Maggot debridement therapy: Utility in chronic wounds and a perspective beyond

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ABSTRACT

Complementary medicinal techniques have a wide history, but the recent focus of modern medicine has been on possible effect mechanisms. Chronic wounds are not only a problem to their patients, but also a burden on the healthcare system due to their extensive costs.

Among complementary therapies, maggot debridement therapy (MDT) is by far the most widely studied method in the field of wound prevention and management. MDT is a very effective treatment technique for chronic wounds and, in addition, is a cheap and easy-to-use method, with only minor, rare adverse effects. Many studies have attempted to clarify the actual effect mechanisms of MDT. MDT works in four ways: debridement, antimicrobial effects, wound healing, and biofilm degradation. These actions are mainly achieved by protein substances exhibiting very variable molecular masses and actions.

MDT has a certain positive effect on chronic wounds and is a strong candidate to maintain a bold presence in the multidisciplinary approach to chronic wound care. Its cost, application simplicity, minimal side effects, and easy-accessibility are major superiorities among other wound-care methods.

Keywords: Lucilia sericata*, chronic wound care, larval debridement, biosurgery.*

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INTRODUCTION

Complementary medicinal techniques used to be controversial applications for medical professionals. Recently, however, multiple studies indicating the probable benefits of these techniques to medicine have widely changed this opinion in a positive way[1-3]. One of these methods, maggot debridement therapy (MDT; also called larval therapy or biosurgery), is the most widely studied complementary medicinal technique in the field of wound prevention and management, and has been included in routine medical applications in many countries worldwide[4]. MDT has a bolder presence among these techniques, as it has been evaluated by many scientific research studies, and its medicinal effects have been observed[4-7]. The mechanisms of MDT action have not been totally examined, but it seems there is a combined and bound mechanistic circle influenced by the maggot itself, patient immunity, wound type, and the infective microorganisms.

Although MDT has recently been accepted worldwide, its usage has an extensive history. While some reports indicate the usage of MDT before 1900, most medical studies using MDT have been completed in the 1900s. As chronic wounds have become more relevant, cheap, effective, and easily applicable methods have been under investigation. To this effect, many studies on MDT have been published[5-9].

Many larval types have been investigated for MDT, but *Lucilia sericata* larvae are the most widely studied and used maggots. The application procedure (free-range, biobag) may slightly change the strength of activity, and it is chosen depending on clinical conditions, wound aetiology, and physicians' opinion. The effects of MDT on venous stasis ulcers, pressure wounds, neuropathic ulcers (diabetic foot ulcers), traumatic, and post-surgical non-healing wounds have been previously investigated, and results were very promising[4,10,11].

Although many researchers have particularly focused on effect mechanisms, there is still a long way to go to total understanding. The effect mechanism of MDT can be divided into four main titles: debridement, antimicrobial effects, wound healing, and biofilm degradation effect[4,10]. These titles are tightly related, and readers should keep in mind that these mechanisms are like a chain reaction, and cannot be evaluated separately. We, herein, review MDT focusing on application, effect mechanisms and clinical indications.

CHRONIC WOUNDS AND WOUND DRESSINGS

Chronic wounds are practically defined as wounds that show an unhealed condition in three months' time. Many aetiologies cause chronic wounds, and they are usually classified into four groups: venous stasis ulcers, ischaemic wounds, diabetic foot ulcers, and pressure injuries (ulcers)[12].

In Western countries, nearly 1% of the population has a chronic wound, and chronic wound care is a serious economic burden on the healthcare system[12]. In the cost modelling of Graves *et al.*[13], it was reported that pressure injuries, diabetic foot ulcers, venous ulcers and artery insufficiency ulcers caused a total annual expense of approximately US$1.65 billion, US$250 million, US$803 million and US$140 million, respectively, with a total amount of US$2.85 billion just in Australia. In the USA alone, US$6–15 million are spent on chronic wound care annually, while chronic wounds sum 2–3% of the total health expenses in the UK and 2% of the total health expenses in Australia[13-15]. Similar rates can be observed in the European Union (2%) and Scandinavian countries (2–4%)[13]. In addition, 50% of patients with a chronic wound that is not treated properly over a one-year period face serious mortality and morbidity[16]. Furthermore, due to various aetiologies, wound sizes, different treatment protocols and patient population heterogeneity, it is believed these numbers may be underestimated.

