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ORIGINAL RESEARCH

Treatment of late bacterial infections resulting from soft-tissue filler injections

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Purpose: Late bacterial infections (LBIs) after esthetic facial augmentation using hyaluronic acid (HA) fillers are relatively rare yet severe complications that are difficult to treat. No adequate treatment standards have hitherto been formulated. We have bridged this gap by formulating a treatment scheme based on the principles of treating foreign-body implantation-related infections and treating bacterial growth in the form of biofilm. The objective of this study was to evaluate the efficacy of a comprehensive scheme for treating LBI complications after facial augmentation using cross-linked HA fillers.

Methods: A total of 22 patients with LBI symptoms at a site of cross-linked HA injection underwent treatment and observation. The comprehensive treatment scheme formulated by Marusza and Netsvyetayeva (M&N scheme) comprised draining the lesion, dissolution of cross-linked HA with hyaluronidase, broad-spectrum antibiotic combination therapy, and use of probiotics. While 17 patients underwent the M&N scheme, the remaining five were treated with other schemes. Statistical analysis of the data was performed using Mann–Whitney *U* and χ^2 nonparametric tests with SAS 9.4 software.

Results: All 17 patients who underwent the M&N scheme experienced resolution of symptoms, with no recurrence of infection at the HA-injection sites.

Conclusion: To treat LBI at a site of cross-linked HA administration, the principles applicable to infections resulting from implantation of a foreign body must be followed. The treatment period should be sufficiently long for complete resolution of symptoms. The efficacy of treatment is considered proven if 2 months have elapsed without recurrence since the symptoms resolved. The M&N scheme is recommended for use as the first therapeutic option for treating LBI related to soft-tissue fillers.

Keywords: bacterial biofilm, hyaluronic acid, soft-tissue filler complications, biofilm treatment

Introduction

Aesthetic medical procedures involving facial augmentation using hyaluronic acid (HA) fillers are commonplace and their number is steadily increasing. In the US, the use of fillers soared from 1.8 million procedures in 2010 to 2.6 million in 2016, according to data from the American Society of Plastic Surgeons.[1] One in every hundred of these patients experiences an infection at a site where a soft-tissue filler has been administered. Inflammatory swelling or nodules usually emerge several weeks to several years after the procedure. Treating infections of this kind poses a serious challenge. If appropriate treatment is not provided at the initial stage of infection (induration, inflammation, and pain), fistulae can form, through which pus and degraded filler pour out.

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In cases of culture-negative pus, some medical practitioners consider the aforementioned symptoms to represent an allergic reaction.[2] The chief arguments against this diagnosis of an allergic reaction are the long time span (often extending from several weeks to several years after the procedure) and the inefficacy of steroidal therapy.[3] Over the last few years, many studies have been published that elucidate the infectious etiology of complications of this kind.[4–8] These studies indicate that the inflammatory swelling or nodules at sites of HA injection are related to bacterial biofilm growth on the surface of the filler, which constitutes a foreign body in the subcutaneous tissue.[4–8] It has also been proven that various fillers, HA included, constitute a foundation upon which bacterial biofilm can form, generating symptoms of late bacterial infection (LBI) complications.[6–8]

In 2014, the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) published guidelines on the diagnosis and treatment of biofilm-related infections. Infections complicating procedures involving tissue fillers were included in the scope of the guidelines.[9] The following typical yet low-intensity inflammatory reactions were considered clinical symptoms of biofilm-related infections: induration, swelling, erythema, pain, and loss of function. Additionally, the following factors were linked to biofilm-related infections: history of a condition predisposing the patient to biofilm growth (ie, a condition involving implantation of a foreign body), infection lasting >7 days, inefficacy of antibiotic treatment, and objective and subjective symptoms of infection that resolve during antibiotic treatment, but recur after its termination.[9]

A biofilm is an aggregation of bacterial cells attached to an artificial surface, embedded in a matrix of extracellular bacterial macromolecules. A biofilm, or sedentary microbial amalgam, may consist of one or many strains. Bacterial cells in a biofilm differ physiologically from planktonic cells (free-floating single cells),[3] including having a significantly lower metabolic rate.

The eradication of a biofilm from a solid surface is difficult. The matrix protects the microbes from many externally applied factors, such as bactericides. Its branched system of tubules ensures nutrient transport and communication among microbes via chemical and physical signaling (quorum sensing). Bacterial cells in a developed biofilm have a shared set of defensive mechanisms that allow the microbial amalgams to function in conditions in which separate cells would likely die.

