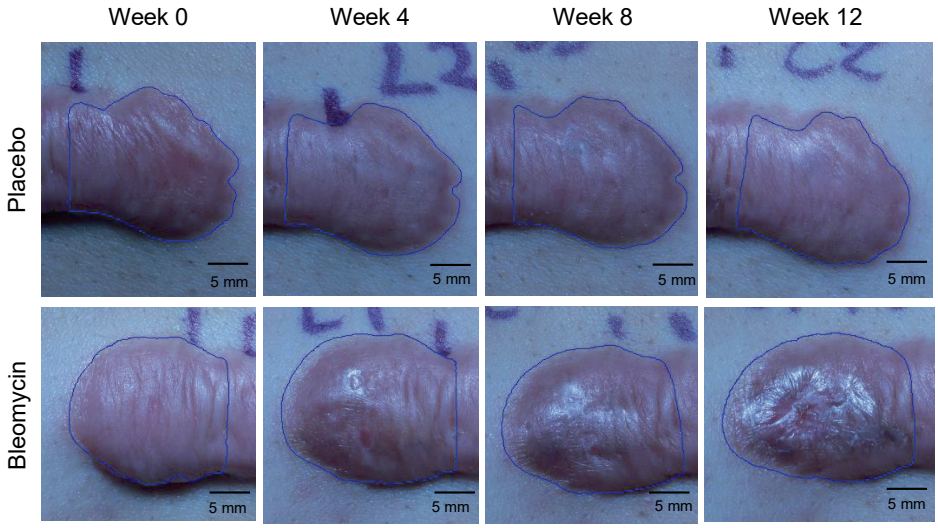
3D-imaging showed a statistically significant reduction in volume ($p< 0.01$) in bleomycin treated lesions, compared to placebo treated lesions (Figure 1-2a; Table 2). The baseline volume of the included lesions was 465.4 mm^3, and was reduced with 20% in the bleomycin treated lesions at follow-up (LSM -91.0 mm^3; CI -122.3, -61.5 mm^3). In contrast, a slight increase in volume of +3% was observed in the placebo treated lesions at follow-up (LSM +13.4mm^3; CI = -17.0, 43.90). Consistent with the volume results, statistically significant changes were observed in lesion height (Table 2).

POSAS

POSAS scores were filled in by the patients and physicians during all visits from baseline up to follow-up. Data on the changes over time are displayed for bleomycin vs. placebo treatment in Table 2, Figure 2c-d, and Table S2. The total POSAS patient score was 47.3 at baseline, and was reduced with 28% (LSM -13.3; CI -17.3, -9.4; p = 0.0344) at follow-up for the bleomycin treated lesions, versus a reduction of 16% (LSM -7,8; CI -11.8, -3.9) for those lesions treated with placebo.

For the patient POSAS, the parameters, itch (-62.2%; LSM -2.8; CI -3.9, -1.7; p = 0.044), thickness (-39.7%; LSM-2.7; CI -3.8, -1.7; p = 0.018) and overall opinion (-28%; LSM -2.3;

CI; -3.2, -1.4; p = 0.0022) showed a statistically significant improvement in the bleomycin treated lesions. Similarly, the total POSAS observer score was 41.0 points at baseline, and was reduced with 20% (LSM -8.4; CI -9.8, -6.9) at follow-up, versus a reduction of 4% (LSM -1.6; CI -3.0, -0.1) for those lesions treated with placebo. For the observer POSAS, the parameters thickness (-32%; LSM -2.3; CI -2.8, -1.7; p=0.01), relief (-23%; LSM -1.7; CI -2.2, -1.2; p = 0.01), surface (-42%; LSM -3.2 CI -3.7, -2.7; p< 0.01) and overall opinion (-21%; LSM -1.5; CI -1.8, -1.2; p < 0.01) were significantly improved at follow-up in the bleomycin treated lesions. The POSAS observer score for pigmentation was 7.7 points at baseline, and was significantly worsened with 7% at follow-up (LSM 0.6; CI 0.2, 1.0; p <0.01).



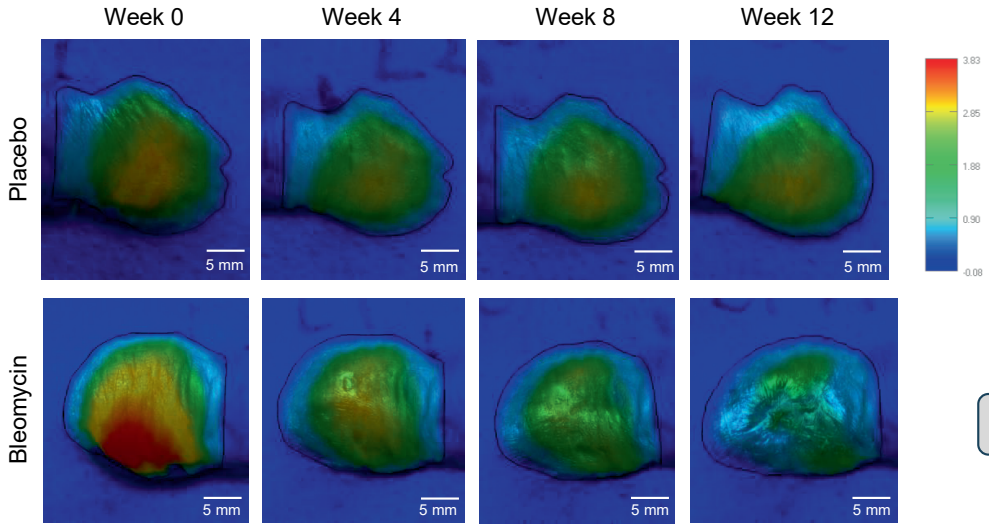


Figure 1 Clinical pictures (1a) and 3D-images (1b) of two keloid lesions which were treated with respectively placebo and bleomycin. A reduction of 46% in volume was detected in the intervention site at follow-up compared to baseline, while the lesion that received placebo did not change.

Patient-reported pain and treatment satisfaction

The patient-reported NRS pain score during EPI treatment was similar for both treatments (bleomycin: LSM 5.4; CI 4.5, 6.3; placebo LSM 5.6; CI 4.5, 6.3; p=0.54). The overall satisfaction of the treatment resulted most frequently in 'satisfied' (69.2%; n=13; Table S3). Moreover, the majority of patients (84.6%; n=13) preferred EPI over conventional needle injections. All patients (100%; n=13) would recommend EPI treatment with intralesional bleomycin to others.

Microcirculation

Cutaneous microcirculation of the lesions was quantified using LSCI. No statistically significant differences in cutaneous microcirculation were observed with bleomycin versus placebo at follow-up (Figure 2b; Table 2).

Residue formation

The extent of drug spillage was evaluated by collecting the residual fluid on the skin surface. In total, 50.0% (±11.8%) of the injected volume with bleomycin and 43.6% (±8.6%) with physiological saline of residual fluid was observed.

Safety and tolerability

Overall, intralesional bleomycin treatment with the EPI was well tolerated (Table S4). No severe adverse events or treatment discontinuations occurred during the study. However, two out of fourteen patients (14%) developed transient superficial necrosis at the injection site, which recovered in approximately four weeks. Furthermore, in the bleomycin treated lesions temporary bruising occurred in two patients (14%), and mild hyperpigmentation was observed in most patients (71.4%%, n=10) at four-weeks follow-up. No infection or ulceration was observed. All adverse events were mild and transient.

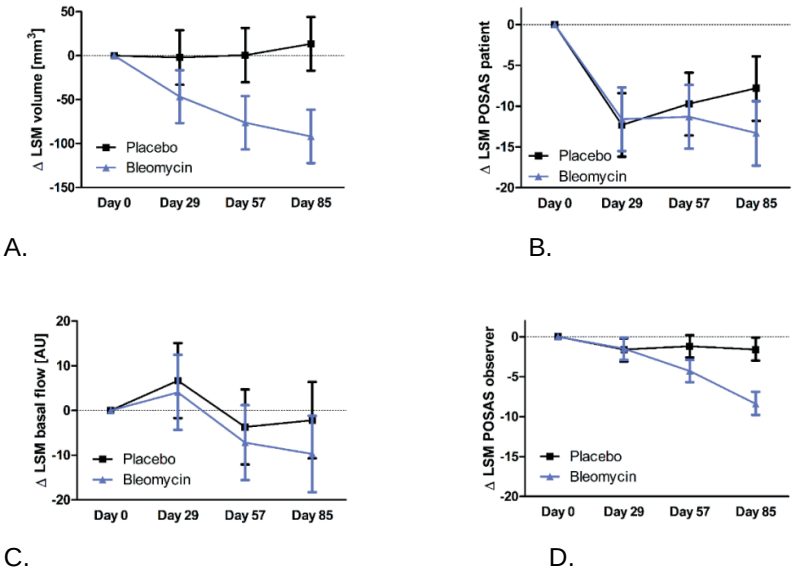


Figure 2.

Results of 3D imaging-, perfusion- and POSAS measurements. Errors bars are defined by LSM +/- upper and lower limits. A) LSM change from baseline in volume [mm^3] by 3D imaging, B) LSM change from baseline in basal flow [AU] by laser speckle contract imaging, C) LSM change from baseline in total POSAS patient score, D) LSM change from baseline in total POSAS observer score.

DISCUSSION

This randomized controlled trial evaluated the efficacy and tolerability of EPI-assisted intralesional bleomycin treatment in patients with severe keloids. We found a significant decrease of 20% in keloid volume after three consecutive bleomycin EPI treatments whereas placebo-treated lesions remained unchanged. Importantly, this decrease was paralleled by a substantial improvement of the POSAS patient and – observer scale of 28% and 26%, respectively after bleomycin treatment. Contrary to our hypothesis, the effect of bleomycin does not occur through permanent changes in microcirculation. Importantly, no severe adverse events occurred. Notably, in the majority of patients mild hyperpigmentation was observed in the bleomycin treated keloids. This phenomenon was previously observed in other intralesional bleomycin studies, but was not regarded as bothersome by most patients.[24] In line with our previous findings, 85% of the patients preferred treatment with the jet injector over conventional needle injections, which supports the use of needle-free injector devices as patient-friendly alternative delivery method in this patient group.[21,22,25]

A previous study of Rijsbergen et al., showed that the 3D imaging technique that was used in our study is an accurate and reliable method for the clinical visualization of HPV-induced skin lesions.[26] No previous trials have been published that used highly sensitive objective 3D measurements in conjunction with patient reported outcomes to assess the clinical effects of intralesional bleomycin treatment in patients with keloids. Khan *et al.* compared six treatments of intralesional bleomycin vs. TCA using conventional needle injections, and found a significant improvement in mean combined POSAS score (sum of patient- and observer score) of 72% vs. 67%, respectively.[27] When intralesional bleomycin was compared with intralesional 5-fluorouracil with or without TCA, mean improvements of 73%, 54% and 55% on the Vancouver Scar Scale were observed after two to six treatments.[28] It is noteworthy to mention that, in the majority of keloid studies, a dosage of 0.375 U intralesional bleomycin was injected every cm^2 using conventional needle injectors or spring loaded jet injectors.[15,27-29] Despite the good efficacy achieved in these studies, bleomycin treatment led to a high rate of treatment discontinuations and mild to

moderate adverse events including ulcerations, necrosis, infection, pain, and hyperpigmentation. Yet, another randomized controlled trial in keloids also found good efficacy without adverse events with a lower dosage of 0.1 U bleomycin per cm^2.[24] This study also showed that with this dosage, no systemic uptake of bleomycin takes place. Therefore, in our study we chose to use the lower bleomycin dose of 0.1 U/cm^2 which we considered to be safer for repetitive administrations.

As a result, bleomycin treatment was generally well-tolerated, with only two patients developing transient superficial necrosis of the treated keloid, which did not lead to treatment discontinuation. However, we recommend to use a concentration of 0.2U/cm², when administering bleomycin via EPI for the treatment of severe keloids due to the spilled volume of 50%.

In the study by Erlendson *et al.*, a single treatment with 5-FU and TCA was administered using an EPI in patients with hypertrophic scars.[30] Remarkably, in their study a lower median procedure-related NRS pain score of 2.0 vs. 5.6 in our trial was observed. However, in general, hypertrophic scars are less painful than severe keloids. In a previous study by our group with intralesional EPI-assisted TCA treatment in keloids, we found a lower mean NRS pain score of 3.8.[23] The higher pain scores in the present study could be related to the burning pain sensation that bleomycin can cause.[31] However, also EPI-assisted injections with placebo resulted in a higher NRS pain score of 5.4. Therefore, the higher injection-related pain scores in our current study is more likely related to our specific patient population that suffered from extremely severe keloids, of which some were already very painful upon palpation.

One of the strengths of this study is the design of the trial. In addition, we incorporated both objective outcomes such as volume reduction measured with a 3D-camera using a standard operating procedure,[26] and subjective outcomes such as POSAS, NRS pain and patient satisfaction. Moreover, to minimize recall bias of adverse events, patients were instructed to take pictures of the treated area and report potential adverse events via an e-diary mobile application on a daily basis.

A theoretical limitation of this study includes a cross-over effect of bleomycin treatment from one side of the lesion to the other side of the split-lesion in larger keloids (≥ 4 cm). Therefore, in all divided keloids an exclusion zone of 1 cm was respected to minimize the potential cross-over effect. Additionally, our study is constrained by a relatively short follow-up time, which limits the evaluation of recurrences. However, a previous meta-analysis has shown already that recurrence rates with bleomycin are low, and therefore we did not prioritize a longer follow-up time.[32] Another limitation is the substantial residual fluid (≈ 50% of the injected volume) observed on the skin after EPI treatments, which was higher than previously reported in other studies (≈ 10-20% of the injected volume).[30,33] This might be related to the rigid nature of the severe keloids that were included in this study that were more difficult to penetrate with EPI.

Our results indicate that intralesional EPI-assisted bleomycin administration is a promising treatment modality for patients with severe keloids. However, since there is a small risk for local adverse events such as transient necrosis, we believe it should primarily be considered if standard of care (intralesional TCA) fails or leads to quick recurrences. Furthermore, intralesional bleomycin cannot be used in pregnant or lactating women, and therefore extra caution is needed when selecting patients for this treatment. Moreover, when performing EPI-assisted intralesional bleomycin treatment, it is important to use protective safety measures such as smoke evacuators and face masks to prevent the inhalation of potentially harmful bleomycin aerosols by patients and practitioners.[34]

Future technical innovation of EPI devices may lead to more efficient, more precise and less painful drug delivery with minimal residue formation. However, until this next generation of devices arrives, addition of local anesthetics such as lidocaine may be considered for decreasing the procedure related pain.
To conclude, in this study we demonstrated that three monthly EPI treatments with bleomycin significantly decreased keloid volume, keloid related symptoms, and was preferred over needle injection by patients with severe keloids. A well-powered

randomized controlled trial with parallel design, extended treatments and longer follow-up time is warranted to confirm our findings.

5. Needle-free electronically-controlled jet injector treatment with bleomycin is efficacious and well-tolerated in patients with severe keloids: results of a randomized, double-blind, placebo-controlled trial.

ACKNOWLEDGEMENTS

The authors thank all participating patients as well as the Dutch clinical network for trials in dermatology (CONNECTED).

Characteristic	N (%)
Sex	
Female	5 (35,7%)
Age	
median (Q1-Q3)	27,5 (23,5 – 35,3)
Fitzpatrick skin type	
1-2	2 (14,3%)
3-4	10 (71,4%)
5-6	2 (14,3%)
Anatomical location *	
Thorax	7 (50%)
Shoulder(s)/ back	4 (28,6%)
Abdomen	1 (7,1%)
Neck	1 (7,1%)
Upper extremities	1 (7,1%)
Etiology*	
Acne	5 (35,7%)
Spontaneous/unknown	5 (35,7%)
Trauma/surgery	2 (14,3%)
Chickenpox	1 (7,1%)
Folliculitis	1 (7,1%)
Previous treatments*	
Intralesional TCA treatments	11 (78,6%)
Intralesional Bleomycin/kenacort treatments	4 (28,6%)
Cryotherapy	4 (28,6%)
(Shave) excision	4 (28,6%)
Clobetasol cream	3 (21,4%)
Vascular or ablative laser treatment	2 (14,3%)
Excision + Brachytherapy	2 (14,3%)
Ciclosporine tablets	1 (7,1%)
Surface in cm^2 of included keloid lesions	
0-10 cm^2	8 (57.1%)
10-30 cm^2	3 (21,4%)
>30 cm^2	3 (21,4%)
Total number of previous treatments mean (±SD	9,8 (±7,7)
Total POSAS observer score at baseline mean (±SD)	41.1 (±8.1)
Total POSAS patient score at baseline mean (±SD)	47.4 (±5.9)
Total number of keloids	
1	1 (7,1%)
2 - 10	7 (50,0%)
10 - 20	3 (21,4%)
20 - 30	2 (14,3%)
> 30	2 (14,3%)

Table 1.