A normal inflammatory process ends with cleansing from infectious agents, and total repair of the injured area. In some conditions, however, the immune reaction somehow continues, and the wound becomes persistent. Although the exact mechanisms have not yet been clarified, persistent infection, hypersensitive reactions, long-term exposure to toxic or foreign bodies, and autoimmunity are potential reasons. Studies indicate that malfunctioning immunologic mechanisms, including cytokines and cell stimulants, cause a non-healing wound[17-19].

The incidence of foot ulcers is rising. Although treatment success rates are promising, relapse rates are very high. Correlating with the rising incidence of diabetes mellitus, diabetic foot ulcers have become a common and important issue that may even lead to amputation. Pressure injuries are another chronic wound type, most prominently observed in long-term hospitalised patients. Studies indicate that in the UK alone, 4% of health expenses are incurred due to pressure injuries[15]. Of note, these wounds are observed usually in high-risk populations such as disabled and/or elderly persons who are often characterised with major co-morbidities (for example, angiopathic, renal and hepatic problems), and nutritional issues, causing a problematic immune status[20-22]. This condition may result with seriously infected ulcers and also exposure to healthcare-associated or nosocomial infectious agents makes the wound "untreatable" due to probable antimicrobial resistance. To date, *Staphylococcus aureus*, coagulase negative staphylococci (CoNS), *Enterococcus faecalis*, *Proteus* species, anaerobic bacteria, and *Pseudomonas aeruginosa* have the highest isolation rates from chronic wounds[12]. It must be noted that there is great variability between infectious agents[15,23].

Biofilm formation created by pathogen agents is another issue of chronic wounds. It causes antimicrobial resistance, provokes or aggravates chronic inflammation, prevents healing, and eventually causes treatment failure, or the necessity for long-term, troublesome, and expensive treatments[12]. Biofilm occurs by the aggregation of microorganisms creating a complex multicellular community, resulting in a continuous chain of hyperinflammation, pathogenic invasion of vascular areas, increased capillary permeability and release of the intravascular contents. In addition, biofilm infections are often polymicrobial, which is also another problem to eradicate infection. More than 14 million biofilm infections occur with a mortality of 350,000 individuals, annually[24,25] and biofilm is formed in over 60% of chronic wounds[26].

There are many types of wound dressings with different specialties designed for various wound types. These dressings include film, hydrogel, foam, hydrocolloid, alginate, hydrofibre, antimicrobial dressings, biologic dressings (MDT), apitherapeutic (honey) products, and tissue engineering products. A good dressing should be easily applicable, aesthetically pleasing, non-painful, cheap, non-allergic, non-toxic, non-traumatising, exude-absorbent, capable of protecting against moisture and warmth, capable of gas transport, preventive of contamination, and capable of necrotic tissue debridement. Aetiologic reason, wound type, and infection status are the main factors considered when choosing the right wound dressing[14,15,23,27].

Biofilm formation results in a limitation of choices, both for treatment strategies and wound dressings. Guidelines agree on the treatment of biofilms as part of a multi-therapeutic design. Debridement is the major approach, but creating a moisture balance, managing host factors (blood sugar regulation, nutrition, et cetera), eliminating the infectious agents and preventing biofilm re-formation by using topical antiseptics, are all crucial. Debridement alone cannot remove all biofilm and there is only a limited time period before biofilm re-formation occurs. In addition, systemic antimicrobials are usually not effective to degrade biofilms[28]. So, in the treatment of biofilms it is critically important to apply a method(s) that provides strong — easily repeatable — debridement, antimicrobial activity that results in suppressing hyperinflammation.

MAGGOTS

For use in MDT, a maggot should facilitate debridement by removing necrotic tissue (not living tissue), show antimicrobial and antibiofilm activity, break the immunity chain of the chronic wound, and stimulate wound healing[4]. Many species were investigated for their ability to achieve these goals. Of these, *Lucilia sericata* larvae have been extensively evaluated and found to be effective[4,10]. *Lucilia cuprina* was also reported, especially for diabetic wounds[29]. Many other species such as *Calliphora vicina*, *Calliphora vomitoria*, *Phormia regina*, *Chrysomya albiceps*, *Sarcophaga carnaria*, and *Hermetia illucens* were indicated to have potential for the same use[30].

