Suggested journals:

~~1. Diabetes Care (16.2) Q1~~
~~2. Journal of Translational Medicine (7.4) Q1~~
3. Advances in Wound Care (5.8) Q1
~~4. IJMS (4.9) Q1~~
5. ~~Journal of Plastic and Reconstructive Surgery (3.2) Q1~~
6. Journal of Tissue Viability (3.374) Q1
7. International Wound Journal (3.1) Q1
8. Wound Repair and Regeneration (2.9) Q1
~~9. Advances in Skin & Wound Care (2.73) Q3 CASE REPORTS~~
~~10. Journal of Surgical Case Reports (2.7) Q3 CASE REPORTS~~
~~11. Plastic, Recons. & Aesthetic Surgery (2.0) Q2 CASE REPORT AND REVIEW~~
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Repurposing of Alpha1-Antitrypsin Therapy for Diabetic Wounds: Case Report and Review of Literature

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Abstract

Background. Alpha1-antitrypsin (AAT) is a circulating tissue-protective glycoprotein that holds immunomodulatory activities without compromising pathogen host-defense. AAT physiologically rises in response to infection, inflammation and tissue injury. Patients with genetic AAT deficiency experience non-smoking lung emphysema, vasculitis, panniculitis and impaired wound healing; they receive lifelong weekly infusions of clinical-grade plasma-derived AAT. According to preclinical studies, AAT enhances wound repair, a process that requires acute inflammation to initiate, and thus be replaced by, inflammatory resolution. Chronic hyperglycemia causes chronic inflammation and, accordingly, impaired wound healing. Since the molecular function of AAT is compromised by glycation, it is hypothesized that AAT augmentation may improve wound healing in diabetic individuals. While presently indicated solely for genetic AAT deficiency, its beneficial attributes prompted multiple off-label trials for treating recently-diagnosed pediatric type 1 diabetes and steroid-refractory graft-versus-host disease. In those and other clinical trials, its safety record is notable. Here, a clinical case study is presented in which a diabetic patient suffered a gunshot wound and had subsequently received AAT augmentation therapy. **Methods**. A 48-year–old male with poorly controlled diabetes was admitted following a gunshot wound to the face, requiring three mandibular repair procedures and a fibula-to-jawbone microvascular reconstruction. Despite conventional care, wounds failed to heal for 3 months; the mandibular site remained purulent and the fibula-site wound developed dehiscence. Upon consent, 5 weekly slow-drip 60 mg/kg AAT infusions were provided, alongside direct topical application. Serum samples were obtained before and after infusions. **Results**. AAT was well tolerated. The leg wound developed granulation tissue and decreased in size, facial and neck fistulas demonstrated excellent response and the larger wounds progressively healed. The patient was discharged after the 5^{th} infusion, able to speak and eat solid food. As expected, both serum AAT and elastase-inhibitory capacity increased upon AAT augmentation; in-vitro, pre-treatment serum inhibited epithelial gap closure, while post-treatment sera samples enhanced re-epithelialization. **Conclusions**. It is suggested that AAT is suited for drug repurposing to treat patients with impaired wound healing, particularly in the context of hyperglycemia. More

clinical studies are urged to create an evidence-based resource for customized AAT application in patients with impaired wound healing.

Keywords: Diabetic wound, drug repurposing, hyperglycemia, inflammation, microvascular reconstruction, tissue repair

Background

Human α1-antitrypsin and diabetes

Protease inhibitors protect tissues from excessive destruction primarily by inhibiting tissue-degrading proteases [1]. Human α1-antitrypsin (AAT) is a 52 kDa circulating glycoprotein that belongs to the family of serine protease inhibitors (SERPIN). It circulates in the blood at steady-state levels of 1-2 mg/ml [2] and rises 4-6–fold upon events of infection, inflammation and/or tissue injury, for the duration of a week or longer. It also rises in the blood during the third trimester of a healthy pregnancy, where it appears to be required to support the stressed tissues and to promote immune tolerance towards the fetus [3–6].

At the molecular level, AAT inhibits inflammatory serine-proteases, such as neutrophil elastase, which would otherwise disrupt tissue junctions so as to allow neutrophils to extravasate and invade, as well as cleave and activate protease-activated receptors (PARs) on the surface of immunocytes, and cleave and activate locally deposited cytokine precursors, such as pro–IL-1β released from necrotic cells [7]. AAT also harbors activities that are independent of its anti-protease function, most probably by binding to local agents [8]. For example, upon binding to local nitric oxide, AAT becomes anti-bacterial and reduces bacterial burden in several sepsis models [9,10]. It is not unexpected, therefore, that the hallmark finding in genetic AAT deficiency (AATD) is spontaneous lung alveolar degradation associated with excessive elastase activity. Interestingly, individuals with AATD also suffer from various types of vasculitis and deep tissue necrosis [11]. They present with dermatological manifestations, such as psoriasis, urticaria and angioedema [12], and suffer from impaired wound healing [13,14].

Patients with genetic AATD are spared from non-smoking pulmonary emphysema by life long weekly infusions of clinical-grade plasma-derived affinity-purified AAT [15]. Under AAT augmentation therapy, patients also experience diminished incidence of lung cancer, superior recovery from bacterial and viral pneumonia and better resolution of skin-related conditions [16,17]. In a case report of an AATD patient that was *not* under AAT augmentation therapy, and had severe postoperative wound healing disruption in both chest and abdominal surgical sites, wounds had closed 10 days after initiation of AAT augmentation [14].