Cultivation and identification of bacterial cultures in so altered a state are very difficult.[10] In addition, the thickness of a biofilm layer on an artificial surface can be 5–1,200 μm, thereby significantly hindering the extraction of representative samples for examination. Whenever typical microbiological culture methods are used, the results are generally false negative. However, the following methods are deemed valid: biopsy with subsequent utilization of peptide nucleic acid fluorescence in situ hybridization or with subsequent three-dimensional confocal laser-scanning microscopy and direct observation using confocal laser-scanning microscopy.[9] Therefore, in recent years, medical biofilm research has progressed from in vitro bacterial cultivation and detection methods to direct in vivo visualization and the utilization of animal models.[10]

Bacteria in a biofilm possess immunity to antibiotics and other antibacterial medications.[11] This immunity is related to their exceedingly slow growth, their resistance to antibiotic penetration, the emergence of resistant phenotypes, and the unfavorable microenvironment within the biofilm.[12] Standard methods for determining resistance to medication that were formulated based on planktonic bacteria do not provide valid results in the case of biofilm. Biofilm-related infections resemble chronic and recurrent diseases, are resistant to conventional antibiotic treatment, and do not yield culture-positive results.[13] The 2014 ESCMID guidelines on the treatment of infections related to foreign-body implantation recommend the use of antibiotics and debridement, in addition to defining the criteria for either removal or retention of the implant. The guidelines recommend that empirical antibiotic therapy should be extensive, involving at least two antibiotics affecting both Gram-positive and Gram-negative bacteria for at least 3 weeks. In most cases, the infected implant should be removed. It should be retained only if its integrity is preserved, there are no fistulae, and the etiological agent of the infection has been identified and is susceptible to antibiotics effective against biofilm-related infections.[9] The objective of this study was to evaluate the efficacy of a comprehensive scheme for treating LBI complications after facial augmentation using cross-linked HA fillers.

Methods

The study was conducted at the Academy of Face Sculpting (Akademia Rzeźbienia Twarzy), Warsaw, Poland in 2012–2017.

Participants

Individuals participating in the study were 22 women aged 30–68 (mean 47.32, median 47.0, SD 10.82) years who had been referred by various cosmetic doctors in Poland. All had symptoms of LBI at sites of cross-linked HA injection. The symptoms had occurred at 1–18 months (mean 3.59, median 2.0, SD 3.86) after the procedure. The patients had been treated with cross-linked HA gel supplied by various manufacturers. The HA was produced by bacterial fermentation, which is legally permitted for use in aesthetic medicine in Poland. The volume of cross-linked HA administered was 1–6 (mean 2.09, median 2.0, SD 1.19) mL. Table 1 shows the relevant data on the participants.

Inclusion criteria for participation in the study were: 1) having undergone an aesthetic procedure with the use of ≥1 mL cross-linked HA gel containing ≥20 mg/mL of stabilized cross-linked HA, which was injected into facial tissue; 2) LBI symptoms of inflammatory swelling or nodules as a complication at one or more sites of injection of cross-linked HA, involving redness, swelling, induration, tenderness, and pus discharge; 3) duration from initial HA administration to LBI occurrence >1 month; and 4) a signed consent form.

Marusza and Netsvyetayeva (M&N) scheme of comprehensive treatment

The M&N scheme involves the following procedures.

1. Puncturing the lesion with an 18 G needle, followed by drainage of pus and fermented cross-linked HA twice a week, until complete resolution.
2. An allergy test is performed before the first administration of hyaluronidase. It entails subcutaneous injection of 20 units of hyaluronidase into the forearm skin, followed by a 30-minute observation period. At least 72 hours must pass before assessing the result to ensure that any negative

Table 1 Description of study participants

Age (years)	Site of HA injection	HA volume (mL)	Time between HA injection and biofilm formation (months)	Site affected by biofilm	Diameter of lesion (cm)
48	Nasolabial folds, corners of mouth	1	6	Right corner of mouth	≤2
49	Tear troughs, nasolabial folds, lips	3	4	All areas treated	≤2
59	Tear troughs, cheeks, chin	6	2	Right cheek	≤2
31	Tear troughs	1	10	Right tear trough	Orbital cavity
37	Nasolabial folds, corners of mouth, lips	2	4	Right nasolabial fold	≤2
47	Nasolabial folds, corners of mouth	1	1	Left nasolabial fold	≤2
53	Tear troughs, nasolabial folds	2	2	Right tear trough	Orbital cavity
38	Cheeks	2	2	Right cheek	≤2
68	Cheeks	2	1	All areas treated	≤2
41	Tear troughs, nasolabial folds, eyebrow ridge	2	3	All areas treated	≤2
58	Cheeks	2	5	All areas treated	≤3
56	Nasolabial folds	1	2	All areas treated	≤2
39	Tear troughs, nasolabial folds	2	2	Left tear trough	Orbital cavity
62	Nasolabial folds, corners of mouth	2	3	All areas treated	≤2
56	Cheeks	2	18	Right cheek	≤2
62	Nasolabial folds	2	2	All areas treated	≤2
30	Tear troughs, both cheeks, lips	4	4	Right tear trough	Orbital cavity
45	Lower face, marionette lines	1	1	Corners of mouth	≤1
35	Wrinkles between eyebrows	1	1	Near right eyebrow, close to nose	≤1
44	Forehead	1	1	Forehead above left eyebrow	≤1
36	Tear troughs, edges of mandible, forehead, temples, lips, nasolabial folds	3	1	Both tear troughs	Both orbital cavities
47	Cheeks, chin	3	4	Chin	≤2