The genus *Lucilia* (Diptera: Calliphoridae) consists of over 160 species that have great importance in forensic entomology[31,32]. *Lucilia sericata* (green bottle fly) is common worldwide, but especially in tropical areas, and has a considerable ability to sense carrion from miles away. In a lifetime, a female maggot leaves nearly 200 eggs that quickly grow to their adult forms by passing through various instars and pupal phases. Maggots (larvae) have a complex body form with 12 segments, dark and light sensors, and secretory glands. As they are "dead eaters", their glands secrete strong proteolytic enzymes, and they can digest nearly half of their body weight in just five minutes. Interestingly, they have a considerable capability to protect themselves from infectious agents, not just in the instar phases, but also in the pupal phase (auto disinfection)[4,10].

MAGGOT DEBRIDEMENT THERAPY

Although scientific reports detailing MDT have mostly been published after World War I, MDT has been practised for many years prior to this date[33,34]. In the 1940s, interest in MDT dropped, but it was later revived in the 1990s[5-9]. The effects of MDT on primarily venous stasis ulcers, pressure injuries, neuropathic ulcers (diabetic foot ulcers), traumatic and post-surgical non-healing wounds, burns, arterial ulcers, Buerger disease, cellulitis, mastoiditis, lymphostasis, osteomyelitis and necrotic tumours have been investigated, and the results were very promising[4]. Many studies have been published to date, and recently, MDT has been accorded an important role in chronic wound care in many countries[35-41].

The species most widely used for this purpose is *Lucilia sericata* larvae[4,10]. Facilities routinely applying MDT produce *L. sericata* in an artificial climate in light/dark condition rooms. At the Instar 2 and 3 stages, maggots are sterilised with various solutions to avoid potential sepsis[36,42-47]. After contamination controls, the maggots are ready to be applied.

MDT can be applied in two forms: "free-range, confinement dressing, cage dressing" or "containment dressing, biobag". These two methods have their respective advantages. For wounds with deep necrotic tissue, free-range dressing is recommended owing to a higher efficiency debridement and a shorter period for application. However, because of patient discomfort, complaints (pain, disturbance), and potential rejection for application, many professionals choose to apply biobags[4,38,48]. A few publications indicate the possibility of bleeding due to free-range dressings, but this remains controversial. Maggot movement on the wound, proteolytic reactions, and fibrinolysis may explain this phenomenon. As such, further studies should be performed in this regard[49,50].

A study investigating MDT from a practitioners' perspective revealed no difference between these two methods[48]. Furthermore, biobag application may lead to patient cooperation and willingness that may result in increased application numbers[4,38,48]. Although this application may result in higher financial expenses, overall, MDT seems to be much cheaper than conventional wound dressing methods[51]. Evidently, it is too hard to interpret the current data with certainty, and it is recommended that healthcare facilities evaluate the data on an individual basis.

The most problematic issue for MDT is patient acceptance. A few studies have reported that patients may experience pain or report disturbance (restless feelings other than pain)[4], but we know these factors are strongly variable. Patient acceptance and pain levels may also vary due to wound type. Steenworde *et al.*[52] reported that these patient cooperation issues did not affect the efficiency of MDT unless the patient stopped therapy. They also stated that "acceptance and willingness" are good ways to avoid cooperation issues.

Several contraindications to MDT such as coagulopathies, allergies to larvae/larval secretions, haemorrhagic abscess, and progressive necrotising wounds were reported. In addition, the respiratory system, head area, fistulae to vital organs, endocrine glands, internal organs and open abdominal injuries are not applicable locations for MDT[4,53].

While there are many studies to highlight the effect mechanisms of MDT, a huge darkness still remains. The mechanisms, to date, can be divided into four titles to facilitate understanding:

Debridement

The first rule of larval debridement is that the larvae are to be fed necrotic tissue, not living tissue. *Lucilia sericata* larvae employ physical and chemical mechanisms to selectively feed on necrotic tissue. It was found that a maggot can break down 25 mg of necrotic tissue in just 24 hours[54]. Mechanical debridement is achieved by scraping the wound area[55,56]. In fact, maggots search for their food, providing another therapeutic advantage. Even in the deepest areas of the wound, maggots eat necrotic tissue, especially when used in free-range dressing[4,10,38]. The chemical mechanism of debridement is achieved by proteolytic enzymes that are secreted in the digestive system of the maggot. These enzymes also play a key role in the antimicrobial effects of MDT[57,58]. In a recent study, a chymotrypsin-like serine protease, identified from *L. sericata,* affects clotting mechanisms by breaking down extracellular matrix proteins

(fibronectin, laminin and collagen IV), indicating chemical debridement[59].