The cellular targets of AAT have been extensively studied. AAT is presently depicted as a primal pro-resolution immunomodulator that affects the behavior of neutrophils, macrophages, dendritic cells and B lymphocytes, but does not directly affect responses of T cells and tumor-directed NK cells [18]. These effects are believed to be mediated through a non-obvious selective divergent modulation of NF-κB subunits [19], allowing p65 to enter the nucleus, unlike in the presence of corticosteroids, and promote the expression of, e.g., p65-obligatory interleukin-1 receptor antagonist (IL-1Ra) [8]. AAT is physiologically oxidized by neutrophil-derived free radicals, allowing activated neutrophils to advance through tissues; this state of AAT is reversible and it regains its tissue-protective function once neutrophils pass on [20].

Pathways affected by AAT have been explored primarily by use of clinical-grade AAT. Thus, AAT was found to promote the production of vascular endothelial growth factor (VEGF) during inflammatory conditions (24). While AAT is not an angiogenic agent, AAT does improve mature blood vessel formation [21,22] and decreases the amount of necrotic tissue in skin flap revascularization studies [21,23]. Accordingly, the gene promoter of AAT is directly induced by hypoxic conditions [24] and it has been proposed that lung emphysema in AATD patients is actually the manifestation of the pulmonary vascular bed failing to regenerate [25–27]. Similar to its impact on revascularization, AAT accelerates epithelial gap closure in various in-vitro epithelial scratch assays [19,28]. By affecting these critical pathways involved in wound repair, AAT accelerates excisional skin wound healing in acute wound models, administered by systemic routes, topical and intradermal infiltration [28,29]. Evidence suggests that the enhanced reparative profile of tissues under AAT-rich conditions is not restricted to the skin, as tissue repair by AAT is also evident in other clinical and preclinical scenarios, such as ischemic heart disease [28,30,31], inner-ear cochlear injury [30,32], cerebral stroke [33–35] and gastrointestinal inflammation [36,37]. Collectively, AAT promotes tissue repair processes at multiple levels [12,38–40].

The impact of AAT on the survival and function of pancreatic islet β-cells is an outstanding example of its tissue-protective attributes [41–43]. AAT promotes insulin secretion and protects β-cells from direct IL-1β–induced apoptosis [41], both in-vitro and in models of islet allografts where it promotes both revascularization [22] and IL-1Ra production [8]. Accordingly, expression of AAT in nonobese diabetic (NOD)

mice significantly reduces insulitis and prevents the development of spontaneous hyperglycemia [44–46]. In accordance with the impact of AAT on immune tolerance [22,40], AAT plays a role in moderating the autoimmune responses associated with type 1 diabetes (T1D), and early intervention with AAT shows promising outcomes in several clinical trials [42,47,48]. Indeed, while individuals with T1D have normal levels of circulating AAT, it appears to be glycated [49–51] and, thus, void of anti-proteolytic function [47,52]. This phenomenon is also present in patients with type 2 diabetes (T2D) [47,53,54]. Interestingly, during gestational diabetes, serum AAT levels are consistently higher compared to normal pregnancies, yet its anti-protease activity is, again, reduced [51].

Wounds under hyperglycemic conditions in relation to α1-antitrypsin

The rapid and escalating inflammatory response during early wound healing is required to trigger processes of decontamination, activate cell migration, attract and direct critical repair-associated immune and non-immune cells, e.g., neutrophils and epithelial cells, and render blood vessels leaky so as to irrigate the injured site with plasma [55]. This aggressive phase of inflammation, though destructive by nature, is indispensable; it is required in order to initiate pathways of inflammatory resolution and prompt the next stage of wound healing, i.e., proliferation of dermal fibroblasts and keratinocytes and the generation of a tissue rich in blood vessels, i.e., the granulation tissue. Remodeling then occurs as fibroblasts convert to myofibroblasts and shrink the wound site, as they deposit extracellular matrix (ECM) proteins to support the evolving structures. Along with apoptosis of the overgrown cell population, the tissue ideally returns to its physiological function [56,57]. Normally, in the early stages of wound healing, M1 macrophages dominate the wound site, releasing pro-inflammatory cytokines, chemokines and proteolytic enzymes, while clearing pathogens and dead tissue. As the healing process advances, a switch towards M2-like macrophages occurs, a phenotype characterized by anti-inflammatory cytokines and growth factors that promote tissue repair, angiogenesis and collagen synthesis [56]. AAT has been shown to promote the differentiation of macrophages towards tissue-protective M2-like macrophages [58–60], agreeing with reduced inflammation in conditions rich in AAT. Incorrect orchestration of the phases of tissue repair may lead to wounds that fail to heal.

These present with a significant risk of infection, are frequently painful, can lead to the severing of tissue or limb, and might be lethal [61]. Indeed, difficult-to-heal wounds are a complex clinical challenge that currently lacks an effective treatment [62].