Patient baseline characteristics

	Bleomycin				Placebo				
	Day 0	Δ Day 29	Δ Day 57	Δ Day 85	Day 0	Δ Day 29	Δ Day 57	Δ Day 85	p-value*
Volume (mm^3)	465.38	-46.64 (-76.78, -16.51)	-76.25 (-106.67, -45.84)	-91.90 (-122.32, -61.48)	465.38	-2.11 (-33.07, 28.85)	0.66 (-30.26, 31.59)	13.44 (-17.01, 43.90)	<0.01
Height (mm)	1.26	-0.14 (-0.24, -0.04)	-0.25 (-0.35, -0.15)	-0.30 (-0.40, -0.20)	1.26	0.00 (-0.10, 0.10)	0.02 (-0.08, 0.13)	0.05 (-0.05, 0.15)	0.0015
Basal Flow (AU)	120.41	4.05 (-4.34, 12.43)	-7.19 (-15.58, 1.19)	-9.73 (-18.28, -1.18)	120.41	6.70 (-1.70, 15.09)	-3.69 (-12.09, 4.70)	-2.18 (-10.73, 6.37)	0.1630
Total POSAS Observer	41.0	-1.5 (-2.9, -0.1)	-4.3 (-5.7, -2.9)	-8.4 (-9.8, -6.9)	41.0	-1.6 (-3.1, -0.2)	-1.6 (-3.1, -0.2)	-1.6 (-3.0, -0.1)	0.0011
Total POSAS patient	47.3	-11.6 (-15.5, -7.7)	-11.3 (-15.2, -7.4)	-13.3 (-17.3, -9.4)	47.3	-12.3 (-16.2, -8.4)	-9.7 (-13.6, -5.9)	-7.8 (-11.8, -3.9)	0.0344

Table 2.

Analysis results of 3D imaging, perfusion and POSAS measurements. Data is presented as LS mean, Δ representing LSM change from baseline, 95% confidence interval and a p-value (Bleomycin-Placebo).

*p-value comparing Δ values at follow-up in control versus intervention.

SUPPLEMENTARY FILES

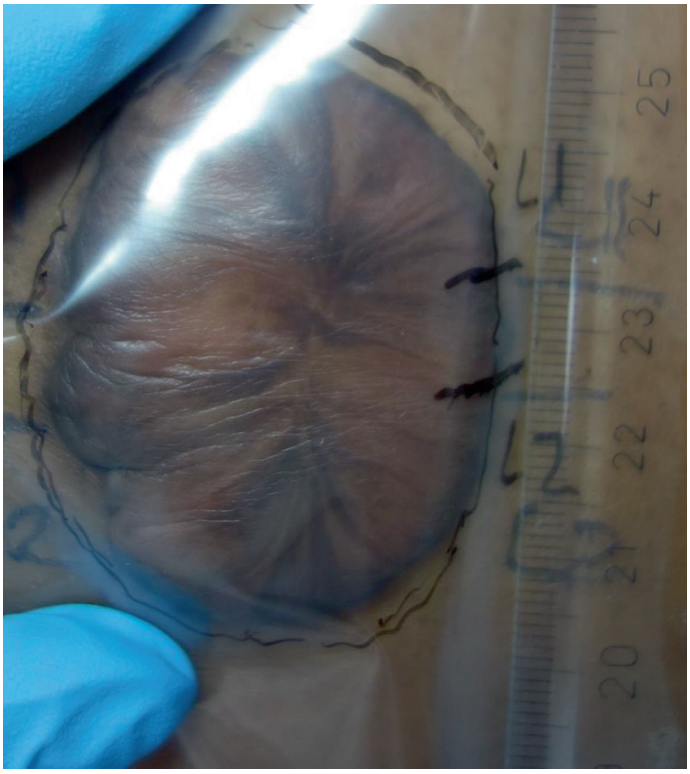


Figure S1

Example of a transparent sheet indicating "lesion 1" and "lesion 2." To prevent any potential carry-over effect, an exclusion zone of 1 cm was respected between the lesions.

5. Needle-free electronically-controlled jet injector treatment with bleomycin is efficacious and well-tolerated in patients with severe keloids: results of a randomized, double-blind, placebo-controlled trial.

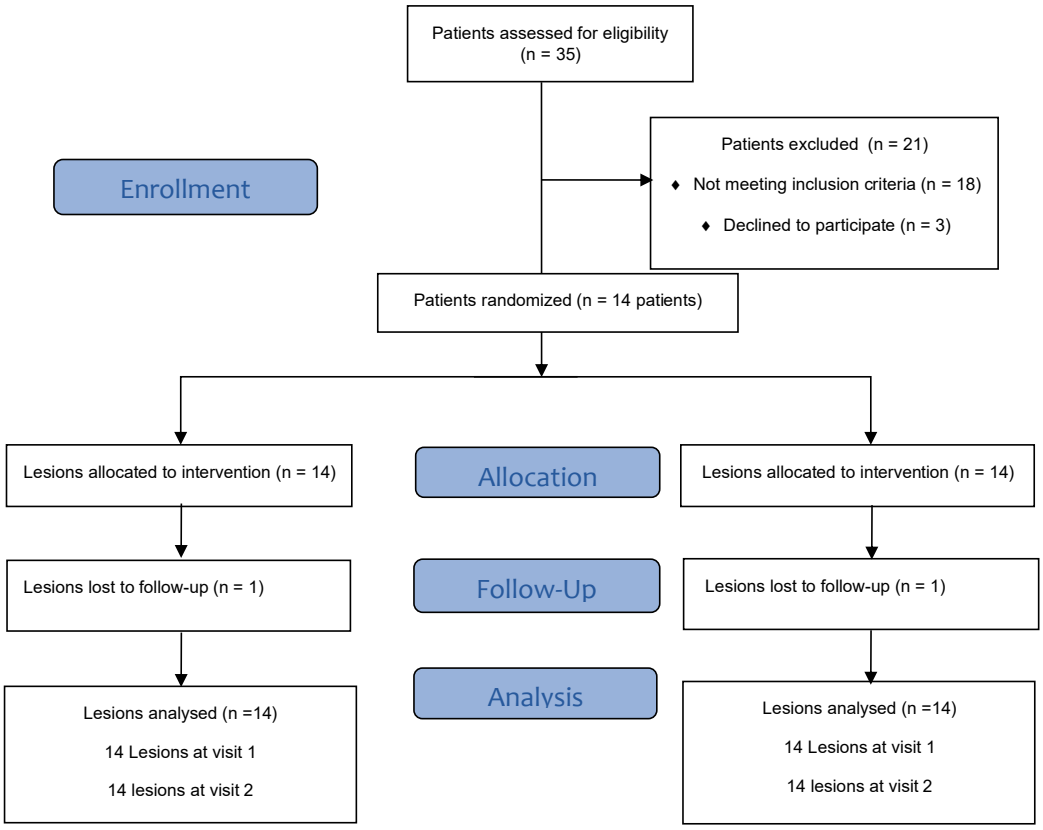


Table S1

Flow diagram of the enrollment, allocation and follow-up of participants and the subsequent data analysis process.

	Bleomycin				Placebo				
	Day 0	**Δ Day 29**	**Δ Day 57**	**Δ Day 85**	**Day 0**	**Δ Day 29**	**Δ Day 57**	**Δ Day 85**	**p-value**
Observer scale Vascularity	6.51	6.30 (5.78, 6.81)	6.30 (5.78, 6.81)	5.32 (4.78, 5.85)	6.51	6.30 (5.78, 6.81)	6.15 (5.63, 6.67)	6.22 (5.69, 6.75)	0.11
Observer scale Pigmentation	6.90	7.33 (6.93, 7.72)	7.40 (7.00, 7.79)	7.48 (7.08, 7.88)	6.90	6.76 (6.37, 7.15)	6.69 (6.30, 7.08)	6.83 (6.43, 7.24)	<0.01
Observer scale Thickness	6.74	5.96 (5.43, 6.49)	5.39 (4.86, 5.92)	4.49 (3.94, 5.04)	6.74	6.31 (5.78, 6.84)	6.67 (6.14, 7.20)	6.35 (5.80, 6.90)	0.01
Observer scale Relief	6.66	6.73 (6.22, 7.24)	5.66 (5.15, 6.16)	4.96 (4.43, 5.48)	6.66	6.37 (5.86, 6.87)	6.37 (5.86, 6.87)	6.38 (5.86, 690)	0.01
Observer scale Pliability	7.17	6.31 (5.83, 6.79)	5.10 (4.62, 5.58)	3.97 (3.47, 4.47)	7.17	6.60 (6.12, 7.08)	6.88 (6.40, 7.36)	7.01 (6.51, 7.51)	<0.01
Observer scale Surface	7.06	6.91 (6.54, 7.28)	6.91 (6.54, 7.28)	6.46 (6.08, 6.83)	7.06	7.07 (6.70, 7.44)	7.07 (6.70, 7.44)	6.66 (6.28, 7.03)	0.30
Observer scale Overall opinion	6.93	6.64 (6.34, 6.94)	6.00 (5.70, 6.30)	5.45 (5.14, 5.76)	6.93	6.50 (6.20, 6.80)	6.78 (6.48, 7.08)	6.76 (6.45, 7.07)	<0.01
Patient scale Pain	3.76	1.85 (1.32, 2.60)	2.20 (1.56, 3.08)	2.77 (1.96, 3.92)	3.76	1.92 (1.36, 2.69)	2.29 (1.63, 3.21)	2.42 (1.71, 3.43)	0.79
Patient scale Itch	7.44	4.39 (2.29, 5.49)	4.54 (3.44, 5.64)	4.65 (3.52, 5.79)	7.44	4.68 (3.58, 5.78)	4.97 (3.87, 6.07)	5.53 (4.40, 6.67)	0.04
Patient scale Color	9.20	7.41 (6.18, 8.64)	7.62 (6.39, 8.85)	7.21 (5.96, 8.46)	9.20	6.55 (5.32, 7.78)	7.20 (5.96, 8.43)	7.48 (6.23, 8.73)	0.43
Patient scale Stiffness	8.57	7.02 (5.96, 8.07)	7.02 (5.96, 8.07)	6.34 (5.27, 7.42)	8.57	6.36 (5.31, 7.41)	7.50 (6.45, 8.55)	7.56 (6.49, 8.63)	0.18
Patient scale Thickness	8.90	7.27 (6.22, 8.32)	6.98 (5.93, 8.03)	6.18 (5.11, 7.25)	8.90	7.55 (6.50, 8.60)	7.55 (6.50, 8.60)	7.80 (6.73, 8.87)	0.01
Patient scale Irregularity	8.88	7.38 (6.44, 8.32)	7.09 (6.15, 8.03)	6.33 (5.36, 7.29)	8.88	7.38 (6.44, 8.32)	7.52 (6.58, 8.46)	8.15 (7.18, 9.11)	0.06
Patient scale Overall opinion	9.02	7.88 (7.05, 8.71)	7.66 (6.83, 8.50)	6.72 (5.86, 7.57)	9.02	7.95 (7.12, 8.78)	8.16 (7.33, 9.00)	8.12 (7.27, 8.98)	0.01

Table S2

Analysis results of POSAS observer- and patients scores. Data is presented as LS mean, Δ representing LSM change from baseline, 95% confidence interval and a p-value (Bleomycin-Placebo).

5.. Needle-free electronically-controlled jet injector treatment with bleomycin is efficacious and well-tolerated in patients with severe keloids: results of a randomized, double-blind, placebo-controlled trial.

Patient satisfaction and preference		**N, N = 13**	**%**
How satisfied are you with the jet injector treatment?	Dissatisfied	1	7.7
	Neutral	1	7.7
	Satisfied	9	69.2
	Completely satisfied	2	15.3
Would you prefer needle injections or jet injections in the future?	Jet injection	11	84.6
	Needle injection	1	7.7
	No opinion	1	7.7
Would you recommend the jet injection to others?	Yes	13	100
	No	0	0

Table S3

Patient satisfaction and preference.

Minor adverse event	N, N = 14	%
Bruising	2	14,3%
Hyperpigmentation	10	71,4%
Superficial necrosis	2	14,3%
Infection	-	-
Ulceration	-	-

Table S4

Adverse events.

5. Needle-free electronically-controlled jet injector treatment with bleomycin is efficacious and well-tolerated in patients with severe keloids: results of a randomized, double-blind, placebo-controlled trial.

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Chapter 6

Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

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6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

ABSTRACT

Objectives The treatment of recalcitrant keloids is challenging. Although intralesional bleomycin using conventional needle injectors (CNI) is effective, it has important drawbacks, such as the need for repetitive and painful injections. Therefore, we aimed to evaluate the effectiveness, tolerability and patient satisfaction of intralesional bleomycin with lidocaine administered with a needle-free electronically-controlled pneumatic jet-injector (EPI) in recalcitrant keloids.

Methods This retrospective study included patients with recalcitrant keloids who had received three intralesional EPI-assisted treatments with bleomycin and lidocaine. Effectiveness was assessed using the Patient and Observer Scar Assessment Scale (POSAS) at baseline and four to six weeks after the third treatment. Additionally, treatment related pain scores numeric rating scale, adverse effects, patient satisfaction and Global Aesthetic Improvement Scale (GAIS) were assessed.

Results Fifteen patients with a total of >148 recalcitrant keloids were included. The median total POSAS physician- and patient-scores were respectively 40 and 41 at baseline, and reduced with respectively 7 and 6-points at follow-up ($p < 0.001$; $p < 0.001$). The median pain scores during EPI-assisted injections were significantly lower compared to CNI-assistant injections, (2.5 vs. 7.0, respectively ($p < 0.001$)). Adverse effects were mild. Overall, patients were "satisfied" or "very satisfied" with the treatments (14/15, 93.3%). The GAIS was "very improved" in one patient, "improved" in nine patients and "unaltered" in four patients.

Conclusions EPI-assisted treatment with bleomycin and lidocaine is an effective, well tolerated, patient-friendly alternative for CNI in patients with recalcitrant keloid scars. Randomized controlled trials are warranted to confirm our findings and improve the clinical management of recalcitrant keloids.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

INTRODUCTION

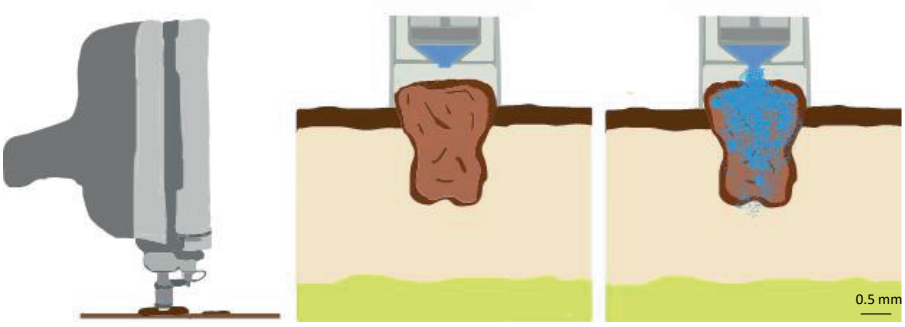
Keloids are hyperproliferative scars that extend beyond the confines of the original wound or trauma and are caused by chronic localized dermal inflammation.[1, 2] Keloids can cause both physical and psychosocial distress.[3] The Quality of Life (QoL) of patients can be severely affected by symptoms such as pain, pruritus, functional impairment and physical appearance.[4]

Keloidal scars are characterized by an overproduction of extracellular matrix components, including collagen, elastin, fibronectin, and proteoglycans.[5] The pathogenesis of keloid scars is not completely understood and entails a combination of genetic and environmental factors, with a higher incidence observed among individuals with darker skin tones.[6-8] Current hypotheses suggest that the reticular dermis plays a vital role in the development of these scars.[9] Trauma or inflammation of the skin can trigger a chronic low grade inflammatory response within this part of the dermis.[2] This inflammatory response involves activation of fibroblasts and a number of cytokines, including IL-6, IL-8, and IL-10, alongside several growth factors. Subsequently, the activation of fibroblasts induces neovascularization and increased deposition of collagen, ultimately leading to keloid formation.[10]

The first line treatment of keloids consists of intralesional needle injections with corticosteroids.[11] Unfortunately, these injections often cause significant procedure related pain and are unsuitable for patients with needle phobia, which occurs in up to 30% of young adults.[9] Furthermore, the firm consistency of the keloid can hamper the intralesional delivery of drugs and may lead to reduced effectiveness.[12] Although treatment with corticosteroids is effective in most patients, there is a high risk for recurrences, which are reported to occur in up to 50% of patients after 12 months.[13] Finally, some patients do not respond to intralesional corticosteroid injections.[14] These limitations underline the need for more effective, safe, and patient-friendly treatment options with long-lasting benefit in this difficult to treat population.