Antimicrobial effect

Maggots exert their antibacterial effects by both "eating" and their bactericidal activity of excretion and secretion (ES). Mumcuoglu[38] described the mechanical eating activity in 2001. In an academic thesis, Dogandemir[60] investigated both sterile and patient-applied whole-body fluids (WBF) via the microdilution method, and found a greater antibacterial effect exerted by gram-positive bacteria than by gram-negative bacteria. It was also noted that there was no effect on *Pseudomonas aeruginosa* and *Candida albicans*. On the contrary, Margolin *et al.*[61] reported antifungal activity, but the study was performed with living maggots, not WBFs.

Bexfield *et al.*[42,43], Barnes *et al.*[36], and Huberman *et al.*[44,45] studied ES and haemolymph fluids, and found many antibacterial proteins of various molecular masses. Teh *et al.*[62] studied the antibacterial effects and compounds of larval extracts, and found fatty acids that may have an inhibitory effect on major bacterial growth. Huberman *et al.*[45] and Kerridge *et al.*[63] also stated that secretory proteins and their molecular masses vary according to bacterial exposure. They noted that after exposure, early secretory proteins have low molecular masses while late ones have high masses.

Chernysh *et al.*[64] identified the protein diptericin (8882 and 9025 Da) in maggot ES, and Kruglikova and Chernysh[46] identified new protein molecules with molecular masses of 1014–9025 Da and 174–904 Da. Antibacterial proteins later identified were lucifensin[65,66] and lucifensin II[67]. Interestingly, lucifensin has recently been investigated for its role against biofilm formation, but further research is still required[57].

Andersen *et al.*[35] studied antibacterial proteins in maggot ES and their sequences via transposon assisted signal trapping (TAST) technique, and compared their findings using The Basic Local Alignment Search Tool (BLAST) system. Proteins showed 28–91% homology with lectin, defensin, attacin, and kitin binding proteins. They also tested the antimicrobial activity of lucifensin, and found that it had potent activity on gram-positive bacteria, but was less effective against gram-negative bacteria and fungi. Recently, Tellez *et al.*[68] identified a new protein named Lucilin, a cecropin-like peptide from *Lucilia eximia*, which shows antimicrobial activity especially against gram-negative bacteria and immunomodulatory activity.

In 2014, Valachova *et al.*[57] revealed the presence in maggot ES of phenyl metalloproteinase, signal peptide protease, three different proteases, and chymotrypsin secretions, stated their amplicons, and, by using BLAST, determined their amino acid sequence homologies with other proteins. In another study, they evaluated larval midgut lysozymes according to molecular mass, amplicons, and antibacterial effects[58].

Pöppel *et al.*[69] detected 47 genetic locations from *L. sericata,* and produced 23 synthetic proteins with antifungal activity, including cecropin, cecropin-like, proline rich, stomoxyn, and defensin. Of particular importance are "elevated during infection (edin)" proteins, whose synthesis is upregulated during exposure to infectious agents. Another interesting finding is the observed synergistic and additive effects between proteins. In addition, they noted that these antimicrobial peptides (AMPs) were synthesised in many body parts of the maggot.

Erdmann *et al.*[70] and Greenberg *et al.*[71] reported that both phenylacetic acid and phenylacetaldehyde produced by commensal *Proteus mirabilis* in the gastrointestinal system of maggots have antibacterial activity. The same mechanism also led to a bacteriostatic effect by wound surface alkalisation[72]. This alkalising effect not only results in bacteriostatic activity, but also creates an optimal environment for antimicrobial enzymes[73].