Under chronic hyperglycemic conditions, the physiological process of wound healing is impaired [63,64]. In patients with diabetes, wound healing is slow and, more dangerously, is prone to become chronic, infected, extend to the bone and risk lower-extremity amputation [65,66]. Diabetic foot ulcers, for example, are responsible for an unacceptable 50-70% five-year mortality rate [66]. The failure to progress to the M2-like phenotype takes an important part in the diabetic wounds [67]. Hyperglycemia affects protein synthesis, migration and proliferation of keratinocytes and fibroblasts, thus creating a non-resolving inflammatory phase marked by the accumulation of unopposed neutrophils and M1 macrophages; concomitantly, the wound site depicts elevated production of tissue-degrading proteases and matrix metalloproteinases [56,62,68], as well as dysregulated angiogenesis, elevated reactive oxygen species (ROS), and persistent bacterial colonization that often forms a biofilm [68]. This loss of healthy tissue homeostasis can be further exacerbated by infection, such as *P. aeruginosa*, which recruits M1 macrophages to the wound bed [65]. Interestingly, AAT has been shown to be particularly effective in reducing the burden of *P. aeruginosa* [69], as observed during studies of inhaled AAT in patients with cystic fibrosis [70]. Hyperglycemia also induces oxidative stress via an excess of advanced glycation end products (AGEs) and the activation of their receptors [63]. AGEs prolong the inflammatory response, impair wound contraction and negatively impact ECM remodeling in the diabetic wound, alongside promoting an imbalance between ROS and antioxidant compounds [68,71,72]. Collectively, under such conditions, AAT is neutralized by both glucose, and ROS.

Off-label AAT studies: clinical indication, patient age, treatment dose and route of delivery

AAT is presently indicated for genetic AAT deficiency at 60 mg/kg slow-drip i.v. infusion once a week, for life, with the goal of preventing pulmonary emphysema [73]. In an attempt to diminish the frequency of infusion sessions, a dose of 120

mg/kg [74,75] and a dose of 240 mg/kg [76] were evaluated and found to be safe in individuals with genetic AATD. The effect of genetic AATD on lungs is usually absent before adulthood, thus, augmentation therapy in the pediatric population has not been tested in the context of AATD. Indications other than genetic AATD were tested as soon as evidence was provided for the tissue protective attributes of AAT, as reviewed elsewhere [77]. In these studies, participants have initial normal concentrations of circulating AAT, lending important information regarding the safety of AAT augmentation in individuals without AATD (**Table 1**).

Three independent clinical trials explored the effects of AAT infusions on individuals with T1D; in all, the treatment represents off-label use per clinical indication, as well as, in some groups, off-label for age and dose plan. The premise was that residual islet mass may be protected by AAT, and that unlike most immunosuppressive agents, AAT is not diabetogenic; thus, all trials were conducted in recently-diagnosed individuals. The pioneering trial was performed at the Barbara Davis Center for Childhood Diabetes, University of Colorado Denver, USA [78]. Twelve T1D subjects that had detectable insulin production received 8 weekly infusions of AAT 80 mg/kg and were then followed for 1 year. The youngest was 12 years old, and no adverse events were observed. Circulating AAT levels were not tested after infusions. According to outcomes, therapy with AAT either increased insulin production or inhibited its chronic decline in some of the patients. Subsequently, a Harvard School of Medicine open-label, dose-escalation study enrolled 8 children ages 8-15 years old and 8 adults ages 16-35 years old [15]; they received weekly AAT 45 mg/kg for 6 weeks and 90 mg/kg for another 6 weeks. The majority of adverse events were instances of hypoglycemia, which are expected when individuals with T1D are treated with standard-of-care insulin during periods of improved blood glucose control. Mean serum levels of AAT were <2.0 mg/ml in 7 of 15 of the subjects within 72 hours after the 90 mg/kg infusion. In Israel, an open-label course of 18 infusions spread over 37-weeks with doses of 40, 60, or 80 mg/kg AAT [48]. Upon completion of the trial, an open-label extension study demonstrated that the administration of multiple repeated AAT infusions (up to 36) to pediatric diabetic patients is safe and well-tolerated for up to 5 years [79]. Subsequently, a phase II, double-blind, randomized, placebo-control trial included two AAT treatment groups, 60 and 120 mg/kg once weekly for 12 weeks, then once

every two weeks for 2 months, and then at the end to the year 6 weekly infusions, totaling 22 infusions [80]. The youngest patient was 8 years old. Most strikingly, at the mark of 1 year, the 120 mg/kg group had 3-fold more patients with HbA1c ≤7%, which is the desired value in diabetic patients [81], and an overall significantly lower mean HbA1c value compared to the placebo group (6.7%±0.9% vs. 8.2±1.4%, respectively). The prospect of involving AAT infusions for addressing diabetes is further reviewed elsewhere [44].

AAT was also tested for preserving insulin secretion in patients undergoing total pancreatectomy with islet auto-transplantation [82]. While pancreatectomy is essential in relieving symptoms of severe or chronic pancreatitis, up to 70% of patients will require lifelong exogenous insulin. Thirteen patients that underwent post-pancreatectomy islet auto-transplantation received 6 weekly 90 mg/kg intravenous AAT from the day before surgery until 28 days after, while 14 patients received treatment with a TNFα inhibitor, and 16 patients underwent islet auto-transplantation alone. Adverse effects were recorded for a year and did not vary between the groups. Interestingly, a significant preservation of insulin secretory response under AAT treatment was observed only among female participants.