Needle-free electronically controlled pneumatic jet injectors (EPI) are an innovative and less painful alternative for intralesional injections with conventional needles.[15] These devices create a high-velocity fluid stream that penetrates the epidermis to inject drugs

intralesionally (Figure 1). In contrast to needle-free spring-driven jet injectors, EPI allow the adjustment of settings such as volume and pressure.[15] A recent study demonstrated the high effectiveness, good tolerability, and patient satisfaction of intralesional EPI-assisted triamcinolone acetonide (TCA) treatment in keloids.[16]



A. B. C.

Figure 1

Illustration of an electronically-controlled pneumatic jet injector-assisted treatment with bleomycin and lidocaine in a keloid scar. (A) Before administering treatment, the electronically-controlled pneumatic jet injector (EPI) hand piece with the injector tip is placed perpendicularly on the keloid scar. (B) A cross-sectioned illustration of the injector tip and nozzle of an EPI-device. The liquid container within the EPI contains a solution with the combination of bleomycin and lidocaine (depicted in blue). (C) Illustration during injection. The EPI device generates a high-velocity jet stream that punctures the epidermis of the keloid, disperses the combination of bleomycin and lidocaine in the mid-deep dermis and creates visible skin papule or blanching.

Bleomycin could potentially be used as an alternative drug for the intralesional treatment of recalcitrant keloids. It is an antineoplastic antibiotic that has been used off-label for various dermatological indications (e.g., hemangiomas, hypertrophic scars, Kaposi sarcoma, and warts) with satisfactory results.[17] The therapeutic effect of bleomycin is attributed to its ability to induce DNA destruction, apoptosis of the cell and inhibition of the collagen synthesis by decreasing a.o. TGF- β1, an important cytokine in immunoregulation, wound healing, angiogenesis and cancer.[18] Previous studies have

demonstrated that Intralesional bleomycin delivered with conventional needles is associated with a significantly lower risk of recurrence compared to intralesional corticosteroids in the treatment of keloids.[19] Lidocaine is usually added to intralesional bleomycin to reduce pain at the injection site and to increase the intracellular uptake of bleomycin.[20]

EPI-assisted intralesional administration of bleomycin with lidocaine, could be a suitable alternative treatment for patients with recalcitrant keloids that have previously failed or discontinued intralesional corticosteroid therapy or experienced a fast recurrence. Therefore, in this retrospective cohort study, we aimed to investigate the effectiveness, safety and patient satisfaction of EPI-assisted intralesional bleomycin with lidocaine in patients with recalcitrant keloids.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

MATERIALS AND METHODS

Study design and population

This retrospective cohort study was performed at the Department of Dermatology at Erasmus University Medical Center (Erasmus MC) in Rotterdam, The Netherlands from November 2022 until May 2023. All adult patients with the presence of ≥1 recalcitrant keloid scar defined as a history of suboptimal treatments (resistance to multiple intralesional TCA injections with conventional needle injectors (CNI) or EPI, needle phobia or needle pain) were eligible for inclusion. The Medical Ethical Research Committee of Erasmus MC in Rotterdam approved the study (MEC-2021-0661). STROBE guidelines were followed. Written informed consent was obtained from all patients for the anonymous use of their clinical data and photographs.

Data collection and outcome measures

Electronical medical records were used for data collection. The primary objective was to assess clinical effectiveness using the Patient and Observer Scar Assessment Scale (POSAS).[21, 22] The POSAS score is a scar assessment tool that measures the quality of a scar.[22] A local standard operating procedure (SOP) for EPI-assisted bleomycin treatment in adult patients with keloids was followed. According to this SOP, the POSAS was used to evaluate the keloids by the treating physicians (V. B.; P. A.) and patients during treatment regular visits to the outpatient clinic at baseline and four to six weeks after the third treatment. The POSAS score consists of six items concerning patient symptoms and clinical characteristics of the keloid. The patient and clinician can score the items from 1 (normal skin) to 10 (worst imaginable abnormality) points. The sum of these items gives the total POSAS score of minimum 6 and maximum of 60 points.

The secondary objectives included tolerability, patient satisfaction and aesthetic appearance. These objectives were evaluated using treatment-related pain scores (numeric rating scale [NRS] range 0–10), adverse effects which were recorded at each visit by the treating physician, a patient satisfaction questionnaire and the Global Aesthetic Improvement Score (GAIS), respectively. The GAIS measures the improvement

of a scar compared to pretreatment, which consists of five degrees: exceptional improvement, very improved patient, improved patient, unaltered patient, and worsened patient.[23] An online questionnaire to measure patient satisfaction was conducted at follow-up and was created with LimeSurvey version 2.06 (LimeSurvey GmbH, Hamburg, Germany).[24]

Treatment

During a regular outpatient visit, a test treatment with intralesional bleomycin combined with lidocaine was administered using respectively an EPI (Enerjet 2.0, Perfaction, Rehovot, Israel) and a CNI (27 gauge) in two similar keloids according to a local SOP. Treatment related pain scores on a numerical rating scale (NRS; range 0−10) during EPI and needle injections and patient preferences were recorded by the treating physician after the test treatment. Depending on pain scores and patient preferences, treatment with either EPI or CNI was chosen as delivery method for the consecutive treatments. All patients who received a test treatment with EPI and CNI, were included in this study. Needle injections with 0.5–3 mL bleomycin mixed with lidocaine (1 USP/mL bleomycin in 5 mg/mL lidocaine and NaCl 0.9%) were used for the intradermal treatment. For EPI an injection volume of 100 µL (device range: 50–130 moL) and pressure level of 3.2 Bar (device range: 2–6 Bar) were pre-selected for each treatment. In firmer keloids, the pressure was increased with 10% per injection until a consistent papule or blanching (clinical endpoint) was visible after injection (Figure 2). Each EPI-assisted injection was administered in a 1 cm^2 surface area. Clinical photographs of all keloid scars were taken at each visit.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

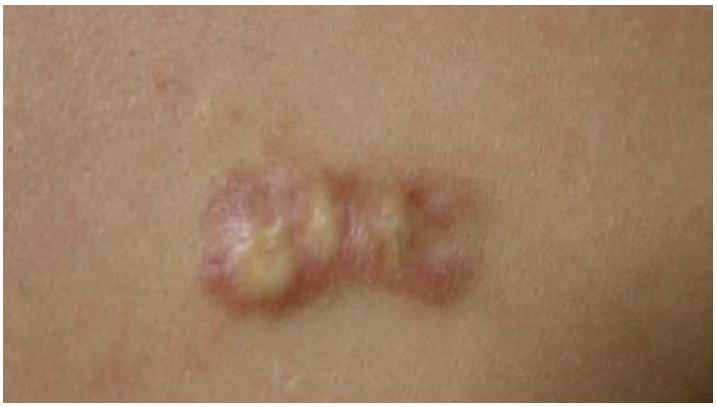


Figure 2

Keloid of a 28-year-old patient, directly after EPI-assisted treatment with bleomycin and lidocaine, with blanching observed as clinical endpoint. The paler regions within the keloid indicate successful administration of medication. EPI, electronically controlled pneumatic jet injector.

Statistical analysis

All data were analyzed using SPSS 28.0 (IB). The Wilcoxon signed-rank test was employed to evaluate the change in median POSAS scores at baseline and follow-up and procedure-related pain scores. Descriptive statistics were presented as median and IQR. A *p* Value of ≤0.05 was considered statistically significant.

RESULTS

Patient characteristics

A total of fifteen patients (eight female) with a median age of 28 (IQR 22−41) years and in total >148 keloids were included (Table 1). The majority of patients (73%, 11/15) had Fitzpatrick skin type three or four. All patients had an extensive treatment history, with at least multiple intralesional triamcinolone acetonide (TCA) treatments by CNIs.

Treatment

Fourteen out of 15 patients (93%) completed the course of three consecutive intralesional EPI assisted treatments and visited the outpatient clinic at a four to six weeks follow-up. One patient (7%) discontinued after two treatments due to a pregnancy wish. Most patients (80%, 12/15) were treated with a pressure ranging from 3 to 4 bar. In the remaining patients (20%, 3/15), a firmer keloid structure required a higher pressure of 4–5 bar to reach the clinical endpoint and achieve dermal distribution. The median interval between treatment was 5 weeks (IQR: 4–6) (Table S1).

Effectiveness

The POSAS observer scores and patient scores were significantly improved at follow-up compared to baseline (Table 2 and Figure 3). The median total POSAS observer score was 40 (IQR 29−51) at baseline, and the median paired difference at follow-up compared to baseline was −7 (−18%; IQR −11 to −3) points ($p < 0.001$). Similarly, the median total POSAS patient score was 41 (IQR 37−47) at baseline, and the median paired difference at follow-up compared to baseline was −6 (−15%; IQR −13 to −3) points ($p < 0.001$). The POSAS observer scale demonstrated a significant improvement in the subcategories “relief” and “overall opinion” ($p = 0.002$; $p = 0.019$), while the POSAS patient scale showed a significant improvement in the subcategories “itch,” “stiffness,” and “thickness” ($p = 0.049$; $p = 0.006$; $p = 0.023$). The GAIS score showed that 10 out of 14 patients (71%) exhibited “improved” or “very improved” treatment outcomes, and four out of 14 patients (29%) showed “unaltered” results (Table S2 and Figure 4).

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

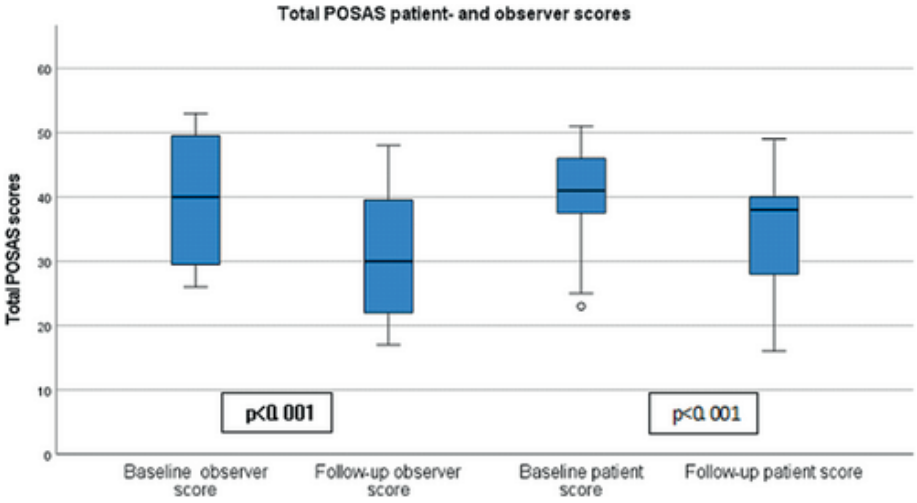


Figure 3

Clinical effectiveness assessed with Patient and Observer Scar Assessment Scale (POSAS) at baseline and after three consecutive treatments with bleomycin and lidocaine using an EPI. Based on the total POSAS scale, patients and physisians reported a significant improvement in the keloids at follow-up. EPI, electronically-controlled pneumatic jet-injector.

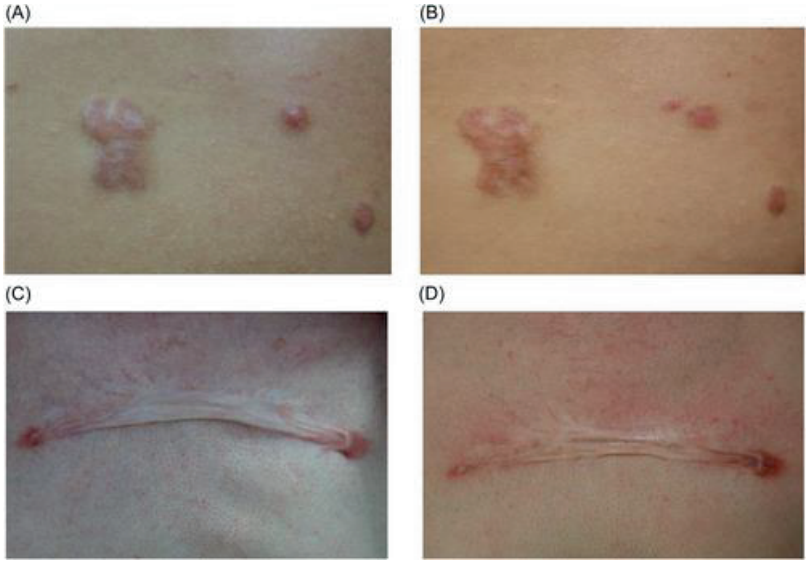


Figure 4

Clinical images of keloids before and after EPI-assisted treatment with bleomycin and lidocaine. (A) Keloid lesions on the shoulder before treatment. (B) Keloid lesions on the shoulder after three EPI-assisted bleomycin treatments. The GAIS was assessed as "improved." (C) Keloid lesion on the chest before treatment. (D) Keloid lesion on the chest after three EPI-assisted bleomycin treatments. The GAIS was assessed as "very improved." EPI, electronically-controlled pneumatic jet-injector; GAIS, Global Aesthetic Improvement Scale

Tolerability

The median NRS pain score during the test treatment was significantly lower with the EPI compared to conventional needle injections (2.0 [IQR 1.5−2.5] vs. 7.0 [IQR 5.5−9.0], $p < 0.001$) (Table S3). The median pain score for all consecutive EPI-assisted treatments was 3.0 (IQR 2.0−5.0). The most frequently (40%, 6/15) reported adverse event was local hyperpigmentation (Table S4). Other local and transient adverse effects after treatment included local pain and sensitivity (13%, 2/15), transient local itching (7%, 1/15), hematoma (20%, 3/15), scab formation (13%, 2/15) and acneiform inflammation of the keloid (7%, 1/15). No severe adverse reactions were reported.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

Patient satisfaction

All patients completed the patient satisfaction questionnaire. All patients recommended the EPI-assisted treatment with the combination of bleomycin and lidocaine to others, and preferred EPI treatment over treatment with hypodermic needles (Table S5). Less pain during treatment was the most frequently mentioned reason for this preference (87%, 13/15). Other reasons for EPI preference included better clinical results (67%, 10/15) and shorter treatment visits (33%, 5/15). Fourteen out of 15 patients (93%) were "satisfied" or "very satisfied" with the EPI-assisted treatments, while one patient rated her/his treatment satisfaction as "neutral" (7%). Eleven out of 15 patients (73%) stated that itching was reduced after treatment, and 12 out of 15 patients (80%) reported that the pain of the keloid was reduced after treatment. Postinjection hyperpigmentation was reported by three patients (20%), which was cosmetically disturbing in two patients (13%). One patient (7%) expressed discomfort due to the noise produced by the EPI device during injection.

DISCUSSION

In this study we show that-EPI assisted treatment with bleomycin and lidocaine is effective in patients with recalcitrant keloids and yielded significantly lower NRS pain scores compared to conventional needle injections. Total POSAS scores after three consecutive treatments were statistically significantly reduced compared to baseline, from both patient and observer perspectives. The sub-categories “itch,” “stiffness” and “thickness” were significantly improved according to the patients, while “pliability” and “overall opinion” with regard to keloid quality were significantly improved according to treating physicians, Only minor adverse effects were observed, of which local hyperpigmentation was most common. Patients were highly satisfied with their treatment and would recommend the treatment to others, presumably because of the reduced injection related pain.