Pöppel *et al.*[74] isolated a novel protein molecule, lucimycin, which showed homology with lucifensin. Despite this homology, lucimycin, unlike lucifensin, showed antifungal activity. Although these researchers studied a limited number of fungi, they observed inhibitory effects on spore germination and mycelial growth for particular species (*Cladosporium herbarium, Alternaria alternata, Lichtheimia corymbifera, Mucor circinelloides, Candida albicans,* and *Trichosporon asahii*). In contrast, no antifungal activity was observed against some species (*Rhizopus oryzae, Aspergillus fumigatus, Aspergillus terreus, Arthoderma benhamiae*).

Another study, published by Polat *et al.*[75], led to a new perspective on MDT. They investigated, *in vivo,* the anti-leishmanial effects of *L. sericata* ES on *Leishmania tropica* infected mice. They reported that fresh and pure ES significantly advanced lesion healing, but this anti-leishmanial effect could not be observed on promastigotes from culture. They suggested that quick oxidation of ES might be the reason for this discrepancy. Daeschlein *et al.*[76] reported that the antibacterial activity of ES against *E. coli* and *S. aureus* decreased over time, which supports this quick destabilisation theory. Sanei-Dehkordi *et al.*[77] supported this data in a study on *Leishmania major*. They found that treatment with *L. sericata* ES led to a considerably lower number of amastigote-infected macrophages in a test group than that in the control group *in vitro,* and observed significant changes in lesion size, indicating that ES inhibits leishmanial virulence.

The common perception is that maggots are more effective against gram-positive bacteria, and that the antibacterial effects on *Pseudomonas* and *Acinetobacter* species are somehow more limited than on other species[35,78,79]. Antifungal and anti-leishmanial effects have also been observed[74,75], but must still be supported by further investigation.

Effects on wound healing

Several studies have reported that MDT promotes wound healing[80-83]. As previously stated, this may be due to both the cleansing of necrotic tissue and antimicrobial effects. However, many researchers reported direct activities of larvae on wound healing. Van der Plas *et al*.[84] studied the cellular effects of *L. sericata* ES, and found that it has inhibitory effects on the proinflammatory immune response (which is a persistent problem in chronic inflammation) without any negative impact on the antimicrobial effects of neutrophils. In addition, decreases in the level of elastase secretion, neutrophil chemotaxis, and hydrogen peroxide generation were observed in a dose-dependent fashion. In contrast, neutrophil chemotaxis and antimicrobial effects were not affected. Another study reported that monocyte proinflammatory and cytokine responses were considerably affected by ES[85]. Tamura *et al.*[86] reported that ES had inhibitory effects on the complement system, resulting in the suppression of persistent inflammation without any negative impact on neutrophil antimicrobial action. It has also been found that proteolytic ES activity has a positive impact on extracellular matrix mechanisms, which also play a role in wound healing[87]. Baumann *et al.*[88] uncovered urate oxidase and allantoin mechanisms in *L.sericata* larvae that regulate pH levels in the wound. This is also needed to ensure the potency of antimicrobial effects and the enzymatic wound healing processes.

Neoangiogenesis is an important part of wound healing, and beneficial effects of *L. sericata* ES on neoangiogenesis have been reported[89]. In contrast, Singorenko *et al.*[90] reported that ES had no direct effect on wound healing. Although they demonstrated transcriptional changes, no positive impact on cell viability, proliferation, migration, or angiogenesis was observed. However, they revealed significant effects on the immune response, which may indirectly modulate wound healing. A recent study has, however, reported that MDT stimulates endothelial cell proliferation and neoangiogenesis in diabetic foot wounds[91]. Zhang *et al.*[92] reported strong evidence that neoangiogenesis was actually stimulated by MDT. They demonstrated a remarkable increase in miR-126 expression (an mRNA that stimulates neoangiogenesis) in MDT-treated patients with diabetic foot ulcers, and confirmed these findings by observing increased miR-126 expression *in vitro* in human umbilical vein endothelial cells. Methodological difference could be a factor in these conflicting results. In addition, wound type and aetiology may have an impact on MDT effects.

Biofilm degradation effect

Biofilm formation is a serious problem that causes antimicrobial resistance, added medical expenses, and additional long-term treatment[93]. Cazander *et al.* published two different studies[37,94], indicating that ES showed a degrading effect on *S. aureus, S. epidermidis, K. oxytoca, E. faecalis, E. cloacae,* and *P. aeruginosa* biofilms on polyethylene, titanium, and surgical steel surfaces. Van der Plas *et al.*[95] found that ES broke down *S. aureus* biofilm without any additional antibiotic treatment. They also noted that linezolid and clindamycin could not degrade biofilm in the first 24 hours.