Being that autoimmunity and functional cell viability in T1D and islet transplantation appeared to be diverted by AAT *without* compromising host-defense, graft-versus-host disease (GVHD) was considered for testing AAT augmentation. Acute GVHD, an unfortunate complication of bone marrow transplantation, might become refractory to systemic steroid rescue therapy in up to 50% of patients, who then have less than 30% chance of survival [83]; in the case of gastrointestinal involvement, loss of AAT via stool correlates with acute GVHD severity [84]. The rationale for use of AAT in acute GVHD with GI involvement is, therefore, both for addressing immune regulation, and gut epithelial integrity. In a recent clinical trial, patients with steroid refractory acute GVHD received twice weekly AAT 60 mg/kg, i.e., double the frequency that is afforded to individuals with genetic AAT deficiency, for up to four weeks [85]. Treatment was well tolerated. Serum AAT levels increased from 1.47±0.1 mg/ml at baseline (N=10, mean±SEM) to an average of 1.97 mg/ml after two weeks (4 infusion sessions, *paired comparison* p=0.004) and then 2.15±0.2 mg/ml at 4 weeks (**Table 1**). In four patients, serum AAT did not rise from baseline in the first 4 weeks. Changes in serum albumin were not observed. Predictably, the

severity of the condition and the high dose of immunosuppression brought about infection and sepsis in several patients, yet they were all observed thirty days after the last infusion of AAT. Surprisingly, within 4 weeks from treatment initiation, the overall clinical response rate to therapy was 65% and complete response rate was 35%. At day 60, a positive clinical response was sustained in 73% of patients without requirement for immunosuppression. Two more trials report consistent outcomes [86,87], and it is presently suggested that AAT may be considered in GVHD management *before* steroid-refractory disease is established [88].

Similar to cell transplantation settings, ischemic heart disease (IHD) represents a sterile inflammatory tissue-destructive condition. Initial evidence for benefits of AAT for IHD patients arose from a study that determined circulating AAT levels in patients entering the ICU; according to that study, patients who had higher AAT levels had a lower incidence of cardiogenic shock and lower mortality rates [89]. Based on positive preclinical outcomes in a mouse model of cardiac ischemia [90], 10 non-AAT deficient patients with acute ST-segment elevation myocardial infarction (STEMI) were enrolled in an open-label, single-dose treatment study of AAT at 60 mg/kg infused intravenously within 12 hours of admission [38]. Patients were followed clinically for 12 weeks; AAT was well tolerated and there were no in-hospital adverse events. Plasma AAT levels increased from average 1.49 mg/m at admission to 2.03 mg/ml after 72 hours. Serum elastase inhibition capacity was not tested. In a post-hoc analysis, data prove that a single dose of AAT given within hours from coronary event shortens the duration of the ischemia-reperfusion injury [91].

As far as off-label testing for routes of AAT treatment, both inhaled and topical AAT have been reported (**Table 2**). Cystic fibrosis (CF) patients have airways that are inflamed, and are marked by the presence of neutrophils that generate excessive levels of elastase that overwhelms the local anti-protease defense mechanism. The airways are also characterized by recurrent *P. aeruginosa* infections [92]. Inhaled AAT has been shown to significantly decrease airway inflammation in CF patients, as demonstrated across several clinical trials [70,93,94]. The rationale for treatment included not only the anti-elastase activity of AAT that is expected to counter excessive elastase activity, but also its anti-inflammatory attributes, its anti-bacterial properties and the intimate relation to an intact lung tissue. As early as 2007, a prospective multicenter, randomized, open-label, parallel group trial provided 52

patients diagnosed with CF with daily depositions of 25 mg inhaled AAT for four weeks [70]. According to study outcomes, sputum AAT levels increased at both 2 and 4 weeks. In addition, AAT inhalation decreased the percentage of neutrophils in the sputum, slightly reduced the levels of free elastase, and, interestingly, significantly reduced *P. aeruginosa* counts. No serious adverse events were observed. Similarly, safety was demonstrated using 77 mg inhaled AAT in 6 healthy subjects and 15 patients with CF [94], as well as using 100 mg and 200 mg inhaled AAT in 20 subjects with CF, once daily for 3 weeks [93]. Of note, the use of inhaled AAT for patients with AATD has been considered, primarily as a measure for improved patient compliance when compared to weekly infusion sessions, as well as for cost considerations, yet the circulating levels obtained by inhalation did not achieve AAT augmentation goals [95,96].

Topical AAT was tested in a rare disorder, Netherton Syndrome, affecting the skin, hair and immune system, in a double-blind, randomized, placebo-controlled trial [97]. AAT gel vs. placebo were applied twice daily for 21 days to two symmetrical areas of 50 cm^2 on the limbs. No adverse effects were observed. Interestingly, transepidermal AAT is presently under consideration for genetic AAT deficiency [98]. In that study, a 3D human epidermis equivalent reconstructed from human primary epidermal keratinocytes was used to show that AAT freely diffuses across epidermis layers in a concentration and time-dependent manner.