Abbreviation: HA, hyaluronic acid.

results are valid, as intolerance may be either the early or late type.[14]

3. Local administration of hyaluronidase directly onto the lesion twice a week for 14–21 days or until complete resolution of swelling and nodules (whichever is longer). The recommended hyaluronidase doses, according to the size of the swelling and nodules, are presented in Table 2.
4. Oral administration of combination antibiotic therapy: 2×400 mg moxifloxacin per os +2×500 mg clarithromycin per os for 14–21 days or until the complete resolution of swelling and nodules (whichever is longer).
5. Probiotic formulation consisting of 1.6 billion CFU of lyophilized strains of *Lactobacillus acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium lactis*, three capsules per day, during the antibiotic therapy and for 1 month after its completion.

Criteria for recovery

These were the absence of symptoms of bacterial infection, such as redness, swelling, hardness, tenderness, and pus discharge, at the site of cross-linked HA administration and an asymptomatic state for 2 years.

Criteria for unsuccessful treatment

These were lack of substantial improvement within the first 14 days after beginning LBI therapy, and relapse, ie, recurrence of LBI symptoms (redness, swelling, hardness, tenderness, or pus discharge) at the site of cross-linked HA administration in the 2-year period after treatment of the original lesions was finished.

Statistical analysis

The SAS 9.4. software suite (SAS Institute Inc., Cary, NC, USA) was utilized for statistical evaluation of the data. A descriptive analysis of the data was performed. This was followed by an analysis using the Shapiro–Wilk test of normality to compare the distribution of the data with the Gaussian distribution. To assess differences between subgroups, the nonparametric Mann–Whitney *U* test (for continuous variables) and the nonparametric χ^2 test (for discrete variables) were applied. $P<0.05$ was considered to represent statistical significance.

Results

Of the 22 patients, 17 (77.27%) were cured using the M&N scheme, while five (22.73%) were cured using other treatment schemes. Of the 17 patients cured with the M&N scheme, five were cured after application of the scheme as the first therapeutic option, whereas for 12 it was applied only after unsuccessful treatment with other schemes.

Based on the treatment schemes and results, the 22 patients were divided into three subgroups: 1) five patients who were treated with the M&N scheme as the first therapeutic option; 2) 12 patients who were treated with the M&N scheme as the second therapeutic option after unsuccessful treatment with other schemes; and 3) five patients who were cured using treatment schemes other than the M&N scheme. Table 3 shows the descriptions of the groups: the group of 17 patients cured using the M&N scheme and subgroups 1–3.

Table 2 Variation in hyaluronidase dose with size of inflammatory facial swelling or nodule

Diameter of swelling or nodule (cm)[a]	≤0.5	≤1	≤1.5	≤2	≤2.5	≤3	In orbital cavity
Each hyaluronidase dose (units)[b]	45	75	105	135	165	195	6–24

Notes: [a]Assessed based on the diameter measured between the opposite rims that were furthest apart. [b]There are routinely six units per injection.

Table 3 Description of the groups

	Age (years)			HA volume (mL)			Time between HA injection and biofilm formation (months)			Treatment duration (days)		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
Patients cured using M&N scheme (n=17)	49.1	49.0	11.5	2.2	2.0	1.2	4.2	3.0	4.2	16.9	14.0	3.6
Subgroup 1	44.8	48.0	11.0	2.6	2.0	2.1	5.0	4.0	3.0	15.4	14.0	3.1
Subgroup 2	50.8	54.5	11.7	2.0	2.0	0.7	3.7	2.0	4.6	17.5	17.5	3.7
Subgroup 3	41.4	44.0	5.5	1.8	1.0	1.1	1.6	1.0	1.3	32.0	14.0	33.0

Abbreviations: HA, hyaluronic acid; M&N, Marusza and Netsvyetayeva.

Subgroup 1

As shown in Table 4, in subgroup 1 there were five patients for whom the M&N scheme was applied as the first therapeutic option after LBI was noticed at a site where cross-linked HA had been administered. For all subgroup 1 patients, resolution of local symptoms was achieved after treatment lasting 14–21 days, with no relapse during the subsequent 2 months. After the 2-month period, all patients underwent remedial therapy with cross-linked HA, with no infectious complications at the site of administration within the subsequent 2 years.