Previously, Bik et al.[16] and Erlendson et al.[25] investigated the effectiveness, tolerability, and patient satisfaction of intralesional EPI-assisted TCA and 5-Fluorouracil, respectively. Notably, in the study by Bik et al., patients who received intralesional TCA with an EPI for their keloids reported an average pain score of 4.3 during the first treatment, which exceeds the median pain score of 2.0 (IQR 1.5−2.5) observed in our study. The lower pain scores in our patients may be attributed to the use of a mixture of bleomycin with lidocaine. The local anesthetic lidocaine, acting as an additional pain-reducing treatment in conjunction with the jet injector, could lead to a remarkable reduction in procedure-related pain (Table S3; NRS pain during needle-assisted bleomycin injection: 7.0). On the other hand, our findings with regard to pain scores show similarity to the findings of the randomized controlled study by Erlendson et al., in which also a median NRS pain score of 2.0 (IQR 2.0−2.0) was found, without the usage of lidocaine. However, in this study also patients with hypertrophic scars were included, which are usually less painful upon injection than (severe) keloids.[26]

In our study we observed a relatively modest decrease in total POSAS patient- and observer scores after three treatments with EPI-assisted bleomycin and lidocaine, with respectively 15% and 18%. This reduction was substantially smaller compared to EPI-assisted TCA in Bik et al. (POSAS reduction of resp. 27% and 34%) and spring loaded jet injector-assisted bleomycin in Saray et al. (complete flattening in 73.3%).[16, 27] These discrepancies could be related to the characteristics of the keloids in our patient population. The patients analyzed in this retrospective study had recalcitrant keloids with a more extensive treatment history, and more severe pain and pruritus at baseline compared with the patients in the studies by Bik et al. and Saray et al. Importantly, some of the patients who were challenging to treat with EPI-assisted TCA, did show good clinical improvement in this study. Moreover, although there is no clear difference between the median total POSAS patient scores before and after treatment in our study, it is important to note the relevant shift in the spread of the interquartile range before and after treatment (Figure 3).

However, in our clinic, for safety reasons, patients are treated with a bleomycin and lidocaine solution of 1 USPE/mL, with a maximum dose of 3 mL. This concentration is lower than 1.5 IU/mL, the concentration that is commonly used in clinical studies with bleomycin, for example, in the study of Saray et al.[27] While increasing the bleomycin concentration could potentially improve efficacy, caution must be exercised due to the high rate of necrosis and ulceration associated with higher doses of bleomycin.[28] In addition, treating physicians regularly observed a considerable amount of spilled volume, noticeable as a residue of the medication on the treated skin. This unintended spill may have led to a lower administered dose than intended, potentially impacting treatment outcomes.

Remarkably, four out of 15 patients did not show any improvement post-treatment. In retrospect, these non-responders had considerably thicker, stiffer and larger keloids than the other patients. Furthermore, all of the non-responders had previously undergone a minimum of five different types of treatments. Presumably, larger and thicker keloids are

more resistant to any type of treatment. However, this observation remains a hypothesis, and further research is necessary to investigate the effect of morphological features on treatment outcomes.

A common adverse effect of intralesional bleomycin injections is local hyperpigmentation at the injection site. This was also observed in our study; six out of 15 patients developed hyperpigmentation (Table S4). Interestingly, only three patients noticed the hyperpigmentation (Table S5). This may be due to the location of the keloids, as those on the shoulders or back may be harder to detect. Furthermore, physicians may be more aware of adverse effects than patients.

This study represents the first evaluation of the effectiveness of EPI-assisted intralesional bleomycin combined with lidocaine treatment in recalcitrant keloid scars. The patients included in our analysis, represent a severely affected patient population who suffer from recalcitrant keloids and, although the sample size is limited, the study findings provide important insight into the effectiveness of this treatment approach in recalcitrant keloids. A strength of our study is the real world setting and patient-oriented approach. As previously noted, patients with keloids often experience a reduced QoL due to the various symptoms that are associated with this disease.[4, 29] By analyzing the patient's perspective on their treatment experiences and outcomes, optimal treatment modalities can be identified that align with the individual patient's needs and preferences.

Limitations of our study are the lack of a control group, and the short follow-up time which precluded the assessment of the recurrence rate of keloids after EPI-assisted bleomycin treatment. Furthermore, the limited sample size of 15 patients may restrict the generalizability of our findings. Although our results are promising, the administration of intralesional bleomycin might be limited in general practice because the off-label status with limited availability. Moreover, jet-injector assisted administration of chemotherapeutics such as bleomycin can cause the formation of potentially harmful aerosols. Therefore, adequate protective safety measures are required, including the use of goggles, gloves, and mechanical room ventilation with FFP-2/FFP-3/N95 masks or

smoke evacuators which can adequately capture these aerosols.[30] Moreover, bleomycin should not be administered to pregnant or lactating women, and should also not be administered by healthcare workers that are pregnant or lactating. For this reason, it is important to inform patients and healthcare personal about the potential health hazards of chemotherapeutics such as bleomycin. In conclusion, we found that needle-free EPI assisted intralesional treatment with bleomycin and lidocaine is effective, well-tolerated and has a high treatment satisfaction in patients with recalcitrant keloids. Future high quality randomized controlled trials are warranted to confirm our results and improve the clinical management of patients with recalcitrant keloid scars.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

ACKNOWLEDGMENTS

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6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

Characteristic	***N* (%)** ***N* = 15**
Sex	
Female	8 (53.3%)
Age, median (Q1–Q3)	28 (22–41)
Fitzpatrick skin type	
1–2	1 (6.7%)
3–4	11 (73.3%)
5–6	3 (20.0%)
Number of lesions	
1–10	8 (53.3%)
10 ≥ 30	4 (26.7%)
>30	3 (20.0%)
Anatomical location	
Abdomen	1 (6.7%)
Shoulder(s)/back	13 (86.7%)
Thorax	7 (46.7%)
Etiology	
Acne	7 (46.7%)
Trauma/surgery	5 (33.3%)
Spontaneous/unknown	4 (26.7%)
Previous treatments	
Brachytherapy	3 (20.0%)
Cryotherapy	2 (13.3%)
Intralesional bleomycin treatments	6 (40.0%)
Intralesional TCA treatments	15 (100.0%)

Characteristic	***N* (%)** ***N* = 15**
Shave excision	5 (33.3%)
Silicon sheeting	4 (26.7%)
Vascular or ablative laser treatment	6 (40.0%)
Pressure EPI	
3–4 bar	12 (40.0%)
4–5 bar	3 (40.0%)
Motivation for EPI + bleomycin treatment[a]	
Needle-phobia	5 (33.3%)
Recurrence after initial efficacy	6 (40.0%)
Severe pain during needle injections	7 (46.7%)
Suboptimal or no results after previous treatments	15 (100.0%)

Table 1. Baseline characteristics.

Abbreviations: EPI, electronically-controlled pneumatic injector; Needle, needle-syringe injection; TCA, triamcinolone acetonide.

[a] Multiple combinations possible.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

	Median (*Q1– Q3*)	Median in difference	*p* Value
Vascularity	7 (5–8)	−2 (−2 to 0)	0.159
Pigmentation	6 (4–8)	1 (0–1)	0.380
Thickness	6 (4–8)	−2 (−2 to −1)	0.354
Relief	6 (5–8)	−1 (−2 to 0)	0.081
Pliability	7 (5–8)	−2 (−3 to −1)	0.002
Surface area	6 (4–8)	−1 (−1 to 0)	0.070
Overall assessment	7 (5–8)	−1 (−2 to −1)	0.019
Total POSAS observer scale	40 (29–51)	−7 (−11 to −3)	<0.001
	Median (Q1–Q3)	**Median in differences**	***p* Value**
Pain	4 (2–5)	−1 (−2 to 0)	0.144
Itching	7 (5–8)	−2 (−3 to 0)	0.049
Color	8 (7–10)	0 (−2 to 1)	0.435
Stiffness	8 (6–8)	−1 (−2 to 0)	0.006
Thickness	8 (7–9)	−1 (−2 to 0)	0.023
Irregularity	8 (6–9)	−1 (−2 to 0)	0.205
Overall opinion	8 (7–10)	0 (0–1)	0.054
Total POSAS patient scale	41 (37–47)	−6 (−13 to −3)	<0.001

Table 2. Clinical improvement assessment using POSAS.

Abbreviation: POSAS, Patient and Observer Scar Assessment Scale.

[a] One patient (*n* = 1) was missing due to the patient's pregnancy wish.

[b] Median in differences is the median change of paired observations before and after treatment.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

SUPPLEMENTARY FILES

Interval between treatments	Median in weeks [Q1 – Q3]
Treatment 1 – treatment 2	5 [4 – 7]
Treatment 2 – treatment 3	4 [4 – 5]
Treatment 3 – follow-up	4 [4 – 5]

Supplement 1. Interval between treatments

	n= 14
GAIS	
Exceptional improved	.
Very improved	1 (7.1%)
Improved	9 (64.3%)
Unaltered	4 (28.6%)
Worsened	.

Supplement 2. Clinical improvement assessment using GAIS

GAIS: Global Aesthetic Improvement Scale

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

	Needle-assisted bleomycin N=15	**EPI-assisted bleomycin N=15**	
Treatment-related pain (NRS)	*Median [Q1 – Q3]*	*Median [Q1 – Q3]*	*P-value*
*Pilot treatment**	7.0 [5.5 – 9.0]	2.0 [1.5 – 2.5]	< 0.001
*Three EPI- assisted bleomycin treatments***		3.0 [2.0 – 4.0]	

Supplement 3. Treatment- related pain

Needle: needle-syringe injections

EPI: electronically-controlled pneumatic injector

NRS: Numerical Rating Scale (range 0-10)

*Intra-patient comparison of needle-assisted bleomycin and EPI-assisted bleomycin

	N (%) n=15
Minor adverse effects observed after treatment	
Hematoma	3 (20%)
Hyperpigmentation	6 (40%)
Minor inflammation in keloid*	1 (6,7%)
Scab formation	2 (13,3%)
Superficial necrosis	1 (6,7%)
Transient itching	2 (13,3%)
Transient pain or sensitivity	1 (6,7%)

Supplement 4. Adverse effects

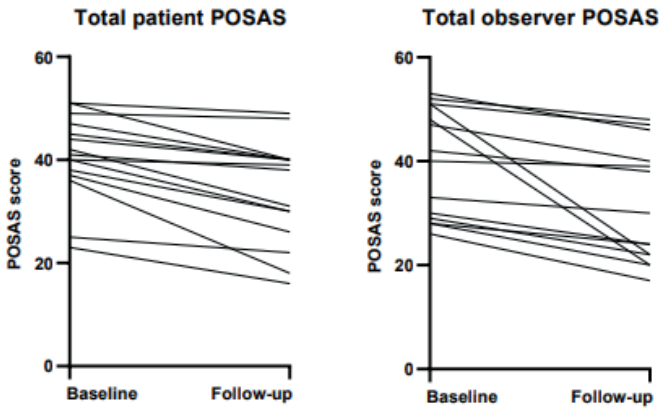
*Adverse effect in patient with history of experiencing similar pre-existing symptoms.

	N (%), n= 15
Satisfied with EPI-assisted bleomycin treatment?	
Strongly agree	5 (33.3%)
Agree	9 (60.0%)
Neutral	1 (6.7%)
(Strongly) disagree	.
Not applicable	.
Reduction of itching?	
Strongly agree	4 (26.7%)
Agree	7 (46.7%)
Neutral	4 (26.7%)
(strongly) disagree	.
Not applicable	.
Reduction of pain?	
Strongly agree	4 (26.7%)
Agree	8 (53.3%)
Neutral	3 (20.0%)
(strongly) disagree	.
Not applicable	.
Recommendation of EPI-assisted bleomycin treatment to others?	
Yes	15 (100.0%)
No	.
Unknown	.
Treatment preference?	
Needle-assisted bleomycin	.
EPI-assisted bleomycin	15 (100.0%)
No preference	.
Reason for preference of EPI-assisted bleomycin over needle-assisted bleomycin?*	
Less painful	13 (86.7%)
Shorter treatment duration	5 (33.3%)
Better results	10 (67.7%)
Disturbed by noise of EPI device?	
Yes	1 (6.7%)
No	14 (93.3%)
Darkening of scar?	
Yes. If yes, cosmetically disturbing?	5 (33.3%), 2(13.3%)
No	8 (53.3%)
Unknown	2 (13.3%)

Supplement 5. Treatment satisfaction questionnaire

EPI: electronically-controlled pneumatic injector, Needle: needle-syringe injection, *Multiple answers possible

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.



A. B.

Supplement 6.

A. Invidual total patient POSAS scores at baseline and after three EPI-assisted treatments with bleomycin and lidocaine

B. Invidual total observer POSAS scores at baseline and after three EPI-assisted treatments with bleomycin and lidocaine.

6. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids.

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Section IV

Perspectives of needle-free jet injector assisted treatment in other populations

Chapter 7

Needle-free jet Injector-assisted triamcinolone treatment of keloids and hypertrophic scars is effective and well tolerated in children.

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ABSTRACT

Background Keloids and hypertrophic scars can cause severe pain, pruritus, and psychological distress. Conventional intralesional corticosteroid treatment with needle injections remains challenging, especially in children with needle phobia.

Objective We aimed to evaluate the effectiveness, tolerability, and patient satisfaction of intralesional treatment with triamcinolone acetonide using a needle-free electronic pneumatic jet injector in children with keloids and hypertrophic scars.

Methods A retrospective study was performed in children with keloids and hypertrophic scars who received intralesional triamcinolone acetonide treatments using an electronic pneumatic jet injector. Effectiveness was evaluated using the Patient and Observer Scar Assessment Scale and Global Aesthetic Improvement Score at follow-up versus baseline. Tolerability was assessed with reported adverse effects and injection-related pain using a visual analog scale. Satisfaction questionnaires were used to evaluate treatment-related patient satisfaction.

Results Six female patients and five male patients aged 5–17 years, with a total of >118 keloids or hypertrophic scars were included. Electronic pneumatic jet injector treatment led to a significant reduction in the total Patient and Observer Scar Assessment Scale observer and patient scores compared with baseline, with a median reduction of 28.9% and 23.8%, respectively ($p = 0.005$; $p = 0.009$). Median visual analog scale pain scores for electronic pneumatic jet injector treatment were significantly lower compared with needle injections, 3.0 versus 7.0, respectively ($p = 0.027$). No severe adverse effects were reported. Overall, 6 patients were ‘satisfied’ and five patients were ‘very satisfied’ with the treatment.

Conclusions Electronic pneumatic jet injector-assisted intralesional triamcinolone acetonide is an effective and well-tolerated treatment for keloids and hypertrophic scars in children. It should be considered as an alternative non-traumatic delivery method, especially in children with needle phobia or severe pain during previous needle injections.

INTRODUCTION

Keloids and hypertrophic scars are pathological scars that can cause considerable pain, pruritus, impairment of movement, as well as severe psychological distress. These types of scars are often extremely difficult to treat, especially in children with needle phobia.[1]

Although keloids and hypertrophic scars (HTS) are considered distinct entities, they have some similarities with regard to their pathophysiology.[2] These excessively growing scars result from chronic inflammation in which several interleukins (ILs, e.g. IL-6, IL-8, and IL-10) and transforming growth factor B1 and B2 play an important role.[3] Moreover, these scars are characterized by abnormal fibroblast proliferation, collagen deposition, and increased angiogenesis.[4] However, differences lie in the fact that keloids extend beyond the boundaries of the original wound, whereas HTS do not.[5]

Keloids and HTS occur very commonly in children or young people aged between 10 and 30 years.[6, 7] Overall, keloids and HTS develop in respectively 0.1–10% and 32–72% of all scars.[8,9,10,11]

First-line treatment includes the application of silicone sheets and intralesional triamcinolone acetonide (TCA) administered with conventional hypodermic needles (CN).[12] Although this can be effective, long-treatment regimens with multiple painful injections are usually needed to reach the desired results. In clinical practice, many children discontinue treatment prematurely because of the painful injections, or refuse treatment entirely because of fear of needles.[13] It is well known that painful and stressful medical procedures can cause a serious burden for children and their parents. Therefore, to prevent traumatic experiences, child-friendly procedures that do not cause pain and stress are highly needed.

In a previous study, children aged between 1 and 14 years with keloids were treated with two to five sessions of intralesional TCA needle injections.[14] This resulted in a mean scar volume reduction of 82.7% compared with baseline ($p < 0.001$). However, the injections

were painful in all patients and side effects (hypopigmentation, skin atrophy, and teleangiectasia) occurred in 21% of the cases.

Needle-free electronic pneumatic jet injector (EPI) technology has been developed as an alternative for conventional needle injections. The main advantages of EPI include less procedure-related pain and a tightly controlled, less operator-dependent delivery of therapeutics to the skin. This novel delivery method may be less painful, less traumatic, and also suitable for children with (extreme) needle phobia.

In a recent study in adults, a significant mean reduction of 34.5% and 26.9% in respectively patient and observer scores measured by the Patient and Observer Scar Assessment Scale (POSAS) was observed.[15] Furthermore, EPI-assisted injections were well tolerated and resulted in low pain scores. In this study, we aimed to investigate the effectiveness, tolerability, and patient satisfaction of EPI-assisted TCA treatment of keloids and HTS in children.