Table 1. Off-label clinical indications for AAT augmentation therapy

Off-label indication	**Pediatric subjects**	**Dose** (frequency, duration)	**Serum AAT** mean mg/ml	**Serum protease**	**Ref.**
T1D	-	**80 mg/kg (weekly, 8 weeks)**	Not tested	Not tested	[78]
	≥8 yrs	**45 mg/kg (weekly, 6 weeks) → 90 mg/kg (weekly, 6 weeks)**	Before last injection: adults, an increase of 0.31±0.12; pediatric, an increase of 0.42±0.22	Not tested	[15]
	≥10 yrs	**40 or 60 or 80 mg/kg (weekly, 12 weeks) → (every 2 weeks, 2 months) → (every month,2 months)**	Not tested	Not tested	[48]
	≥14 yrs	**40 or 60 or 80 mg/kg (weekly, ≤36 weeks)**	Not tested	Not tested	[79]
	≥8 yrs	**60 or 120 mg/kg (weekly, 12 weeks) → (every 2 weeks, 2 months) - gap - (weekly, 6 weeks)**	Not tested	Not tested	[80]
Islet transplant	-	**90 mg/kg (weekly, 6 weeks)**	Not tested	Not tested	[82]
Steroid refractory acute GVHD	-	**60 mg/kg** (×2 per week, 4 weeks)	Baseline: 1.47 Week 2: 1.97 Week 4: 2.15 Week 8: 1.66	Not tested	[85]
	-	**90 mg/kg → 30 mg/kg** (every other day**,** 6 doses) → **120 mg/kg** (weekly, 7 weeks)	Not tested	Not tested	[87]
	-	**90 mg/kg → 30 mg/kg** or **60 mg/kg** (days 3, 5, 7, 9, 11, 13 and 15)	Not tested	Not tested	[86]
Cardiac disease	-	**60 mg/kg** (within 12 hrs from ICU admission)	Baseline: 1.49 72 hrs: 2.03	Not tested	[38]

Table 2. Off-label route of administration

Indication	Pediatric population	Route	Ref.
AATD	-	Inhalation	[96]
AATD COPD exacerbation	-		[99]
Cystic Fibrosis	≥8 yrs		[70,93, 94]
Netherton Syndrome	-	Topical (rAAT*)	[97]

* rAAT, recombinant AAT

Case study: Off-label AAT for complicated wound in diabetic individual

A 48-yr-old non-identified male was brought to the Emergency Department of Barzilai University Medical Center, Ashkelon, Israel (BUMCA) with a severe avulsive gunshot wound to the lower face. He was fully awake and responsive (Glasgow Coma Scale on arrival 15), but, due to the extensive face injury, unable to speak. He was thoroughly examined according to the ATLS guidelines, and intubated to prevent airway compromise. After total body CT scan, facial bones reconstruction demonstrated complete absence of mandibular bone from the angle of the mandible on the right, to the molar teeth area on the left, in addition to severe soft tissue deficit (**Fig. 1**). Following tracheostomy, wounds were debrided from shrapnels and necrotic tissue and sutured with drains according to the principles of damage control.

While at the ICU, the patient was identified by his family and his medical history revealed uncontrolled diabetes and obesity. Past medical records indicated poor glycemic control (random measurement blood glucose of 644 mg% and HbA1c 14.6%). Strict glycemic control was initiated. One week following the initial injury (**Fig. 1,** 7 Oct.), the patient demonstrated worsening neck swelling, purulent discharge from wounds, fever and rising numbers of WBC. A second exploratory surgery was scheduled for extensive lavage and fine debridement of necrotic tissues, while patient scans were used to produce a 3D reconstruction model of his native mandible; a patient-specific titanium plate (PSTP) was printed for stabilization of facial fractures. Two weeks following admission, the patient underwent fracture

fixation with PSTP and revision of wounds (**Fig. 1A**). Discharged from the ICU to the unit, he continued to exhibit poor wound healing with persistent formation of intraoral and skin fistulas, despite broad antibiotic coverage and routine wound lavage. Throughout the hospital stay, wound cultures were positive for *Candida auris*, *Citrobacter koseri*, *Enterococcus faecalis*, *Clostridium difficile*, *Proteus mirabilis* and *Staphylococcus MRSE*. Despite combined advanced antibiotic regimens, infection persisted without resolution. During the entire time, the patient was on strict glycemic control.

Two and a half months following admission, fibula osteocutaneous free flap was harvested to reconstruct the mandible and the soft tissues of the face (**Fig. 1B**); during the surgery, further wound debridement was performed, yet wounds persisted with discharge and positive cultures. Two additional surgical interventions were required for closure of oral fistulas. However, overall condition showed marginal improvement with regular wound irrigations. The fibula harvest site exhibited an extremely slow course of healing, with residual necrotic areas, and had subsequently developed wound dehiscence.

Following multidisciplinary team discussion, treatment was initiated with AAT infusions (Glassia®,1 g / 50 ml solution, Kamada LTD, Ness Ziona, Israel; weekly 60 mg/kg slow-drip infusion sessions, a course borrowed directly from AATD treatment protocols, for 5 weeks), supplemented with topical application to both neck and leg wounds. Aside from standard wound care, no other treatment during this period was applied to the wounds. Within days, small gaps between stitches had sealed, and two weeks later, the purulent discharge in the neck fistula started to dry out and the patient exhibited overall marked improvement in wound healing. Soon after the 5th infusion, four months from the gunshot injury, the patient was discharged with complete neck and leg wound closure. His condition at the 6-month time point, as a regular outpatient under long-term follow-up, is depicted in **Figure 2**. He can speak, and eat solid foods.

Serum samples were collected prior to AAT initiation, and then again at 1 and 4 days from infusion. Samples were used to determine AAT levels by commercial ELISA according to manufacturer's instructions (ICL, Portland, OR, USA) and elastase inhibition capacity using commercial kit, according to manufacturer's instructions (Sigma-Aldrich, St. Louis, MO, USA) (**Table 3**). As expected, circulating

AAT levels increased upon infusion. Notably, serum capacity to inhibit elastase in-vitro was markedly enhanced 1 day from infusion, and less so on day 4 from infusion.