Subgroup 2

As shown in Table 5, in subgroup 2 there were 12 patients for whom the M&N scheme was applied as the second therapeutic option after unsuccessful treatment with other treatment schemes. The original unsuccessful biofilm treatments were as follows. Four patients were treated with the M&N scheme, but with ciprofloxacin instead of moxifloxacin. Resolution of symptoms was achieved, followed by remission during the 2-month observation period. After remedial administration of cross-linked HA, three patients experienced relapse of symptoms at previously affected sites, while the fourth experienced a relapse at another site. Another three patients were treated with the M&N scheme, but with clarithromycin monotherapy in two cases and a combination of clarithromycin with amoxicillin and clavulanic acid in the other case. Spontaneous relapse (without remedial HA administration) occurred in all three patients after the original symptoms had resolved. Four other patients were treated with a variety of antibiotics, but without hyaluronidase, and there was a lack of initial symptom resolution at the site of the original biofilm. The final patient of the 12 had four symptom relapses after each remedial HA administration, despite having been comprehensively treated with the M&N scheme three times. The scheme was successful only after the patient had a tooth with a periodontal cyst extracted and was treated for chronic odontogenic sinusitis.

Of the 12 patients in subgroup 2, eight received remedial cross-linked HA after resolution of symptoms with the M&N scheme, and remission was observed during the following 2 months. No relapses occurred in the following 2 years. The other four patients did not wish for another application of cross-linked HA as part of remedial therapy.

Subgroup 3

As shown in Tables 6 and 7, in subgroup 3 there were five patients who were cured after using schemes other than the M&N. The biofilm treatments and results were as follows. For the first patient, resolution of symptoms of the original biofilm was achieved after a single administration of 40 units of hyaluronidase alone (not in accordance with the hyaluronidase dosage in the M&N scheme). Following a 1-month remission period, a relapse caused by remedial cross-linked HA administration was observed. Treatment of the relapse involved administration of hyaluronidase alone at the dosage of 135 units recommended in the M&N scheme (first time for this patient according to the M&N scheme, and no other aspects of the M&N scheme were involved). For the second patient, resolution of symptoms of the original biofilm was observed after the sole use of a hyaluronidase dose according

Table 4 Description of participants treated with M&N scheme as first therapeutic option

Age (years)	Site of HA injection	HA volume (mL)	Nodule diameter (cm)	Time between HA injection and biofilm formation (months)	Site affected by biofilm	Relapse	HA injection after recovery	No biofilm relapse 2 years after recovery
48	Nasolabial folds, corners of mouth	1	≤2	6	Right corner of mouth	No	Yes	Yes
49	Tear troughs, nasolabial folds, lips	3	≤2	4	All areas treated	No	Yes	Yes
59	Tear troughs, cheeks, chin	6	≤2	2	Right cheek	No	Yes	Yes
31	Tear troughs	1	Orbital cavity	10	Right tear trough	No	Yes	Yes
37	Nasolabial folds, corners of mouth, lips	2	≤2	4	Right nasolabial fold	No	Yes	Yes

Notes: Comprehensive treatment in accordance with the M&N scheme: puncture of the lesion and drainage of pus and fermented HA. Orally administered combination antibiotic and probiotic therapy: 2×400 mg moxifloxacin + 2×500 mg clarithromycin + probiotic formulation consisting of 1.6 billion CFU lyophilized strains of *Lactobacillus acidophilus, L. delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium lactis*, three capsules a day. Duration of antibiotic therapy: 14–21 days or until the complete resolution of swelling and nodules (whichever is longer). Duration of probiotic treatment: during the antibiotic therapy and 1 month after its termination. Duration of locally administered hyaluronidase treatment: 14–21 days or until the complete resolution of swelling and nodules (whichever was longer). Hyaluronidase dosage: according to Table 2.
Abbreviations: M&N, Marusza and Netsvyetayeva; HA, hyaluronic acid; CFU, colony-forming unit.

Table 5 Description of participants successfully treated in accordance with the M&N scheme after unsuccessful treatment with other schemes