METHODS

This retrospective cohort study was conducted at the outpatient clinic of the Dermatology department of the Erasmus Medical Center, Rotterdam, The Netherlands. Children with keloids or HTS who were previously treated with TCA using an EPI between February 2021 and April 2023 were included. This study was approved by the Medical Ethical Committee of Erasmus Medical Center (MEC-2021-0661). STROBE guidelines were followed. For all patients, written informed consent for usage of their medical data was obtained.

Our primary objective was to evaluate effectiveness using the POSAS and GAIS scales.[16, 17] The POSAS score is a scar assessment tool that measures the quality of a scar.[16] Both the patient and the clinician need to fill in the POSAS score that consists of six items concerning patient symptoms and clinical characteristics of the keloid. The patient and clinician can score the items from 1 (normal skin) to 10 (worst imaginable abnormality). The sum of these items gives the total POSAS score of a minimum 6 points and a maximum of 60 points. The GAIS measures the improvement of a scar compared to pre-treatment, which consists of five degrees: exceptional improvement, very improved patient, improved patient, unaltered patient, and worsened patient.[17]

Our secondary objective was to evaluate tolerability and patient satisfaction. Tolerability was evaluated with treatment-related pain scores and records of documented adverse effects. Patient satisfaction was evaluated at follow-up with an electronic questionnaire (Limesurvey 2.06; GmbH, Hamburg, Germany). Parents of patients aged ≤12 years completed the POSAS patient scale and satisfaction questionnaire with input from their child to the best of their ability. Patients aged >12 years completed the POSAS patient scale and satisfaction scale themselves.

Patients were eligible for EPI treatment with TCA if they were aged 4–18 years, presented with one or more keloid(s) or HTS, had experienced moderate-to-severe injection-related

pain scores with CN (>VAS 4) OR had needle-phobia OR had a therapeutic history with suboptimal results using CN.

A local standard operating procedure for EPI-assisted treatment in children was followed. Prior to the first full treatment, an optional test treatment was performed where intralesional TCA with respectively a CN (27 gauge) and EPI was administered in two similar HTS or keloid scars (±1 cm2) in a random order. Treatment-related pain scores (VAS: range 0–10) with both injection techniques were evaluated.[18] Intralesional triamcinolone 10–40 mg/mL (Kenacort; Bristol Myers Squibb, New York, NY, USA) was administered using the Enerjet 2.0 device (Enerjet 2.0; Sinclair, Rehovot, Israel) on every subsequent visit, and repeated every 4–6 weeks. The TCA concentration used for the treatment was based on scar severity and the judgment of the treating physician. The need for subsequent treatments was discussed between the treating physician, child, and their parents every visit. An injection volume of 100 µL (device range: 50–130 µL) was administered in every squared centimeter of the scar, with a maximum treatment surface of 20 cm2 per session. A pressure setting of 2 Bar (device range: 2–6 Bar) was selected, and was increased by 10% until the clinical endpoint (papule or blanching) was observed (Electronic Supplementary Material [ESM]). A follow-up was scheduled 4–6 weeks after the last treatment for scoring of the final POSAS and GAIS scores.

A standardized form was utilized to extract data from the electronic patient files. Data were analyzed using SPSS version 28 (IBM, Armonk, NY, USA) and were presented as medians with interquartile ranges (IQRs). Hereafter, ordinal data (POSAS, GAIS, and VAS) were analyzed using a Wilcoxon signed-rank test. $P < 0.05$ was considered as statistically significant.

RESULTS

Demographics

Eleven patients (six female) with a median age of 12 years (IQR: 9–12 years) and a total of more than 118 keloids or HTS were included in this retrospective cohort study (Table 1). Seven patients with keloids and four patients with HTS were treated with intralesional TCA using an EPI. The majority of patients (82%, 9/11) had previously received multiple CN-assisted TCA injections. Reasons to initiate EPI-assisted treatments were needle phobia (45%, 5/11), severe procedure-related pain during CN (82%, 9/11), and/or suboptimal results after previous treatments (82%, 9/11).

Treatment

A median of 4 (IQR: 3–7, n = 11) EPI-assisted intralesional TCA treatments were administered in total (Table 1). Pressure settings varied between 2.8 and 4.6 Bar (20–65% of maximum pressure).

Effectiveness

Clinical improvement was observed in all patients, both from a patient and physician perspective (Table 2, Figs. 1 and 2). The follow-up period varied from 6 weeks to 12 months. The median total POSAS observer score was 38.0 (IQR: 30.5–40.5) at baseline, and decreased significantly with a median of −11.0 points (−28.9%; IQR: −13.5 to −7.5) at follow-up ($p = 0.005$, n = 11). The median total POSAS patient score was 43.0 (IQR: 38.0–47.0) at baseline, and changed significantly with a median of −10.0 points (−23.8%; IQR: −20.0 to −5.0) at follow-up ($p = 0.009$, n = 11). The overall aesthetic clinical effectiveness using the GAIS scale at follow-up was assessed as ‘improved’ and ‘very improved’ compared with baseline in respectively 73% (8/11) and 27% (3/11) of the patients.

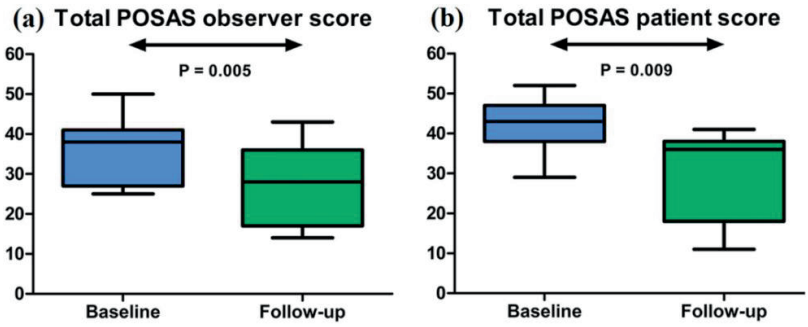

Figure 1.

Clinical effectiveness assessed with the Patient and Observer Scar Assessment Scale (POSAS) at baseline and at follow-up. a Based on the total POSAS observer scale, treating physicians observed a significant improvement at follow-up compared with baseline. b Based on the total POSAS patient scale, patients observed a significant improvement at follow-up compared with baseline

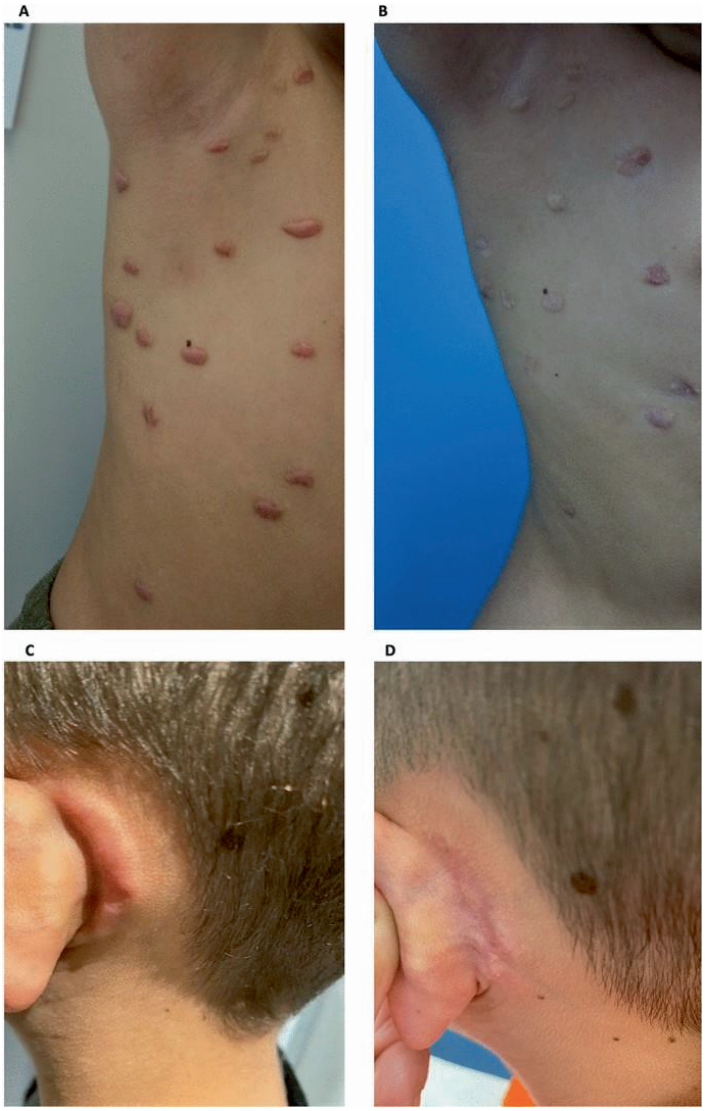

Figure 2.

Clinical results of electronic pneumatic injector-assisted triamcinolone acetonide in keloids. A 12-year-old male patient with multiple keloids that occurred after Mollusca contagiosum (a), which demonstrated 'improved' results after electronic pneumatic injector treatments at follow-up (b), compared with baseline. Another 12 year-old male patient with a keloid that occurred after surgery (c), which demonstrated 'very improved' results at follow-up (d), compared with baseline.

Procedure-Related Pain

Seven patients participated in the pilot treatment (Fig. 3). Median procedure-reported VAS pain scores were 3 (IQR: 3–4; n = 11) and 7 (IQR: 6–8; n = 7), with respectively the EPI and CN (p = 0.027). Visual analog scale scores were 4 (IQR: 3–4; n = 11) during the consecutive EPI treatments.

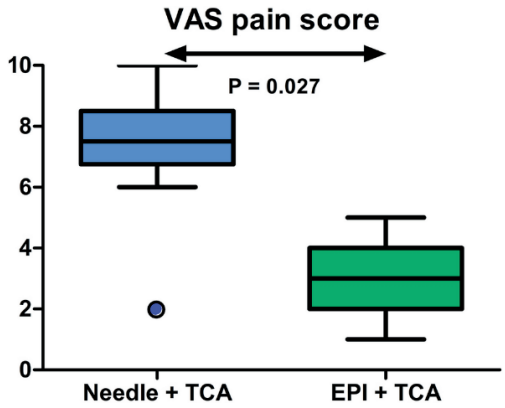

Figure 3.

Procedure-related visual analog scale (VAS) pain scores. Procedure-related VAS pain scores during the pilot treatment were significantly lower with electronic pneumatic injector (EPI)-assisted injections compared with needle injections. TCA triamcinolone acetonide.

Adverse Events

In three patients, mild adverse reactions (hyperpigmentation: n = 1, hypopigmentation: n = 1, and white macules: n = 1) were reported. No other general side effects of TCA such as subcutaneous atrophy, teleangiectasia, and local infection were observed. The 1- to 2-mm white macules at the injection site may have resulted from intralesional TCA precipitations, and were resolved spontaneously at follow-up. No severe adverse reactions were reported.

Patient Satisfaction

Eleven patients completed the patient satisfaction questionnaire (ESM). All patients stated that they 'agreed' or 'strongly agreed' to have experienced an overall improvement of the scar(s) at follow-up compared with baseline. All patients preferred the EPI treatment over CN. Two patients of respectively, 5 and 9 years of age, experienced the injection-related noise of the EPI as mildly disturbing.

DISCUSSION

In this retrospective cohort study, we evaluated the effectiveness, tolerability, and treatment satisfaction of needle-free EPI-assisted intralesional TCA injections in children with HTS and keloids. We found a significant reduction of 28.9% and 23.8% in the POSAS-observer and POSAS-patient scores, respectively. Importantly, EPI resulted in significantly lower procedure-related pain scores compared with CN [3 (IQR: 3–4) vs 7 (IQR: 6–8), respectively ($p = 0.027$)]. This difference in scores markedly surpasses the minimal clinical important difference threshold of 1 point.[18] Furthermore, all children preferred needle-free EPI treatment over CN.

Electronic pneumatic jet injector-assisted intralesional treatment offers a standardized, accurate, and reproducible treatment with the possibility to repetitively deliver volumes up to 0.1 mL of therapeutic drugs to the skin.[19] In our experience, EPI-assisted treatments are also less time consuming than CN-assisted treatments. This is especially important to take into account when aiming to provide non-traumatic care in children with multiple keloids and HTS requiring frequent and repetitive treatment.

Although our findings are promising, there are also some potential drawbacks of EPI-assisted TCA treatment that need to be considered. First, 2 out of 11 children reported that they experienced the noise of the EPI as mildly disturbing. However, it was noted that these children were able to manage the discomfort by using an iPad and headphones to distract themselves. Therefore, it is recommended to prepare children for the noise that the EPI generates, to reduce anxiety associated with the treatment. Additionally, to further improve non-traumatic care, it is important to proactively involve children in their disease, treatment, and prognosis.[20]

Another drawback may lie in the relatively high costs of the EPI device and disposables needed for every treatment. However, we previously treated these severely affected children with multiple keloids or HTS with conventional needles under general anesthesia. Therefore, it is worth noting that although the costs of an EPI device are

substantial, they are considerably lower than the cost related to repetitive treatments under general anesthesia, which also carries significant additional safety risks. Lastly, a substantial amount of residue on the skin was observed directly after EPI-assisted injections. However, a previous ex-vivo study demonstrated that EPI-assisted injections distribute more evenly and consistently in the dermis than CN-assisted injections.[19] For this reason, a smaller dose might be needed with EPI. Eventually, this smaller dose may also declare a smaller number of mild adverse events compared with Acosta et al.[14]

The strengths of this study include the real-world treatment setting using a local standard operating procedure with a predefined treatment interval and device settings. Another strength is the usage of both patient- and physician-reported outcomes. We used a child-centered approach, by proactively involving the children with their treatment. Moreover, we consistently used a VAS tool for the assessment of patient symptoms (pain and itch) and procedure-related pain, which was easy to understand for our population.

The limitations of this study are the retrospective design, the lack of a control group, and a small sample size. A follow-up time of 4–6 weeks has been maintained. However, a longer follow-up time is needed to evaluate the long-term effect of this treatment. Moreover, selection bias might play a role in the patient satisfaction and pain scores, as most patients had needle phobia.

CONCLUSIONS

Our findings indicate that needle-free, EPI-assisted intralesional TCA treatment is an effective and well-tolerated treatment for keloids and HTS in children. It should be considered as an alternative delivery method, especially in children with extensive skin involvement, needle phobia, or severe pain during previous conventional needle injections.

Variable	Value, n (%), N = 11
Gender	
Female	6 (55)
Age	
4-6 years	1 (9)
6-10 years	3 (27)
10-14 years	2 (18)
14-18 years	5 (45)
Fitzpatrick skin type	
1-2	3 (27)
3-4	6 (55)
5-6	2 (18)
Number of lesions	
1-10	6 (55)
11-30	2 (18)
>30	3 (27)
Anatomical location	
Extremities and trunk	3 (27)
Inguinal	1 (9)
Mastoid/ear	2 (18)
Scapulae/back	2 (18)
Scapulae/ back and other location (extremities/trunk)	3 (27)
Etiology	
Acne	4 (36)
Complicated Varicella Zoster	1 (9)
Mollusca contagiosa	1 (9)
Trauma	5 (45)
Previous treatments[a]	
Multiple conventional needle bleomycin treatments	1 (9)
Multiple conventional needle TCA treatments	9 (82)
PDL laser	1 (9)
Silicon sheeting	2 (18)
Surgical excision + i.l. TCA treatment	2 (18)
Topical Corticosteroids	2 (18)
Motivation for EPI + treatment[a]	
Needle-phobia	5 (45)
Suboptimal or no results after previous treatments	9 (82)
Severe pain during needle injections	9 (82)
Pressure EPI in bar, device range 2-6 Bar (% pressure)	
2.8 – 3,2 Bar (20-30%)	5 (45)
2.8 – 4 Bar (20-50%)	4 (36)
3.2 – 4.6 Bar (30-65%)	2 (18)

Concentration TCA in mg/ml[b]	
10 mg/ml	2 (18)
20 mg/ml	11 (100)
40 mg/ml	2 (18)
Number of needed EPI treatments	
1-3	2 (18)
3-5	4 (36)
5-7	2 (18)
7-9	3 (27)

Table 1 Patient characteristics (n=11)

[a] Multiple combinations possible

[b] All patients received triamcinolone acetonide 40 mg/ml diluted 1:1 with NaCl during the pilot treatment. Depending on the observed results, at the consecutive treatments a higher or lower concentration was administered.