The impact of serum on human epithelial gap closure assay was performed, in which the effects of patient serum samples were compared to sera obtained from three healthy individuals (approved by the Ethics Committee of Soroka Hospital SOR 1106-2018, ensuring adherence to ethical guidelines for biomedical research). Briefly, in-vitro epithelial gap closure assay was performed using A549 cells. Cells were grown to confluence in 24-well plates and uniform linear wounds were inflicted using a sterile 200-µl pipette tip, thus creating a cell-free area, as described elsewhere (37,53). Cultures were washed twice with complete RPMI 1640 supplemented with 2.5% FCS (both from Biological Industries Inc., Beit Haemek, Israel). Treatments were introduced directly onto cells at 1:10 dilution in 2.5% FCS; control conditions included 2.5% media alone. Images were acquired immediately after wounding and 24 hours later using a photomicroscope (Zeiss), cell-free areas were digitally marked and analyzed by ImageJ; outcomes are presented as cell density within the gap area at initial wounding. Data are presented as mean±SEM. As shown in **Table 3**, the density of cells that had repopulated the epithelial gap at 24 hours in the presence of healthy sera was greater than the number of cells under patient serum sample 0, i.e., hyperglycemic serum pre-AAT therapy. One day post-infusion, the number of cells that had repopulated the gap was greater than healthy controls, and after 4 days from infusion, greater than day 1 from infusion, though the only statistically significant difference was between control sera and day-4 patient serum sample ($p=0.019$, student t-test).

Figure 1

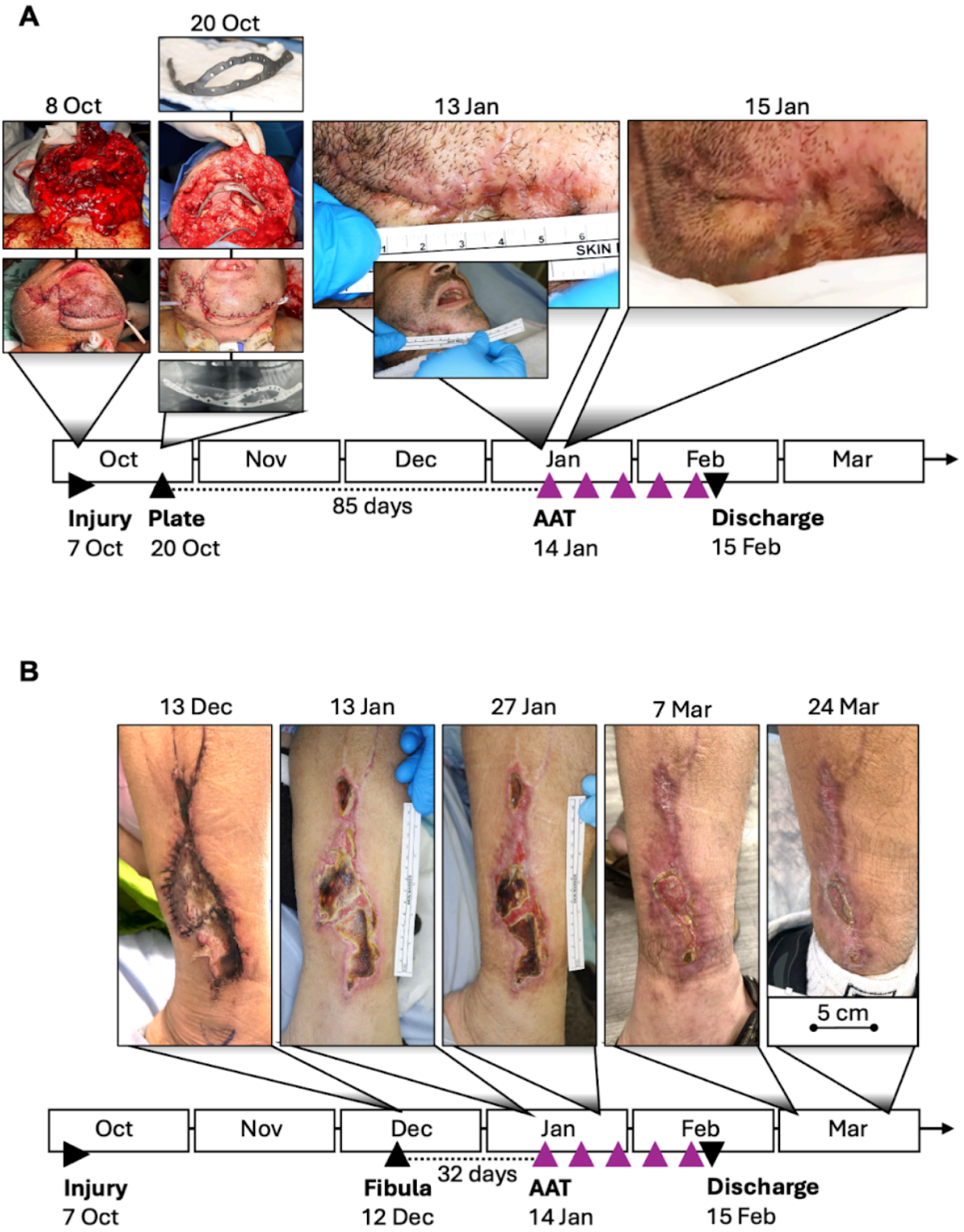

Figure 1. Timeline and representative images. Chronological representation from left to right. (**A**) Facial injury. *Dashed line*, time between plate implantation and initiation of AAT infusions. (**B**) leg wound. *Dashed line*, time between fibula-to-jaw surgery and initiation of AAT infusions.