Age (years)	Site of HA injection	HA volume (mL)	Nodule diameter (cm)	Time between HA injection and biofilm formation (months)	Site affected by biofilm	First therapeutic option, antibiotics	First therapeutic option, hyaluronidase	Relapses, n
47	Nasolabial folds, corners of the mouth	1	≤2	1	Left nasolabial fold	Ciprofloxacin 2×500 mg, clarithromycin 500 mg, Trilac 3×1 caps per os; duration in accordance with M&N scheme	Yes, in accordance with M&N scheme	1
53	Tear troughs, nasolabial folds	2	Orbital cavity	2	Right tear trough	Ciprofloxacin 2×500 mg, clarithromycin 500 mg, Trilac 3×1 caps per os; duration in accordance with M&N scheme	Yes, in accordance with M&N scheme	1
38	Cheeks	2	≤2	2	Right cheek	Cefalexin 2×500 mg for 10 days per os, then doxycycline 100 mg for 10 days per os	No (patient refused)	–
68	Cheeks	2	≤2	1	All areas treated	Ciprofloxacin 2×500 mg, clarithromycin 500 mg, Trilac 3×1 caps per os, duration in accordance with M&N scheme	Yes, in accordance with M&N scheme	1
41	Tear troughs, nasolabial folds, eyebrow ridge	2	≤2	3	All areas treated	Ciprofloxacin 2×500 mg, clarithromycin 500 mg, Trilac 3×1 caps per os, duration in accordance with M&N scheme	Yes, in accordance with the M&N scheme	1
58	Cheeks	2	≤3	5	All areas treated	Ciprofloxacin 2×500 mg, clarithromycin 500 mg, Trilac 3×1 caps per os for 14 days. Then, during hospitalization: metronidazole 3×500 mg, amoxicillin–clavulanate 3×2.1 g IV, hydrocortisone IV 200 mg for 7 days	No (doctor did not apply scheme)	–
56	Nasolabial folds	1	≤2	2	All areas treated	Ciprofloxacin 2×500 mg, Clarithromycin 500 mg, Trilac 3×1 caps per os, duration in accordance with M&N scheme	Yes, in accordance with M&N scheme	4
39	Tear troughs, nasolabial folds	2	Orbital cavity	2	Left tear trough	Ciprofloxacin 2×500 mg, clarithromycin 500 mg, Trilac 3×1 caps per os, duration in accordance with M&N scheme	Yes, in accordance with the M&N scheme	1
62	Nasolabial folds, corners of the mouth	2	≤2	3	All areas treated	Cefalexin 4×500 mg, clindamycin 3×300 mg per os for 14 days, then incision and draining and gentamicin 3×80 mg IV for 5 days	No (doctor did not apply the scheme)	–
56	Cheeks	2	≤2	18	Right cheek	Clarithromycin 500 mg, amoxicillin–clavulanate 3×1 g, Trilac 3×1 caps per os	Yes, in accordance with M&N scheme	1
62	Nasolabial folds	2	≤2	2	All areas treated	Clindamycin 4×300 mg per os for 9 days, then metronidazole 2×500 mg per os, abscess incision from the side of the mouth, and drainage of the lesion thrice	No (doctor did not apply scheme)	–
30	Tear troughs, both cheeks, lips	4	Orbital cavity	4	Right tear trough	Clarithromycin 500 mg, Trilac 3×1 caps per os, duration in accordance with M&N scheme	Yes, in accordance with M&N scheme	1

Notes: Comprehensive treatment in accordance with the M&N scheme: puncture of the lesion and drainage of pus and fermented HA. Orally administered combination antibiotic and probiotic therapy: 2×400 mg moxifloxacin + 2×500 mg clarithromycin + probiotic formulation consisting of 1.6 billion CFU lyophilized strains of *Lactobacillus acidophilus, L. delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium lactis*, three capsules a day. Duration of antibiotic therapy: 14–21 days or until complete resolution of swelling and nodules (whichever is longer). Duration of probiotic treatment: during antibiotic therapy and 1 month after its termination. Duration of locally administered hyaluronidase treatment: 14–21 days or until complete resolution of swelling and nodules (whichever was longer). Hyaluronidase dosage: according to Table 2.
Abbreviation: M&N, Marusza and Netsvyetayeva; HA, hyaluronic acid; IV, intravenous.

No symptom resolution	Relapse, site affected by biofilm	Relapse, nodule diameter (cm)	Relapse, cause	Relapse/no symptom resolution, comprehensive treatment in accordance with the M&N scheme	Relapse/no symptom resolution, duration of treatment in accordance with the M&N scheme (days)	Relapse/no symptom resolution, treatment with hyaluronidase	HA injection after recovery	No biofilm relapse 2 years after recovery
–	Same area	≤2	Administering HA	Yes	14	Yes	No	Yes
–	Same area	Orbital cavity	Administering HA	Yes	14	Yes	Yes	Yes
Yes: infected HA not completely removed (nodule 5 mm in diameter remained)	–	–	–	Yes	21	No	Yes	Yes
–	New area: over the right eyebrow and the right cheek	<1 cm	Administering HA in the eyebrow area, new site	Yes	21	Yes	Yes	Yes
–	Same area	≤2	Administering HA	Yes	21	Yes	Yes	Yes
Yes	–	–	–	Yes	21	Yes	Yes	Yes
–	Same area	≤2	Administering HA, each time	Yes, as well as tooth extraction and treatment of both infected mandibular sinuses	21	Yes	No	Yes
–	Same area	Orbital cavity	Spontaneous	Yes	14	Yes	Yes	Yes
Yes	–	–	–	Yes	14	Yes	No	Yes
–	Same area	≤2	Spontaneous	Yes	14	Yes	Yes	Yes
Yes	–	–	–	Yes	21	Yes	No	Yes
–	New area: tear trough under the left eye	Orbital cavity	Spontaneous	Yes	14	Yes	Yes	Yes