Needle: needle-syringe injection

EPI: electronically-controlled pneumatic injector

TCA: Triamcinolone Acetonide

	Baseline (n=11)	**Follow-up compared to baseline (n=11)**	
	Median (Q1 – Q3)	***Median in difference[a] (Q1 - Q3)***	***P-value***
Total POSAS	38.0 (30.5 – 40.5)	-11.0 (-13.5 – -7.5)	0.005
Observer scale			
Subcategories			
Vascularity	5.0 (5.0 – 7.0)	-1.0 (-2.5 – -0.5)	0.011
Pigmentation	4.0 (2.0 – 6.0)	0.0 (-2.0 – 0.0)	0.453
Thickness	6.0 (5.0 – 8.5)	-2.0 (-2.5 – -2.0)	0.018
Relief	7.0 (4.5 – 8.0)	-2.0 (-2.0 – -1.0)	0.052
Pliability	6.0 (5.0 – 8.0)	-2.0 (-3.0 – -1.5)	0.010
Surface area	7.0 (5.0 – 8.0)	-2.0 (-2.5 – -1.0)	0.018
Overall assessment	7.0 (5.5 – 8.0)	-2.0 (-3.0 – -1.0)	0.005
Total POSAS	43.0 (38.0 – 47.0)	-10.0 (-20.0 – -5.0)	0.009
Patient scale			
Subcategories			
Pain	5.0 (2.0 – 5.0)	-1.0 (-3.0 – 0.0)	0.135
Itching	7.0 (4.5 – 8.0)	-1.0 (-2.5– 0.0)	0.038
Color	9.0 (6.0 – 10.0)	-1.0 (-2.5 – -0.5)	0.005
Stiffness	7.0 (5.5 – 8.0)	-1.0 (-3.0 – 0.0)	0.028
Thickness	8.0 (8.0 – 9.0)	-2.0 (-3.5 – -2.0)	0.003
Irregularity	9.0 (8.0 – 9.0)	-3.0 (-4.0 – -1.0	0.006
Overall opinion	8.0 (7.0 – 9.0)	-1.0 (-3.5 – 0.0)	0.007

GAIS	**N (%)**
Exceptional improved	.
Very improved	3 (27%)
Improved	8 (73%)
Unaltered	.
Worsened	.

Table 2 Clinical improvement assessment using POSAS and GAIS

* Median in differences is the median change of paired observations before and after treatment

GAIS: Global Aesthetic Improvement Scale

POSAS: Patient and Observer Scar Assessment Scale 2.0

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Chapter 8

General discussion

Keloids are fibroproliferative scars that are regarded as chronic inflammatory skin disorders.[1] Patients with keloids constitute a highly heterogenous group of individuals, having mild to very severe keloids. Severe keloids can be extremely resistant to treatment, generally cause significant pain, itch and restriction of movement, resulting into a marked decrease in quality of life.[2]

Over the past decades, multiple treatment approaches have been suggested for keloids, with the gold standard being intralesional treatment with corticosteroids using conventional needles.[3] This treatment can be effective, but is often associated with procedure-related pain. Unfortunately, minimally invasive, efficacious and patient-friendly alternatives to conventional needle-injections are currently not common in clinical practice. In addition, severe forms of keloids also referred to as 'recalcitrant' keloids, may be extremely difficult to treat, with high recurrence rates posing a major clinical problem. In the past decades, electronically controlled needle-free injectors were developed that may help to overcome many problems related to the use of conventional needles.

As there are multiple knowledge gaps with regard to the treatment of keloids and hypertrophic scars with needle-free jet injectors, the aim of this thesis was not restricted to a single type of research. We designed and performed studies from bench to bedside: preclinical, literature, prospective and retrospective clinical studies. In this general discussion we aim to integrate the most important findings of this thesis and elaborate on perspectives for keloid treatment, leading the way to more high-quality research and ultimately the improvement of patient care in clinical practice.

MAIN FINDINGS OF THIS THESIS

Keloidal scars

Keloidal scars belong to one of the most common chronic inflammatory skin disorders, especially in darker Fitzpatrick skin types 4 through 6. Impairment in quality of life seems to be higher in severely affected patients, which are described in the literature as patients with keloids larger than 10 cm^2 or when multiple keloids are present. In **chapter 4**, we investigated which keloid properties may result in keloids being categorized as 'severe'. We found that the efficacy of certain treatments for keloids may be negatively influenced by keloids that are larger in size, located on the chest and extremities, or have a lower Vancouver Scar Scale (VSS) score, longer duration of the keloid prior to treatment, and a more extensive treatment history.

Although keloids form a serious burden, yet no effective, safe and minimally painful treatment for this group of difficult to treat keloids is available. In this thesis we sought to find an efficacious, safe and patient-friendly treatment for patients that suffer from (severe) keloidal scars.

Currently, needle injections with triamcinolone acetonide (TCA) are the standard treatment for keloids. However, this therapy with TCA is often suboptimal due to adverse effects like skin atrophy, hypo- and hyper pigmentation and a high risk of recurrences because of the temporary effects of TCA.[4] Therefore, treatments with a safer profile, that offer longer term effects are warranted. Bleomycin is an antibiotic chemotherapeutic agent that causes apoptosis of keratinocytes and fibroblasts and has an antiangiogenic effect.[5] This mechanism of action also results in less recurrences in keloids.

However, needle-injections with bleomycin in keloids are painful, making patients reluctant to undergo treatment. Therefore, needle-free drug delivery devices such as needle-free jet-injectors (NFI) represent an attractive option for treating keloids.

Needle-free jet injectors in dermatology

A needle-free jet injector (NFI) is a drug delivery device that enables intradermal drug delivery of liquid therapeutics.[6] NFI have been used for several indications as alternative to conventional hypodermic needle injections (CNI), including needle-free mass vaccination and self-administration of insulin by patients with Diabetes.[7] NFI may overcome several limitations associated with CNI. For example, NFI can be used for patients with (extreme) needle-phobia, cause less injection-related pain and do not harbor a risk of needle-stick injuries.

In the past decades, NFI have also been used for dermatological indications. In this thesis we first performed a systematic review **(chapter 2)** that summarizes and critically evaluates the available evidence for the efficacy and tolerability of intralesional treatment using NFI to treat several dermatological indications.[8] Thirty-seven articles, involving 1911 participants were finally included in the review.

Two types of jet-injectors were used to treat dermatological indications: spring-loaded jet-injectors (SLI) that act with pressure generated by a mechanical spring, and electronically controlled pneumatic jet-injectors (EPI) that generate pressure by compressed gasses (e.g. CO_2 or air). Several drugs, with varying characteristics (e.g. different viscosities and molecular masses) including triamcinolone acetonide (TCA), bleomycin and 5-fluorouracil (5-FU) were successfully delivered intralesionally. The most evidence was found for NFI-assisted treatment of keloids and other types of scars (hypertrophic, atrophic and burn scars) using various drugs. Other indications in which NFI were used were alopecia areata, hyperhidrosis, nail diseases, non-melanoma skin cancer, common warts, local anesthesia, and aesthetic indications. However, the quality of the included studies was generally low. Only two high quality RCTs were identified in our review. These studies showed that jet injections with a combination of 5-FU and TCA and jet injections with saline are efficacious, safe, and well-tolerated in respectively hypertrophic scars and atrophic acne scars.[9,10] Additionally, our review suggested that jet injector treatment might result in less injection related pain than CNI for certain indications.

Two studies, a prospective- and retrospective cohort study, were identified that investigated the effectiveness and tolerability of intralesional jet injections to treat keloids with respectively bleomycin, and jet injections with a combination of 5-FU, triamcinolone and lidocaine.[11,12] Both cohort studies showed high effectiveness and tolerability with NFI-assisted jet injections to treat keloids. However, the study of Saray et al. in which NFI-assisted treatment with bleomycin was given, methodological quality lacked.[12] Moreover, a SLI was used in this study, while our previous ex vivo studies show that EPI result in a more favorable biodistribution (consistent and accurate dermal drug delivery) compared to SLI in normal skin.

No RCTs that investigated the efficacy, safety and patient satisfaction of NFI-assisted jet-injections in keloids were found. We concluded that high-quality RCTs are warranted to support evidence-based recommendations concerning the usage of NFI-assisted treatment of keloids in clinical practice.

The efficacy and safety of intralesional EPI-assisted bleomycin to treat severe keloids

Bleomycin owes its working mechanism to cell apoptosis and its anti-angiogenic effect. It binds to DNA strands via electrochemical attraction and cleaves these DNA strands by oxidizing metal ions which creates free radicals.[13] In the dermis, bleomycin specifically induces cell apoptosis of keratinocytes and fibroblasts, and thereby suppresses the synthesis of collagen.[14] The efficacy and safety of intralesional bleomycin in dermatological indications including keloid was investigated prior to our clinical studies in a previous systematic review that was outside the scope of this thesis.[15] This review showed that the intralesional drug delivery of bleomycin was efficacious and safe to treat keloidal scars. Intralesional bleomycin for keloids was investigated in three studies. Importantly, in the study of Neini *et al.* no recurrences were observed at 3 months follow-up.[16] Other studies that were outside the scope of this review, also found low recurrence rates with intralesional bleomycin treatment in keloids.[5] The adverse events were all mild to moderate and included transient symptoms of erythema, hyperpigmentation, pain, superficial necrosis and superficial ulceration. Contraindications of bleomycin are

bleomycin intolerance, peripheral vascular diseases, pregnancy or lactation, and Raynaud phenomenon.

The promising efficacy and safety profile of intralesional bleomycin administered with EPI according to previous pre-clinical studies led to the design of a randomized controlled trial (RCT). The efficacy, safety, and patient satisfaction of EPI-assisted bleomycin treatment in keloids are described in **chapter 5.** In this RCT we administered three treatments with EPI-assisted bleomycin in severe keloids. We found a reduction in keloid volume of 20% in bleomycin treated lesions, compared to a slight increase with placebo. Similarly, we found a significant reduction in keloid related symptoms with EPI-assisted bleomycin. However, there was no significant change in blood flow with bleomycin compared to placebo, and therefore we do not expect permanent changes in microcirculation with the used bleomycin dosage. Adverse events were mild and included hyperpigmentation, transient necrosis and hematoma. We concluded that EPI-assisted bleomycin treatment is an efficacious, safe and well tolerated treatment for patients with severe keloids.

Up to date, no other studies have been published that use highly sensitive 3D measurements to assess changes in volume after intralesional bleomycin treatment in keloids. Therefore, we cannot directly compare our findings with previous results from literature. However, a previous RCT of Khan *et al.* compared six treatments of intralesional CNI-assisted bleomycin with triamcinolone in keloids and found a significant improvement in mean total POSAS of 72% vs 67%, respectively.[17] The BLEOJET study shows some resemblance to the RCT of Erlendson et al., but administered a single treatment with 5-Fluorouracil using EPI to treat hypertrophic scars.[9] A volume reduction of 33% in scar volume was detected with a 3D-camera in the hypertrophic scars that were treated with 5-Fluorouracil. Although this is considerably more than observed in our study, it is imported to highlight the significant differences between hypertrophic scars and severe keloidal scars, of which the latter is more often not responsive to treatment.

Interestingly, injection-related pain scores with EPI-assisted bleomycin were still relatively high. NRS Pain scores were 5.4 and 5.6, in respectively the bleomycin and placebo treated

lesions (p = 0.54). Because injection related pain scores were also high in the placebo treated lesions, we concluded that these high injection-related pain scores may be inherent to this severely affected population who suffer from severe keloids of which some were already painful upon palpation. However, we still hypothesized that the usage of a needle-free EPI was clinically meaningful because the majority of patients preferred EPI over conventional needles. Based on the results of this study, we decided to add lidocaine to the EPI-assisted treatment with bleomycin in the subsequent real-world study. The results of this study are shown in **chapter 6**. The POSAS patient- and observer-scores were respectively 41 and 40 at baseline, and reduced with respectively 6 and 7-points at follow-up ($p < 0.001$; $p < 0.001$). Moreover, we found that the addition of lidocaine to bleomycin was beneficial, since it leads to minimal injections related pain scores (NRS pain 2.0 (IQR 1.5−2.5)). Adverse events were mild and included hyperpigmentation, transient local pain, sensitivity or itching, hematoma, scab formation and acneiform inflammation of the keloid. All patients preferred EPI treatment over treatment with needles. This study shows similarity to the study of Bik et al. In this study the effectiveness, tolerability, and patient satisfaction of intralesional EPI-assisted TCA were investigated.[18] In this cohort study injection related NRS pain scores were assessed as slightly more painful (mean NRS pain 4.3 ± 1.9), and adverse events were hematoma, local itching, mild skin atrophy, superficial deposition of TCA, and transient pain, sensitivity or burning. Unlike observed in studies that investigated bleomycin treatment, no necrosis occurred.

The clinical application of needle-free jet injections to treat children with keloids and hypertrophic scars

In general, extra caution with regards to new therapies is warranted when treating children. However, at the same time there is an urgent need for innovative child-friendly therapies to treat children with painful hypertrophic- and keloid scars. First-line treatment of children with keloids and hypertrophic children includes the application of silicone gel or sheets and intralesional TCA administered with conventional needles.[19] However, many children refuse needle-injections because of needle-phobia or discontinue treatment prematurely because of the painful needle injections.[20] Previous results show that EPI-assisted treatment with EPI and TCA in keloids was effective on short term, and well tolerated in adult patients with recalcitrant keloids. In **chapter 7** we therefore performed a retrospective study to evaluate the effectiveness, tolerability and patient satisfaction of needle-free EPI-assisted TCA treatment in children with keloids and hypertrophic scars.[21] Eleven patients aged 5-17 years, with a total of >118 keloids or hypertrophic scars were included in this study. We observed a significant reduction of 28.9% and 23.8% in respectively the POSAS-observer and POSAS-patient scores, respectively. Adverse events in our study were limited to mild adverse reactions (hyperpigmentation: *n* = 1, hypopigmentation: *n* = 1, and white macules: *n* = 1) were observed. No moderate or adverse events such as subcutaneous atrophy, telangiectasia, and local infection occurred. Due to a limited sample size, we could not perform sub analysis for keloids and hypertrophic scars. EPI caused significantly lower injection-related pain scores compared to needle-injections (3 (IQR: 3–4) vs 7 (IQR: 6–8), respectively (p = 0.027)). This difference clearly surpasses the minimal clinical important difference (MID) of one point.[22] Moreover, all children preferred EPI-injections over needle-injections.

In a previous study of Acosta *et al.*, children between 1 and 14 years old with keloidal scars were treated with intralesional TCA administered using conventional needles.[23] In this study two to five treatments resulted in a mean scar volume reduction of 82.7% compared to baseline. However, the needle injections were (very) painful in all cases. Another study by Hamrick *et al.,* found that TCA injections before and after excision is effective and well-tolerated in children.[24] However, in this study procedure related pain

scores were not reported. It is well established that painful and stressful procedures can cause serious burden in the pediatric population, and should be avoided if possible. With our study, we showed that EPI-assisted intralesional TCA treatment can be an effective, and child-friendly alternative treatment for minors with keloids or hypertrophic scars.

Biodistribution of electronic pneumatic jet-injections in severe keloids

The biodistribution of a drug refers to how a therapeutic is distributed in the skin after it is administered. This distribution of therapeutics in the skin is directly related to the efficacy of a certain treatment. Key factors that influence the biodistribution of a drug include fluid specific properties (e.g. size and molecular mass), skin characteristics, and the drug delivery technique.

A previous ex-vivo study in human skin, and an in-vivo study in pigs showed promising results with regards to the biodistribution of intralesional EPI-assisted jet injections.[25] The EPI-assisted injections resulted in more consistent, standardized and equally distribution of injections into the dermis, compared to SLI-assisted and CNI-assisted injections. However, no previous studies investigated the biodistribution of EPI-assisted jet injections in keloidal skin. We hypothesized that the biodistribution of EPI-assisted jet injections in rigid keloidal scars may differ from the distribution in normal skin due to differences in skin characteristics (e.g. rigidity, elasticity, and porosity). Therefore, we performed an *ex vivo* study that evaluated the biodistribution of EPI-assisted jet-injections and CNI-assisted jet-injections in severe keloids. Our results showed that the biodistribution of drugs in keloids demonstrated a high variability, compared to normal skin using both EPI and CNI. In summary, we observed large heterogeneity with regards to the biodistribution in severe keloids with both needles and an EPI. This may partly explain why there is a large variability in treatment efficacy among severe keloids.