Figure 2

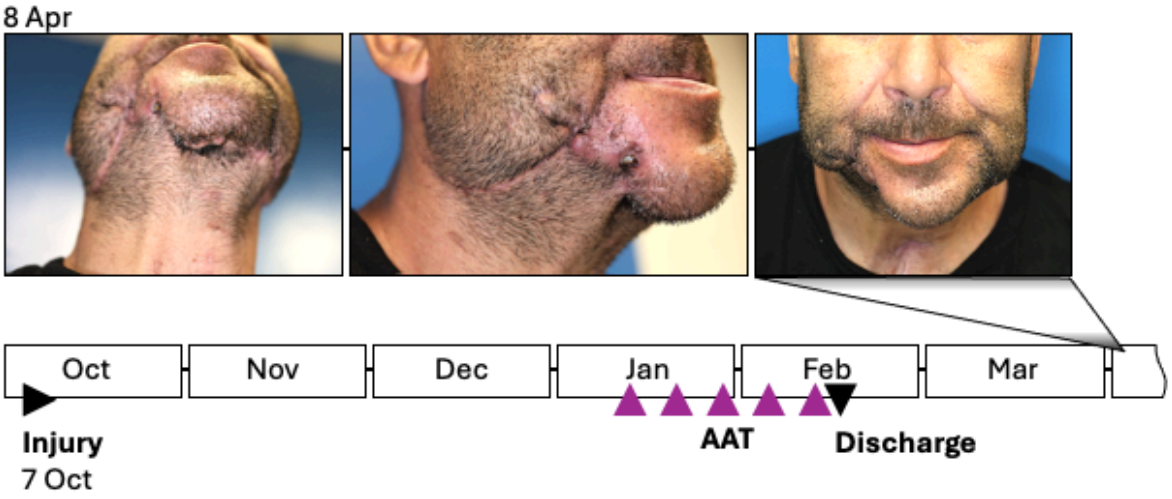

Figure 2. ***Timeline and representative images post-discharge***. Chronological representation from left to right.

Table 3

		Time from infusion	α1-antitrypsin mg/ml	Elastase activity	24-hr Epithelial cells / gap
	CT (N=3)		1.16 ± 0.16	66%	235 ± 50
Patient	Sample 0	0 days	1.46	66%	197 ± 148
	Sample 1	1 day	1.52	8%	451 ± 138
	Sample 2	4 days	1.51	57%	530 ± 267

Discussion

Every traumatic face injury varies significantly. In particular, a gunshot causes comminuted fractures of face bones, tooth injuries, nerve damage, and tissue loss, leading to substantial aesthetic and functional impairment. Tissues that come into direct contact with the intense force of a projectile are significantly impaired in their function compared to a normal tissue tear, and pathogen contamination occurs not unlike that which predominates in thermal burn injuries. The mechanism of a gunshot wound is intricate; upon entry, the bullet will create a small temporary cavity due to the transfer of kinetic energy. As the bullet travels, it displaces and compresses tissues, resulting in the formation of a larger temporary cavity around the wound channel that disrupts organs and bony structures, while a powerful hydraulic shockwave causes the rupture of blood vessels, nerves, and vital structures both near and far. The trajectory of the bullet subsequently forms a lasting wound channel, whose dimensions and configuration are contingent upon the caliber, shape, and angle of impact. When the bullet exits it usually produces a substantial uneven wound. Opportunistic infections and scarring are prevalent, and the recovery process is a lengthy and demanding journey that includes frequent surgical interventions, physical therapy, and psychological assistance.

A gunshot wound in someone with uncontrolled long-term diabetes has an even larger impact on tissue regeneration capacity. If in a healthy individual the wound healing process would require a week or two, the healing process of a gunshot wound could extend to a few months, and the added aspect of underlying diabetes would further extend the time required for healing to 6-8 months or more, increasing the risk for serious complications. The October 7th, 2023 massacre in Israel resulted in a large number of casualties over a short period of time, affecting a diverse population of individuals, many of which were diagnosed with underlying diabetes.

In the presented case, the diabetic patient was challenged not only with a gunshot wound and its associated recurring microbial infections, but also with a significant surgical wound on the right leg where a segment of the fibula was removed for restorative grafting, and with several surgical wounds from superficial and oral cavity reconstructive procedures. The condition of the patient in the presented case was further complicated by a consistent pus discharge from the right neck wound. Wounds were maintained in a relatively clean condition by irrigation

with povidone iodine and saline combined with clindamycin [100]; however, as soon as irrigation was discontinued, infections recurred. In parallel, the titanium printed plate implant began to erode through the skin, and leg wound dehiscence began to develop. Thus, off-label AAT augmentation therapy was applied. At the end of 5 weeks of AAT augmentation therapy, totalling a 4-month stay, the patient was discharged with full functional recovery, including the ability to speak and to eat solid foods.

Meticulous glycemic control during such a complicated rehabilitation process is extremely challenging. The patient was placed on an appropriate diet and, accordingly, lost weight (from 119 kg at admission to 91 kg at discharge, average 1.4 kg per week; based on patient height 176 cm, from BMI 38.4 to BMI 29.4, respectively). The levels of HbA1c were lower than previous values logged in his medical records (as low as 6.9% during hospitalization). However, it must be noted that the patient received blood transfusions during the fibula surgery; blood transfusions can decrease HbA1c in patients with diabetes, rendering the marker, in this specific case, false [101]. While it is possible that the metabolic state of the patient improved under tight glucose monitoring, a short duration of improved glycemic control might not suffice in restoring wound repair capacity as multiple inflammatory pathways have been epigenetically imprinted in a manner that is presently considered difficult to reverse [102,103]. These epigenetic changes affect a multitude of cell types (e.g., macrophages and endothelial cells), and a large variety of pathways (e.g., inflammation, angiogenesis and tissue repair).