Table 6 Participants (subgroup 3) successfully treated by application of schemes other than M&N, description of initial treatment

Age years	Site of HA injection	HA Volume (mL)	Nodule diameter (cm)	Time between HA injection and biofilm formation (months)	Site affected by biofilm	First therapeutic option, antibiotics	First therapeutic option, hyaluronidase	Relapses, n
45	Lower face	1	≤1	1	Corners of the mouth	No	Immediate administration of 40 units of hyaluronidase	1
35	Wrinkles between eyebrows	1	≤1	1	Near right eyebrow, close to nose	No	In accordance with M&N scheme	–
44	Forehead above the left eyebrow	1	≤1	1	Forehead above the left eyebrow	Extended antibiotic therapy involving several doctors over 3 months. The patient had been treated with the following medications among others the patient did not remember: gentamicin 3x80 mg IM, ceftriaxone 2 g IV	No	–
36	Both tear trough valleys	3	Both orbital cavities	1	Both tear trough valleys	Clarithromycin 1x500 mg, Trilac 3x1 cap, duration in accordance with M&N Scheme	In accordance with M&N Scheme	–
47	Chin	3	≤2	4	Chin	Clarithromycin 1x500 mg, Trilac 3x1 cap, duration in accordance with M&N Scheme	In accordance with M&N Scheme	–

Notes: Comprehensive treatment in accordance with M&N scheme: puncture of the lesion and drainage of pus and fermented HA. Orally administered combination antibiotic and probiotic therapy: 2x400 mg moxifloxacin + 2x500 mg clarithromycin + probiotic formulation consisting of 1.6 billion CFU lyophilized strains of *Lactobacillus acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium lactis*, three capsules a day. Duration of antibiotic therapy: 14–21 days or until complete resolution of swelling and nodules (whichever was longer). Duration of probiotic treatment: during antibiotic therapy and 1 month after its termination. Duration of locally administered hyaluronidase treatment: 14–21 days or until complete resolution of swelling and nodules (whichever was longer). Hyaluronidase dosage according to Table 2.
Abbreviations: HA, hyaluronic acid; IM, intramuscular; IV, intravenous; M&N, Marusza and Netsvyetayeva.

Table 7 Participants (subgroup 3) successfully treated by application of schemes other than M&N, description of relapses and recovery

Age years	No symptom resolution	Relapse, site affected biofilm	Relapse, nodule diameter (cm)	Relapse, cause	Relapse/no symptom resolution, comprehensive treatment in accordance with M&N Scheme	Relapse/no symptom resolution, treatment duration in accordance with M&N Scheme (days)	Relapse/ no symptom resolution, treatment with hyaluronidase	HA injection after recovery	No biofilm relapse 2 years after recovery
45	–	Same area	≤2	Administering cross-linked HA gel	Hyaluronidase alone	No antibiotics applied	Hyaluronidase alone in accordance with M&N scheme, (Table 2)	Yes	Yes
35	–	–	–	–	–	–	–	No	Yes
44	Yes	–	–	–	No, lesion removed with laser, leaving scar tissue	No antibiotics applied	No Hyaluronidase applied	No	Yes
36	–	–	–	–	–	–	–	Yes	Yes
47	–	–	–	–	–	–	–	No	Yes

Notes: Comprehensive treatment in accordance with M&N scheme: puncture of the lesion and drainage of pus and fermented HA. Orally administered combination antibiotic and probiotic therapy: 2x400 mg moxifloxacin + 2x500 mg clarithromycin + probiotic formulation consisting of 1.6 billion CFU lyophilized strains of *Lactobacillus acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *Bifidobacterium lactis*, three capsules a day. Duration of antibiotic therapy: 14–21 days or until complete resolution of swelling and nodules (whichever was longer). Duration of probiotic treatment: during antibiotic therapy and 1 month after its termination. Duration of locally administered hyaluronidase treatment: 14–21 days or until complete resolution of swelling and nodules (whichever was longer). Hyaluronidase dosage according to Table 2.
Abbreviations: HA, hyaluronic acid; M&N, Marusza and Netsvyetayeva.

to the M&N scheme for 28 days (no other aspects of the M&N scheme were involved). For the third patient, resolution of symptoms of the original biofilm was achieved after laser surgery (the initial lesion was removed with laser, leaving scar tissue). For the final two patients, resolution of symptoms of the original biofilm was observed after the application of a modified M&N scheme, with clarithromycin monotherapy as the only deviation.