PERSPECTIVES

In this thesis the clinical application of intralesional treatment with bleomycin and triamcinolone acetonide using an EPI was explored. In the last paragraphs of this chapter an outline of current challenges in research, current developments in keloid treatment and emerging needle-free technologies will be discussed.

Challenges in research on keloid treatment

According to the Dutch guidelines for keloidal scars, mild keloids should be treated with silicon plasters, non-ablative laser therapy (e.g. Pulsed Dye Laser or Nd-Yag laser) or intralesional triamcinolone administered by conventional needle injections.[26] However, up to date there is no (inter)national evidence-based guideline for the treatment of severe keloidal scars. Due to the lack of standardization, patients that are referred to a dermatologist are likely to receive a different treatment compared to a plastic surgeon or general practitioner. This may be the result of the lack of strong evidence for one particular treatment modality. Ideally, future research should be multidisciplinary organized in order to achieve a standardized algorithm for (severe) keloid treatment.

Moreover, current research is varying with regards to the outcome assessment measures. Some studies use volume reduction as outcome measure, while others report on scar assessment scales, such as POSAS, VSS or JSW.[27] Ideally, there should come consensus on which scar assessment tool to use for keloids. This uniformity could contribute to easier comparisons between treatments used among different studies.

Another challenge is the high heterogeneity among keloid patients. Keloid characteristics such as size, duration of keloids and location of keloids can play an important role on treatment efficacy, and should therefore be properly mentioned in studies, while this is a point that is frequently lacking in clinical studies.

Research gaps in current literature concerning EPI-assisted treatment

In the previous studies we investigated the efficacy and safety of EPI-assisted jet-injections in keloids with different research methodologies. Firstly, we evaluated the literature to identify the current evidence with regards to EPI-assisted injections to treat dermatological indications. Hereafter, we performed both explorative *ex-vivo* and *in vivo* studies with small groups of patients with keloids. However, to improve treatment options for patients with recalcitrant keloids and make real impact in clinical practice more high-quality research is needed. Future research should focus on studies with larger sample sizes and longer follow-up. This may attribute to evidence for an international treatment guideline.

Collaborations

The need for a high quality RCT with a large sample size and sufficient follow-up time after EPI-assisted bleomycin in recalcitrant keloids faces some difficulties. Firstly, exact numbers concerning the prevalence of patients with severe keloids are lacking. However, it is known that the prevalence of all keloids including mild to severe keloids is around 0.1% in European countries. Therefore, to perform large trials it is necessary to collaborate with other expert centers with sufficient experience of keloid treatment with EPI. Moreover, EPI are currently relatively expensive, and are particularly used in countries with a high socio-economic status. Therefore, there is a need for the development of tailor-able, handheld jet-injectors that are more affordable for hospitals in countries with lower socio-economic status. In order to develop these jet-injectors, physicians need to collaborate with engineers to design and develop more efficient tunable jet-injector.

The future of intralesional treatment administered with EPI for keloid scars

In our research, we explored EPI-assisted drug delivery in *ex vivo* keloids and we evaluated clinical endpoint in keloids patients *in vivo*. Additionally, an *in vivo* keloid study could be performed to explore the spatiotemporal profile of bleomycin administered with an EPI, to closely investigate EPI-assisted injections of keloids in clinical practice.[30] Next, an ex-vivo study which compares fluids with different characteristics (e.g.

bleomycin, triamcinolone, lidocaine, and 5-Fluorouracil) could provide more insight in differences in biodistribution and splash back in keloids. Ideally, a high-speed camera may be used to compare the jet stream backflow and splatter in slow motion when injected on *ex vivo* keloid skin and to *in vivo* keloid tissue.

In future clinical studies bleomycin should preferably be administered in combination with lidocaine. In our real-world data study, we observed much lower pain scores, compared to the RCT in which we treated patients solely with bleomycin. Moreover, lidocaine increases the intracellular uptake of bleomycin.[28] Eventually, to change clinical practice and make EPI-assisted bleomycin a serious alternative to treat recalcitrant keloids, larger high quality randomized controlled trials are needed. Ideally, this will be a RCT with a long-term follow-up and parallel design including multiple arms: 1) CNI and triamcinolone, 2) CNI and bleomycin with lidocaine and 3) EPI with bleomycin and lidocaine. This would allow to compare the efficacy in terms of recurrence with i.l. bleomycin versus triamcinolone, and compare the addition of an EPI compared to CNI. A parallel design in contrast to a split-lesion design will be more appropriate for patients to score patient reported outcome measures such as NRS pain-, and POSAS tool. Significant differences in efficacy, safety and patient satisfaction will eventually lead to more efficacious, safe and patient-friendly treatment for recalcitrant keloids according to international guidelines. Also, EPI treatment with 5-Fluorouracil (and TCA) should be explored in patients with recalcitrant keloids, since high efficacy and safety was found in patients with hypertrophic scars.[9]

Other applications to be explored for EPI

Previous work demonstrated satisfactory effectiveness, tolerability and treatment satisfaction with EPI-assisted corticosteroids in adult patients with keloids.[18] Therefore, EPI-asisted treatment with TCA was offered to children who would normally receive intralesional corticosteroid treatment using conventional needles for their keloids or hypertrophic scars. We concluded that EPI-assisted TCA is an effective and child-friendly treatment for keloids and hypertrophic scars. Therefore, we would suggest to perform

studies with EPI-assisted treatments for other indications in which normally needles would be required, such as local anesthesia before local surgical procedures.

Moreover, EPI-assisted treatment could be beneficial in the intralesional treatment of inflammatory diseases such as alopecia areata, lichen simplex, hidradenitis suppurativa, prurigo nodularis and common warts. Lastly, in the future there could be a place for the intralesional treatment with chemotherapeutics such as bleomycin or 5-fluorouracil, but also immunotherapy with checkpoint inhibitors such as anti-programmed cell death-1 therapy (aPD-1) or hedgehog inhibitors such as vismodegib to treat non-melanoma skin cancer.[29-31] However, the evidence for intralesional treatment with these therapeutics in basal cell carcinoma and squamous cell carcinoma is currently limited, and therefore should only be considered if local excision is not an option, e.g. in elderly patients with multiple non-melanoma skin cancer lesions who do not wish further excisions.

Indications for needle-free drug delivery beyond dermatology

The advantages of needle-free drug delivery are not limited to the treatment of skin diseases. More than half of children and almost a quarter of the adult population fears needles.[20] This is one of the reasons patients can be reluctant to receive vaccinations. For this reason, there is an urgent need for needle-free vaccination options.

Suggestions for engineering improvements for next generation NFI devices

As stated before, it is crucial that clinicians and engineers collaborate and synergize to develop even more effective, safe and patient-friendly next generation jet-injectors. In this thesis, we performed studies in an ex-vivo and in-vivo setting, enabling the exploration of issues of current EPIs. Firstly, we observed increased residue formation on the skin in both ex vivo and in vivo studies with keloid tissue, compared to previous research with ex vivo healthy skin samples. Therefore, we would suggest the addition of a system that can measure skin thickness and rigidity, which can regulate settings such as volume, pressure and jet-injection speed automatically based on a feedback system. Recently, there has been growing interest in EPIs that use laser energy. The first EPI, the Mirajet that is actuated by laser energy was commercialized in 2022.[32] This mechanism

uses optical energy, which creates a liquid bubble in the EPI that drives a micro-jet to penetrate the epidermis and dermis. The benefits of this laser-induced NFI is the accurate injection of a small volume (0.0003 mL) into a precise depth in the dermis.[33] Furthermore, injections can be administered at a high frequency and with minimal injection-related pain. We would welcome the development of handheld devices based on this mechanism, which eventually could also be used in a home-setting for patients. This could significantly alleviate waiting time for an already overcrowded outpatient clinic waiting list, and eventually may contribute to reducing the rising costs of healthcare in general. Moreover, with EPI-assisted drug delivery of chemotherapeutics a smoke evacuator is necessary to capture aerosols is needed. A smoke evacuator is not available in all settings, which complicates ease of use. For this reason, a strong droplet evacuator could be integrated in the EPI to directly capture all aerosols during injection. Lastly, patients especially in the pediatric population can experience the noise of the EPI as disturbing. To make this treatment as child-friendly as possible, we would suggest to develop an EPI that makes negligible noise.

General conclusions

This thesis presents the evaluation of an alternative patient-friendly treatment for patients with recalcitrant keloids using a needle-free electronic pneumatic jet-injector (EPI). The evaluation encompasses different types of research, including preclinical and clinical studies.

The studies outlined in this thesis show the clinical applicability of EPI to treat several dermatologic indications, and specifically (recalcitrant) keloids. We found notably lower treatment efficacy in older keloids, keloids located on the chest, extremities, auricle, and shoulder, larger keloids, lower baseline Vancouver Scar Scale score, and keloids with history of recurrence. This thesis showed that EPI-assisted bleomycin is an effective and tolerable alternative option to treat patients with recalcitrant keloidal scars. The addition of lidocaine to EPI-assisted bleomycin results in a minimally painful and patient-friendly treatment. Furthermore, our results indicate that using EPI and triamcinolone acetonide to treat keloids and hypertrophic scars in children offers several benefits. However, both needle- and jet injections demonstrate large heterogeneity in biodistribution in severe keloids, leading to variability in treatment efficacy. Therefore, a minimally invasive needle-free drug delivery treatment that ensures more consistent biodistribution in severe keloids is needed.

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Chapter 9

English and Dutch summaries

SAMENVATTING

Hoofdstuk 1 is de algemene inleiding van dit proefschrift. In dit hoofdstuk wordt gericht op het belang van een innovatieve, effectieve, veilige en patiëntvriendelijke behandeling voor keloïden. Keloïden zijn snelgroeiende littekens die ontstaan na inflammatie of trauma. Deze fibroproliferatieve littekens kunnen zorgen voor een verminderde kwaliteit van leven door symptomen zoals pijn, jeuk en bewegingsbeperking. De patiëntenpopulatie met keloïd is divers en keloïden kunnen beperkte tot ernstige klachten veroorzaken. Vooral patiënten met ernstige keloïden kunnen veel klachten ervaren. Desondanks zijn deze keloïden moeilijk te behandelen. In dit hoofdstuk wordt ingeleid waarom naaldvrije injecties met bleomycine potentieel een goed alternatief zouden kunnen zijn in deze patiëntenpopulatie. De huidige standaardbehandeling bestaat uit conventionele naald-injecties met triamcinolon acetonide (TCA). Echter, behandeling met naaldinjecties en TCA kent verschillende beperkingen. Enerzijds is toediening van medicatie middels naaldinjecties suboptimaal. Naaldinjecties kunnen pijnlijk zijn en zijn niet geschikt voor patiënten met een naaldenfobie. Ook bestaan keloïden, en met name ernstige keloïden vaak uit zeer stug weefsel. Hierdoor kan hoge weerstand in keloïd worden verwacht, wat succesvolle medicatie toediening belemmert. Tevens gaat behandeling met naaldinjecties gepaard met hoge interoperabiliteit, wat kan leiden tot verschil in effectiviteit. Anderzijds leidt behandeling met TCA vaak tot recidief van het keloïd vanwege de tijdelijke effecten van TCA. Derhalve is een behandeling met langdurige effecten gewenst. In tegenstelling tot TCA kan behandeling met bleomycine een langduriger effect bewerkstelligen.

Hoofdstuk 2 beschrijft het klinische bewijs voor de effectiviteit en veiligheid van naald-vrije jet-injectoren voor de behandeling van dermatologische aandoeningen. Zowel traditionele veer geladen jet-injectoren, als innovatievere elektronische pneumatische jet-injectoren (EPI) worden gebruikt binnen de dermatologie. In tegenstelling tot de traditionele jet-injectoren, is het met de EPI mogelijk om de druk en het volume van de injecties aan te passen. Dit maakt dat je met een EPI een gecontroleerde geneesmiddelenafgifte kunt bewerkstelligen. De bevindingen van dit hoofdstuk tonen aan

dat naald-vrije jet-injectoren worden gebruikt voor verscheidende dermatologische indicaties, zoals recalcitrante wratten, huidkanker en pathologische littekens zoals keloïd. Het meeste bewijs werd gevonden voor goede effectiviteit en veiligheid voor de behandeling van pathologische littekens, waaronder keloïd, hypertrofische littekens en atrofische littekens. Echter, had het merendeel van deze studies een matige methodologische kwaliteit. Derhalve is er behoefte aan klinische studies met hoge methodologische kwaliteit die de effectiviteit en veiligheid van EPI in keloïd onderzoeken.

Hoofdstuk 3 toont de biodistributie van TCA toegediend middels een naald-vrije jet-injector en conventionele naald in keloïdale huid en gezonde huid. De biodistributie werd in deze studie onderzocht middels een innovatieve beeldvormende techniek, namelijk de 3D-Fluorescent Imaging Cryomicrotome System (3D-FICS). Deze techniek maakt het mogelijk om het geïnjecteerde TCA in hoge resolutie en 3D te visualiseren. De 3D techniek biedt de mogelijkheid om de vorm van het geïnjecteerde TCA te analyseren. Echter, was de vorm van de geïnjecteerde vloeistof met zowel de naald- als de jet-injector erg heterogeen, en kon er geen patroon worden herkend. De resultaten van deze studie toonde ook aan dat de heterogeniteit van het geïnjecteerde volume in keloïd met zowel jet-injecties als naald-injecties groter is in vergelijking met het geïnjecteerde volume TCA in gezonde huid. Mogelijk zou dit een verklaring kunnen zijn voor het verschil in effect tussen keloïden met dezelfde behandeling.

Hoofdstuk 4 beschrijft aan de hand van bestaande literatuur welke keloïd gerelateerde karakteristieken kunnen leiden tot recalcitrantie. De resultaten van dit onderzoek lieten zien dat de locatie, duur, grootte, therapeutische voorgeschiedenis en ernst van het keloïd mogelijk invloed hebben op het effect van behandeling. Met name keloiden gelokaliseerd op de oorschelp, schouder, extremiteiten, grotere keloïden, keloïden met uitgebreide therapeutische voorgeschiedenis en lage baseline Vancouver Scar Scale score zouden potentieel meer kans geven op recalcitrantie. Echter, is dit bewijs gelimiteerd door beperkingen in het aantal en de kwaliteit van de beschikbare studies.

Hoofdstuk 5 toont de resultaten van een dubbelblind, gerandomiseerd en gecontroleerd onderzoek in patiënten met ernstig keloïd. Patiënten werden in dit onderzoek driemaal behandeld met intralesionale bleomycine toegediend met een EPI in de helft van de keloïdale laesie, en met fysiologisch zout in de andere helft van de laesie. In dit onderzoek werden zowel objectieve uitkomstmaten zoals volume (gemeten met een 3D camera) en bloedstroom (gemeten met een Laser Speckle Contrast Imaging techniek), als subjectieve uitkomstmaten zoals keloïd gerelateerde symptomen en injectie gerelateerde pijn onderzocht. In de bleomycine behandelde laesies werd een reductie in keloïd volume en keloïd gerelateerde symptomen gevonden, wat niet werd gezien in de laesies die waren behandeld met fysiologisch zout. Er werd geen significant verschil in bloedstroom gezien tussen de bleomycine en placebo behandelde laesies. Bijwerkingen die optraden waren tijdelijke necrose, haematoom en hyperpigmentatie. Opvallend is dat de procedure gerelateerde pijn met een NRS van 5 relatief hoog was. Echter, was deze pijnscore niet significant verschillend vergeleken met laesies die waren behandeld met fysiologisch zout.

Hoofdstuk 6 toont de resultaten van een real-world data studie, waarin intralesionale EPI behandeling met bleomycine in de klinische praktijk werd geëvalueerd. In deze studie werden patiënten met recalcitrante keloïden geïncludeerd die drie behandelingen hebben gehad met bleomycine en lidocaïne, toegediend met een EPI. Er werd een klinische verbetering gezien in deze moeilijk te behandelen populatie, welke werd geobjectiveerd met de Patient and Observer Scar Assessment Scale (POSAS). De bijwerkingen die werden geobserveerd waren eveneens necrose, haematoom en hyperpigmentatie. Verder ging de injectie gepaard met minimale pijn, die significant als een stuk lager werd beoordeeld door patiënten dan naaldinjectie met bleomycine en lidocaïne. Het vragenlijstonderzoek toonde aan dat alle patiënten behandeling met EPI prefereerden boven behandeling met naalden.