Pseudomonas aeruginosa causes problems, especially in intensive care units, due to both its ability to create biofilms, and its multidrug resistance[12]. Although the anti-pseudomonal effect of MDT is currently controversial, Cazander *et al.*[37,94] observed a degrading effect of ES on *P. aeruginosa* biofilms, and Pöppel *et al.*[69] indicated genetic markers for antimicrobial effects against *P.aeruginosa*. Brown *et al.*[96] identified DNAase-1 in *L. sericata* ES, which had a destructive effect on *P. aeruginosa* biofilms.

FUTURE PERSPECTIVE

MDT itself is in use of clinical practice; however, researchers can be divided into two sections about this subject: i) those who practise MDT in their studies in favour of clinical perspective; and ii) those who perform studies in a molecular and proteomic vision. These two “cults” actually walk on different paths to the same target. Although MDT has a great scientific value in clinical practice due to serial studies reported worldwide, only a few researchers focused at the molecular and protein levels. Recently, several studies published have especially chosen the subject area of specific molecules isolated from medicinal maggots and their potential beneficial effects as antimicrobials, wound healers, and so on. Recent studies of Pöppel *et al.*[59,69] showed recombinant technology can be a tool for gaining particular molecules singularly to use for medicinal purposes, which also opened a path to transgenic molecules[97]. In another study, Gordya *et al.*[98] reported anti-biofilm activity of *Calliphora vicina* AMPs by significantly destroying the matrix and eliminating the bacteria. These data may lead medical societies to produce new wound dressings containing components such as these particular AMPs and transgenic molecules for especially the occasions such as biofilms, where the clinicians have limited options. Despite it being hard to obtain high amounts of purified proteins directly from maggots, thanks to recombinant science, these molecules are still strong candidates for future drug researches[99].

CONCLUSIONS

As a complementary medicinal technique, MDT stands as a “scientifically proven” method. Although there are still unclarified issues, the use of MDT is undisputed. Chronic wounds are a common problem and MDT acts as another therapeutic option for patients.

Antibacterial activity against gram-positive bacteria is clearly observed, but effects on other particular bacteria (*Acinetobacter* spp and *Pseudomonas aeruginosa*) and fungi remain still controversial[35,78,79]. Previously stated studies indicate many mechanisms and molecules in action, but it seems these are only single drops in a vast ocean. Recently, Franta *et al.*[100] found many secreted enzymes of *L. sericata*

that have the potential to play a role in several effect mechanisms. It should be noted that these mechanisms and molecules do not just work alone, but work synergistically. In addition, it seems there is a "provocation and adaptation" mechanism in which peptides are selectively secreted, depending on pathogen exposure or peptide type (molecular mass and activity), and the maggot somehow "adapts" to what it encounters. Some studies indicate that the exact antimicrobial effect comes forward in the situation of this so-called "provocation and adaptation," and observation of the effects on these controversial species may be achieved in this period. In addition, transgenic maggots may be more cost-effective and improve patient outcomes by secreting particular wound-healing peptides[97]. Further investigations are necessary.

Chronic wounds have many aetiologic reasons that affect the success rate of MDT. Most reasons are systemic diseases (for example, diabetes mellitus) with co-morbidities (for example, chronic renal failure) that also negatively impact the immune system of the patient. This situation strongly limits the applicability of systemic drugs, and forces medical professionals to avoid the possible side effects. In addition, chronic wounds such as venous stasis and diabetic foot ulcerations actually have a vascularisation pathology, which may also limit the benefits of systemic treatments. MDT has another advantage in that it is applied locally, and has no systemic effect. This makes MDT especially appealing for patients with systemic co-morbidities[101].

In conclusion, MDT satisfies nearly all expectations for optimal wound care: fighting infection, debridement, provoking wound healing, and neoangiogenesis[4,89]. Its low-cost, noninvasive nature, and absence of systemic effects make this method a good option. MDT is not an exact wound treatment method, but can be an important part of a multidisciplinary approach to treat chronic wounds. There is a black hole in its mechanisms, and by highlighting them, the area of use might widen.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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