Statistical analysis

For subgroups 1 and 2, there were no significant differences in the median age (χ^2=–0.90, P=0.37), median volume of HA administered (χ^2=0.10, P=0.92), or median duration between cross-linked HA administration and LBI symptoms (χ^2=1.58, P=0.11). For subgroups 1 and 3, there were no significant differences in median age (χ^2=0.83, P=0.40) or median volume of HA administered (χ^2=0.42, P=0.68). However, there was a significant difference in median duration between cross-linked HA administration and LBI symptoms (χ^2=2.09, P=0.04), with subgroup 1 having a longer median duration than subgroup 3.

When comparing the group of 17 patients cured using the M&N scheme with the group of five patients cured with other schemes (subgroup 3), there were no significant differences in median age (χ^2=1.52, P=0.12) or median volume of HA administered (χ^2=0.54, P=0.58). However, there was a significant difference in median duration between cross-linked HA administration and LBI symptoms (χ^2=2.07, P=0.03), with the group of 17 patients having a longer median than subgroup 3.

Discussion

When developing the scheme for treating LBI complications at sites where cross-linked HA fillers were administered, we assumed that the complications were related to bacterial growth in the form of biofilm on the surface of the filler.[3] We also adopted the view that cross-linked HA introduced into facial soft tissue constituted a foreign body. As such, we used a comprehensive compound approach for the development of the treatment scheme. We modeled this approach on principles used in other medical disciplines regarding foreign-body implantation-related infections and bacterial growth in the form of biofilm. These principles are: first, draining and removal of necrobiotic tissues; second, complete removal of the foreign body on which the biofilm has formed; and third, empirical antibiotic therapy in the form of combination therapy for an extended period. As a result of this approach, development of antibiotic resistance can be avoided and the entire spectrum of bacteria that could be etiological agents can be accounted for. Unless the entire infection is eradicated, biofilm microbes may survive and cause a relapse once antibiotic treatment is terminated.[9] This occurred in some of the patients in our study: ineffective treatment and spontaneous relapses occurred in each subgroup when any lesions had been left in soft facial tissue.

In compliance with the first rule, drainage of pus along with the bacteria-contaminated and fermented HA filler should be performed. We suggest draining the lesions as the first procedural stage, twice a week until complete resolution. The second rule is to remove the foreign body completely, ie, the basis upon which the biofilm has formed. The intended effect can be accomplished by application of hyaluronidase directly onto the lesion. This is the method utilized by the majority of practitioners of aesthetic medicine. According to other study authors, the recommended dosage ranges between 1.5 and 75 units and is dependent on the specific complication site.[15–17] Successful treatment relies on the complete dissolution of nodules. Based on our practical experience, the volume associated with each hyaluronidase administration, which is conducted twice a week, should be proportional to the size of the lesion (assessed based on its diameter, measured between the opposite rims that are farthest apart) and ranges from 6 to 195 units per administration. The estimated duration of therapy should be adequate for the intended effect, and no shorter than 14 days.

The third rule relates to the use of combination antibiotic therapy of proven efficacy against the type of bacteria forming the biofilm. Given this, we sought efficacious empirical therapeutic options with the broadest possible bactericidal spectra, as the etiological agents of infection could not be determined for any of the participants. The antimicrobial medication regimen needed to be efficacious against both Gram-positive and Gram-negative aerobic bacteria, as well as against anaerobes. In addition, medications had to be orally administrable and certified for the treatment of skin and subcutaneous tissue infections.

According to the relevant sources, these criteria are met by moxifloxacin and clarithromycin.[18–27] Moxifloxacin is a chemotherapeutic agent belonging to the fluoroquinolone group. It differs from ciprofloxacin in terms of its in vitro effectiveness against Gram-positive aerobic bacteria, strong bactericidal properties against Gram-negative aerobic bacteria, and simultaneous effectiveness against anaerobes. It also affects atypical mycobacteria related

to dermatological infections. In addition, it penetrates the biofilm environment well and exhibits its bactericidal properties within the biofilm. The effectiveness of moxifloxacin monotherapy has also been proven against biofilm formed by staphylococci.[18–21] Moxifloxacin can also enter infected vesicles, muscles, and subcutaneous fatty tissue. Therefore, it is a reasonable option for treating skin and skin-structure infections.[22]