Hoofdstuk 7 toont de resultaten van een real-world data studie met vragenlijst onderzoek in kinderen met keloïd en hypertrofische littekens die behandeld zijn met een EPI en TCA. In dit onderzoek vonden we dat klinische verbetering optrad met deze naald-

vrije behandeling en dat de behandeling goed werd getolereerd. De naald-vrije behandeling resulteerde in een significant lagere injectie gerelateerde pijnscore in vergelijking met naaldinjecties. Alle geïncludeerde patiënten prefereerden naald-vrije EPI behandeling over behandeling met naalden. Gezien de meerderheid van de patiënten onder de achttien jaar kampt met een angst voor naalden of naaldenfobie, is deze naald-vrije behandeling specifiek van toegevoegde waarde in deze populatie.

Hoofdstuk 8 is de algemene discussie van dit proefschrift, waarin onze bevindingen in een groter perspectief zijn geplaatst. Het behandelt huidige uitdagingen in de behandeling van recalcitrant keloïd en gaat in op de voor- en nadelen van behandeling met intralesionale bleomycine toegediend met een EPI. Ook wordt besproken bij welke dermatologische indicaties EPI van toegevoegde waarde zou kunnen zijn. Tot slot worden suggesties gedaan om in de toekomst de naald-vrije behandeling in keloïden te optimaliseren.

SUMMARY

Chapter 1 is the general introduction of this thesis. This chapter focuses on the importance of an innovative, effective, safe and patient-friendly treatment for keloids. Keloids are pathologically-growing scars that develop after inflammation or trauma. These fibroproliferative scars can cause a reduced quality of life due to symptoms such as pain, itching and restriction of movement. The patient population with keloids is diverse and keloids can cause limited to serious complaints. Patients with severe keloids in particular can experience many symptoms. Nevertheless, these keloids are difficult to treat. This chapter introduces why needle-free injections with bleomycin could potentially be a good alternative in this patient population. The current standard treatment consists of conventional needle injections with triamcinolone acetonide (TCA). However, treatment with needle injections and TCA has several limitations. On one hand, administering medication through needle injections is suboptimal. Needle injections can be painful and are not suitable for patients with needle phobia. Keloids, and especially severe keloids, also often consist of very stiff tissue. As a result, high resistance in keloid can be expected, which hinders successful drug administration. Treatment with needle injections is also associated with high interoperability, which can lead to differences in effectiveness. On the other hand, treatment with TCA often leads to keloid recurrence due to the temporary effects of TCA. Therefore, a treatment with long-lasting effects is desirable. In contrast to TCA, treatment with bleomycin can achieve a longer lasting effect.

Chapter 2 describes the clinical evidence for the effectiveness and safety of needle-free jet injectors for the treatment of dermatological conditions. Both traditional spring loaded jet injectors and more innovative electronic pneumatic jet injectors (EPI) are used in dermatology. Unlike traditional jet injectors, the EPI allows to adjust the pressure and volume of the injections. This means that you can achieve controlled drug release with an EPI. The findings of this chapter demonstrate that needle-free jet injectors are used for a variety of dermatological indications, such as recalcitrant warts, skin cancer and pathological scars such as keloid. Most evidence was found for good effectiveness and

safety for the treatment of pathological scars, including keloid, hypertrophic scars and atrophic scars. However, the majority of these studies had moderate methodological quality. Therefore, there is a need for clinical studies with high methodological quality that investigate the effectiveness and safety of EPI in keloid.

Chapter 3 shows the biodistribution of TCA administered via a needle-free jet injector and conventional needle in keloidal skin and healthy skin. The biodistribution was investigated in this study using an innovative imaging technique, the 3D-Fluorescent Imaging Cryomicrotome System (3D-FICS). This technique makes it possible to visualize the injected TCA in high resolution and 3D. The 3D technique offers the possibility to analyse the shape of the injected TCA. However, the shape of the injected fluid with both the needle and jet injectors was very heterogeneous, and no pattern could be recognized. The results of this study also showed that the heterogeneity of the injected volume in keloid with both jet injections and needle injections is greater compared to the injected volume of TCA in healthy skin. This could possibly explain the difference in effect between keloids with the same treatment.

Chapter 4 describes, based on existing literature, which keloid-related characteristics can lead to recalcitrance. The results of this study showed that the location, duration, size, therapeutic history and severity of the keloid may influence the effect of treatment. In particular, keloids located on the ear, shoulder, extremities, larger keloids, keloids with extensive therapeutic history and low baseline Vancouver Scar Scale score would potentially be more likely to develop recalcitrance. However, this evidence is limited by limitations in the number and quality of available studies.

Chapter 5 shows the results of a double-blind, randomized and controlled study in patients with severe keloid. In this study, patients were treated three times with intralesional bleomycin administered with an EPI in half of the keloidal lesion, and with physiological saline in the other half of the lesion. In this study, objective outcome measures such as volume (measured with a 3D camera) and blood flow (measured with a Laser Speckle Contrast Imaging technique), as well as subjective outcome measures such

as keloid-related symptoms and injection-related pain, were examined. In the bleomycin treated lesions a reduction in keloid volume and keloid related symptoms was found, which was not seen in the lesions treated with saline. No significant difference in blood flow was seen between the bleomycin and placebo treated lesions. Side effects that occurred were temporary necrosis, hematoma and hyperpigmentation. Interestingly, the procedure-related pain with an NRS of 5 was relatively high. However, this pain score was not significantly different compared to lesions treated with saline.

Chapter 6 shows the results of a real-world data study, in which intralesional EPI treatment with bleomycin was evaluated in clinical practice. This study included patients with recalcitrant keloids who received three treatments with bleomycin and lidocaine administered with an EPI. Clinical improvement was seen in this difficult-to-treat population, which was objectified with the Patient and Observer Scar Assessment Scale (POSAS). The side effects observed also included necrosis, hematoma and hyperpigmentation. Furthermore, the injection was associated with minimal pain, which was rated significantly lower by patients than needle injection with bleomycin and lidocaine. The questionnaire survey showed that all patients preferred treatment with EPI over treatment with needles.

Chapter 7 shows the results of a real-world data study with a questionnaire in children with keloids or hypertrophic scars treated with an EPI and TCA. In this study we found that clinical improvement occurred with this needle-free treatment and that the treatment was well tolerated. The needle-free treatment resulted in significantly lower injection-related pain scores compared to needle injections. All included patients preferred needle-free EPI treatment over treatment with needles. Given that the majority of patients under the age of eighteen suffer from a fear of needles or needle phobia, this needle-free treatment is specifically of added value in this population.

Chapter 8 is the general discussion of this thesis, which places our findings in a larger perspective. It addresses current challenges in the treatment of recalcitrant keloid and discusses the advantages and disadvantages of treatment with intralesional bleomycin

administered with an EPI. It also discusses in which dermatological indications EPI could be of added value. Finally, suggestions are made to optimize needle-free treatment for keloids in the future.

Chapter 10

Appendices

Abbreviations

List of co-authors

List of publications

About the author

PhD portfolio

Dankwoord

ABBREVIATIONS

CCT	clinical controlled trial
CI	confidence interval
3D-FICS	3D-fluorescent imaging cryomicrotome system
EPI	electronically-controlled pneumatic jet injector
5-FU	5-fluorouracil
GAIS	global aesthetic improvement scale
HTS	hypertrophic scars
i.l.	Intralesional
IQR	interquartile range
LSCI	laser speckle contrast imaging
NFI	needle-free jet injector
NRS	numerical rating scale
POSAS	patient and observer scar assessment scale
RCT	randomized controlled trial
SD	standard deviation
SLI	spring-loaded jet injector
TCA	triamcinolone acetonide
VSS	Vancouver scar scale

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M. Haedersdal
Department of Dermatology, Bispebjerg Hospital, University of Copenhagen, Copenhagen,
Denmark

LIST OF PUBLICATIONS

IN THIS THESIS

Bekkers V.Z, Bik L., van Huijstee JC, Wolkerstorfer A, Prens E.P., van Doorn M.B.A. Efficacy and safety of needle-free jet injector-assisted intralesional treatments in dermatology-a systematic review. Drug Deliv Transl Res. 2023 Jun;13(6):1584-1599. doi: 10.1007/s13346-023-01295-x. PMID: 36884194

Bekkers V.Z, Khan F., Aarts P., Zdunczyk K.M., Prens E.P., Wolkerstorfer A., Rissmann R., van Doorn M.B.A. Needle-free electronically-controlled jet injector treatment with bleomycin and lidocaine is effective and well-tolerated in patients with recalcitrant keloids. Lasers Surg Med. 2024 Jan;56(1):45-53. doi: 10.1002/lsm.23737. PMID: 37933762.

Bekkers, V.Z., Van Eijsden, C., Yin, Q, Wolkerstorfer A., Prens E.P., van Doorn M.B.A. Needle-Free Jet Injector-Assisted Triamcinolone Treatment of Keloids and Hypertrophic Scars is Effective and Well Tolerated in Children. Clin Drug Investig 44, 51–57 (2024). https://doi.org/10.1007/s40261-023-01332-0. PMID: 38093082

Bekkers VZ,* Yin Q,* Roelofs MCM, Dobbe JGG, de Vos J, Bloemen PR, Aalders MCG, Gibbs S, Lapid O, Niessen FB, van Doorn MBA, Wolkerstorfer A. The biodistribution of triamcinolone acetonide injections in severe keloids: an exploratory three-dimensional fluorescent cryomicrotome study. Arch Dermatol Res. 2024 Jun 8;316(7):368. doi: 10.1007/s00403-024-03041-w. PMID: 38850361

Bekkers V.Z,* Barsoum P,* Yin Q, Niessen F, van Zuijlen P, Lapid O, van Doorn M, Wolkerstorfer A. Effect of Keloid Properties on Treatment Efficacy, a Systematic Review. Dermatol Surg. 2024 Jun 14. doi: 10.1097/DSS.0000000000004256. PMID: 38874219.

Bekkers V.Z.,* Zdunczyk K.M.,* Bik L. Ten Voorde W., Aarts P., Oerlemans F., Bohoslavsky R. Haedersdal M., Prens E.P., Rissmann R., Van Doorn M.B.A. Needle-free electronically-controlled jet injector treatment with bleomycin is efficacious and well-tolerated in patients with severe keloids: results of a randomized, double-blind, placebo-controlled trial. Clin Exp Dermatol. 2024 July 20th. doi: 10.1093/ced/llae254. PMID: 39030712

OTHER PUBLICATIONS

Bik L, Wolkerstorfer A, **Bekkers V.Z.**, Prens EP, Haedersdal M, Bonn D, van Doorn MBA. Needle-free jet injection-induced small-droplet aerosol formation during intralesional bleomycin therapy. Lasers Surg Med. 2022 Apr;54(4):572-579. doi: 10.1002/lsm.23512. Epub 2021 Dec 21. PMID: 34931319; PMCID: PMC9303553.

Zdunczyk K.M., **Bekkers V.Z.** , Van der Kolk T., Broekhuizen K., van Doorn M.B.A., Rissmann, K. van der Maaden. Taking a deep dive into the skin – a comprehensive literature review on the use of needle free jet injectors for intra- and transdermal drug delivery. [In Preparation]

Bekkers V.Z., Bik L. , Van Doorn M.B.A. Naald-vrije injecties. NTvDV. 2022 Nov; jaargang 32; nummer 10: 53-55 NTvDV_10_2022_LR.pdf (nvdv.nl)

ABOUT THE AUTHOR

Vazula Zulfra Bekkers was born on July 19, 1997, and raised in Rotterdam by her Surinamese mother, Halimoennisa Kasiemkhan, and Dutch father, Frenk Bekkers. She graduated from Wolfert Dalton's Gymnasium in Rotterdam in 2015.
From 2015 to 2018 she pursued her bachelor's degree in Medicine at the Vrije Universiteit (VU) in Amsterdam. In the weekends she returned to Rotterdam to accompany her cousin as her caregiver and close friend. During her master's degree in Medicine and PhD in dermatology, Vazula studied extracurricular courses in the bachelor's programs of Philosophy and Health Economy Policy and Law at Erasmus University. She also served her fellow students as the master commissioner on the board of the Co-Raad VU from 2019 to 2020. She moved back to Rotterdam in 2020, where she started investigating aerosol formation of bleomycin with needle free jet injectors at the dermatology department of Erasmus Medical Center (EMC) under supervision of dr. Liora Bik and dr. Martijn van Doorn. In 2021, she finished her clinical rotations with the final rotation at the plastic surgery department of the Groene Hart Hospital in Gouda. Motivated by the potential of needle-free jet-injectior-assisted drug delivery in dermatology, she completed her master degree in Medicine with research on this topic in 2021. Supported by the dermatology department of EMC and the Centre for Human Drug Research in Leiden, she formally continued her research by working on her doctoral thesis on needle-free jet injector-assisted drug delivery in severe keloids under the supervision of dr. Martijn van Doorn, prof. dr. Robert Rissmann and prof. dr. Errol Prens. In 2024 she also worked as a psychiatry resident in GGZ Rivierduinen in Leidschendam, while finishing her doctoral thesis in dermatology.
On June the 29th of 2022 Vazula married with Tarik Küçük, who she met at the Medical Faculty of EMC. They now enjoy a happy life together in Rotterdam in the company of their two cats, Sonna and Mia.

PORTFOLIO

Name PhD Student: Vazula Z. Bekkers

Erasmus MC Department: Dermatology

Research School: NIHES

PhD period: December 2021 – October 2024

Promotors: E.P. Prens & R. Rissmann

Copromotor: M.B.A. Van Doorn

Training program	**Year**	**Workload**
General courses		
PhD introduction session	2022	0.2 ECTS
Review of mathematics and introduction to statistics	2022	1.5 ECTS
Biostatistics 1	2022	4.5 ECTS
Good clinical practice: BROK	2022	1.5 ECTS
LimeSurvey	2022	0.5 ECTS
Scientific integrity	2023	0.3 ECTS
Biomedical Writing for PhD candidates	2023	2.0 ECTS
Conferences attended		
Research meetings and journal clubs, Dermatology, Erasmus MC, The Netherlands	2021 - 2024	2.0 ECTS
Skintermezzo meetings, Dermatology, Erasmus MC, The Netherlands	2021 - 2024	1.0 ECTS
PhD Weekend Dermatology, Utrecht, The Netherlands	2022	1.0 ECTS
Wetenschapsvergadering, Rotterdam, The Netherlands	2022	1.0 ECTS
EADV annual conference, Milano, Italy	2022	1.0 ECTS
ASLMS: 24 Hours of Lasers and Energy-Based Devices in Cutaneous Applications	2022	1.0 ECTS
ESLD: Laser and Energy-based Treatments teaching course	2022	1.0 ECTS

ISID annual conference, Tokyo, Japan	2023	1.0 ECTS
SCARS annual conference, Berlin, Germany	2023	1.0 ECTS

International presentations

EADV annual conference, Milano, Italy: *Efficacy and tolerability of needle-free jet injectors for intralesional treatment in dermatology - a systematic review and methodological assessment (poster presentation)*	2022	1.0 ECTS
ISID annual conference, Tokyo, Japan: *Needle-free jet injections with bleomycin to treat severe keloids: a patient-friendly, effective and safe treatment (poster presentation)*	2023	1.0 ECTS
SCARS annual conference, Berlin, Germany: *Needle-free bleomycin treatment using an electronic-pneumatic jet-injector in keloids (oral presentation)*	2023	1.0 ECTS
EADV annual conference, Amsterdam, The Netherlands: *Topical keloid treatments (oral presentation)*	2024	1.0 ECTS

Teaching

Resident education, Department of Dermatology, Erasmus Medical Center, Rotterdam, The Netherlands: *1. Keloids and energy-based treatment 2. EPI-assisted treatmens*	2022 - 2023	0.5 ECTS
Resident education, Department of Dermatology, Amsterdam University Medical Center, Amsterdam, The Netherlands: *electronic pneumatic jet-injections with bleomycin to treat keloids*	2024	0.3 ECTS
Trial education, Department of Dermatology, Erasmus MC, The Netherlands: *training in study related tasks*	2022 – 2023	2.0 ECTS

(EPI, 3D-camera, Laser Speckle Contrast Imaging, POSAS scoring)		
Supervising master thesis of Fatima Khan	2022	2.0 ECTS
Supervising master thesis of Maud Roelofs	2023	2.0 ECTS
Supervising master thesis of Paul Barsoum	2023	2.0 ECTS

Other

Study protocol for a future study: The efficacy and safety of intralesional combination treatment with bleomycin and triamcinolone vs. triamcinolone monotherapy using an electronic pneumatic jet injector: a randomized, double-blind, controlled trial	2023	2.0 ECTS

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