The activity of elastase, as tested in the presence of patient serum, and the effect of the same serum on in-vitro re-epithelialization, are of immense potential for bedside evaluation [104–106]. Here, elastase activity was markedly diminished one day after AAT infusion, but was not as affected on day 4 after infusion. This finding agrees with the fact that non-glycated AAT is required to directly bind to elastase for elastase inhibition to take place, and the half-life of AAT upon infusion (**Fig. 3**) is such that on day one its levels are maximal, but then it declines in a sharp manner and is significantly lower on day 4 [107]. In contrast to the inhibition of the enzymatic activity of elastase by serum AAT, the capacity of serum to influence re-epithelialization most-probably gains from a multitude of factors that have been generated by the presence of AAT in the circulation over several days, and might not

require the initial high concentration of AAT; this would explain the advantage of patient serum on epithelial cell density both on day 1 and, to a greater extent, day 4 post-infusion. It may be possible to elucidate this theory by directly supplementing patient serum from prior to AAT infusion with clinical-grade AAT in-vitro, and test whether it was necessary to have AAT circulate for the duration of the study. In addition, this serum profile is worthy of further investigation in order to explore the composition of pro-resolution and resolution-limiting agents that result from systemic exposure to AAT. Indeed, advantages of AAT augmentation for patients with genetic AATD extend beyond pulmonary emphysema [12].

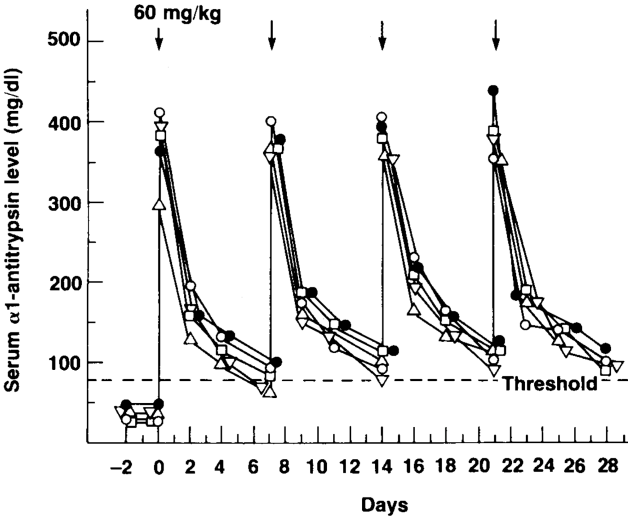

Figure 3. Serum AAT profile during augmentation therapy. Serum levels of AAT during short-term therapy in five patients with genetic AAT deficiency. Each set of post-treatment values denotes levels at 30 minutes, two days, four days, and seven days after each infusion. The dashed line represents the alleged protective threshold level of circulating AAT. Adapted with permission from the seminal work of Weres et al. [107].

In light of the immunomodulatory attributes of AAT, as well as its capacity to promote re-epithelization and revascularization, future studies should also measure systemic inflammatory markers, e.g., ESR and CRP. In addition, the microenvironment of wounds should be characterized, e.g., microbial load, pH, fibroblast activity and relevant cytokine levels, including TGFβ, VEGF and PDGF. These parameters may shed light on the mechanisms in which AAT facilitates wound healing in individuals with limiting underlying conditions. Studies should also consider topical AAT treatment prior to systemic infusions; topical AAT does not negate subsequent infusion therapy and would be substantially less expensive to execute. Based on preclinical data, it would require most probably less than a week for the potential of topical AAT to exert visible changes to a wound under hyperglycemic conditions (*unpublished observations*).

In conclusion, considering that wounds are each unique and the patients are highly diverse in regards to ongoing medications and degree of past and present glucose control, the design of a clinical trial that addresses repurposing of AAT for the benefit of wound repair is expected to suffer from patient over-selection at the

expense of full representation of this important unmet medical need. Thus, more clinical studies are urged in order to create an evidence-based resource that will offer recommendations for the customized application of AAT in patients with delayed wound healing. Taken together, given its remarkable safety record in a variety of off-label applications, it is suggested that AAT is suited for drug repurposing, and that it may be considered for adjunct treatment of patients with wounds that are slow to heal.

Competing interests

The authors declare that they have no competing interests.

Funding

The study was funded by The Diane Lynn Family Foundation to E.C.L.

Authors' contributions

AA and IV have equal substantial contribution to the conception and design of the work, acquisition, analysis of data, interpretation of data and revision of the work; AY has substantial contribution to the conception and design of the work; IF performed acquisition and analysis of data and helped draft the work; YI has substantial contribution to the conception and design of the work, performed acquisition of data and helped draft the work; AN has substantial contribution to the conception and design of the work, performed acquisition of data and helped draft the work; TE performed acquisition of data; SC has helped draft the work and substantively revised the work; ECL has substantial contribution to the conception and design of the work, analysis of data and its interpretation, funding and drafting of the work. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

We thank the patient and his family for their outstanding cooperation. Written informed consent for publication of clinical details and clinical images was obtained from the patient. A copy of the consent form is available for review by the journal editor.

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