Clarithromycin is a semisynthetic macrolide antibiotic with bactericidal properties against Gram-positive and atypical bacteria. In addition, it has the unique property of destroying biofilm structures irrespective of the type of bacteria comprising them.[23–27] Many authors have recommended eradicating biofilm using combination therapy in which clarithromycin is the second antibiotic, as it can increase the effectiveness of the first antibiotic.[23–27] In cases of biofilm formed by methicillin-resistant *Staphylococcus aureus*, clarithromycin acts synergistically with daptomycin to remove the biofilm from titanium implants, which cannot be achieved with daptomycin monotherapy.[23] Fujimura et al also described the destruction of *S. aureus* biofilm on titanium implants using combination therapy involving clarithromycin with cefazolin or vancomycin.[24] Gander et al indicated that a combination of clarithromycin with moxifloxacin considerably increased the destructive impact on staphylococci biofilm in an in vitro model.[25] Lastly, the combination of ciprofloxacin and clarithromycin displays considerable synergy in the destruction of *Pseudomonas aeruginosa* biofilm in rat mucosal tissue.[26,27]

The duration of the antibiotic therapy should be related to the clinical response and last at least 7 days after the complete resolution of symptoms and disappearance of palpable lesions in the soft tissue. Subsequent remedial administration of cross-linked HA after the resolution of symptoms should occur no earlier than 2 months after successful treatment.

The introduction of probiotics into the scheme was justified by the results of our previous study, which showed that treatment with physiological skin flora decreased the risk of LBI after cross-linked HA administration.[28] We are aware that our choice of probiotics was not perfect for ensuring an appropriate composition of the physiological skin flora during and after empirical broad-spectrum antibiotic usage. However, the formulation that we used is the only probiotic registered in Poland as a medication, rather than as a mere supplement. We have only recently begun a search for specific probiotic formulations adjusted for the location and type of infection, as well as the antibiotic therapy used.

By adhering to this therapeutic approach, we achieved satisfactory efficacy in treating LBI after cross-linked HA administration. One exception occurred in a case of one patient of 17, who had four relapses following each attempt at remedial administration of cross-linked HA, despite rigorous application of the M&N scheme. This was related to a concurrent uncured periodontium infection and to odontogenic sinusitis. As such, we wish to stress the importance of appropriate establishment of eligibility before treatment.[29] It is essential always to diagnose and eradicate any other infections before administering cross-linked HA, in line with the presurgery rules used before cardiology and orthopedic surgeries involving foreign-body implantation. This minimizes the risk of complications from aesthetic medical procedures, including biofilm-related LBIs at sites where cross-linked HA has been administered.

An interesting observation was made during our study regarding the significant difference in the median duration from cross-linked HA administration to LBI-symptom development between the 17-patient group cured using the M&N scheme and the five-patient group cured using other schemes (subgroup 3). For the former group, the mean duration was 4.2 (median 3.0, SD 4.2) months, while for the latter group it was 1.6 months (median 1.0, SD 1.3). This suggests that quickly growing bacteria (which generate symptoms in a shorter time frame) are easier to eradicate. Nevertheless, due to the insufficient size of the five-patient subgroup 3 and our lack of knowledge about the microbial species that caused the infections, we are unable to make a more informed comment regarding this phenomenon, and it requires further study.

In conclusion, it is worth underscoring that the recommended scheme is designed for empirical treatment of LBI complications related to bacteria in a biofilm. These complications manifest >4 weeks after the procedure, the etiological microbes are unidentifiable using standard microbiological methods, and their original source is rarely identified. The etiology of LBI complications is the subject of our future research. It is suggested, but with the caveat of a lack of rigorous evidence, that etiological microbes have low virulence, unlike those causing early bacterial complications. Early bacterial complications, which are usually caused by *S. aureus* or *Streptococcus pyogenes*, are severe and thus require other treatment schemes. Their occurrence is directly related to inappropriately performed skin disinfection at the HA-administration site or to infringements of other rules related to aseptic and antiseptic methods. It is essential to follow these rules carefully with respect to all procedures that involve compromising skin integrity.

Conclusion

In LBI therapy for a site of cross-linked HA administration, the principles used in other medical disciplines regarding for-

eign-body implantation-related infections must be observed. The therapy ought to be of sufficient duration for symptoms to resolve without relapse in the following 2-month period. This ensures that cross-linked HA can be administered safely as part of remedial therapy after treatment of LBI. The M&N scheme is recommended as the first therapeutic option for treating LBI complications related to soft-tissue fillers.

Ethics approval and consent to participate

All participants provided written informed consent. The research was approved by the Ethics Committee at the Department of Microbiology of Warsaw Medical University. This study was conducted in accordance with the Declaration of Helsinki.

Consent for publication

The authors hereby certify that all work contained in this article is original. All authors contributed to this research, claim full responsibility for the contents of this article, and have read and approved the final manuscript.

Availability of data and material

All available data are presented in the tables.

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Author contributions

All authors contributed toward data analysis, drafting and critically revising the paper, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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