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ARTICLE

Photodynamic therapy with intralesional methylene blue and 635nm light-emitting diode lamp in hidradenitis suppurativa. A retrospective follow-up study in 7 patients and review of the literature.

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Hidradenitis supurativa is a chronic inflammatory skin disease which has an estimated prevalence of 1 %, characterized by the formation of recurrent painful suppurative nodules and abscesses in flexural areas of the body. It is believed that its pathogenesis involves an aberrant, genetically-determined activation of innate immunity against bacterial comensal flora of intertriginous areas. It has been found that formation of antibiotic-resistent bacterial biofilms is a common finding in hidradenitis lesions. Photodynamic therapy with different compounds and light sources has demonstrated its efficacy in a number of infectious diseases such as nail mycosis and chronic periodontitis. We retrospectively report our experience in the treatment of hidradenitis with photodynamic therapy using intralesional methylene blue and a 635 nm light-emitting diode light in 7 patients. Two patients received one session whereas 5 patients received two sessions. At one month follow-up good response was achieved in 6 patients. After 6 months, 5 patients (71 %) maintained remission of the disease in the treated area. In view of the results and literature review, we regard methylene blue as an ideal photosensitizer for photodynamic therapy in this disease.

Introduction

Hidradenitis suppurativa (HS) is a chronic autoinflammatory disorder with a variable prevalence estimated in 1 % of the general population (1). It affects intertriginous skin areas with rich apocrine sweat glands' content (2). It is characterized by the formation of nodules and abscesses, with chronic pain and mucopurulent discharge. HS is a disabling condition, which relentlessly progresses, causing fistulas, hypertrophic scars and contractures. The oldest, and simplest staging system for HS is the Hurley classification. Stage I is characterized by lesions without sinus tract formation. Stage II manifests as lesions with sinus tract formation, but with limited scarring. Stage III is defined as multiple lesions, with more extensive sinus tracts and scarring. Recent studies suggest that HS is a multifactorial disease with a genetic basis (3). An aberrant response of the keratinocytes and innate immunity cells to commensal bacteria produces a mild inflammatory cascade in genetic susceptible individuals. The follicular epithelium responds with an increased production of keratin, hyperplasia and cyst formation (1). Infundibular hyperkeratinization leads to follicular occlusion. Consequently, the hair follicle ruptures, and spills its content (corneocytes, bacteria, and keratin) into the surrounding dermis, ensuing a greater inflammatory response and secondary bacterial colonization of the contiguous tissue and evoking the formation of characteristic lesions. It has been postulated that affected patients have an exaggerated response to the exposed materials from broken hair follicles. This phenomenon has been related to an increased activity of the innate immunity (3,4). In a minority of patients with HS, mutations of gamma-secretase have been identified. These mutations are expected to reduce protein function and they could be responsible of the cystic hyperkeratosis and the formation of epidermal cysts. But in addition, gamma-secretase mutations are also connected to defective Notch signalling, which at the same time plays a role in innate immunity control (1,3-6).

Review of prospective investigations suggests a synergistic relationship between impaired innate immunity and microbial factors and some authors even consider HS to belong into the expanding spectrum of bacterial biofilm-based disorders (7-9). Although no uniform pattern of microflora has been observed in HS patients, commensal flora from non-affected controls significantly differs from that in HS patients: coagulase negative staphylococci and anaerobic bacteria are the most frequently found types of bacteria in HS patients (7-11). Other potential causes of HS include endocrine and environmental factors, such as obesity, smoking and mechanical stress, all of which contribute to a pro-inflammatory state (3,12-14).

Treatment of HS is a challenge, as there is no curative option yet (15). Based in the inflammatory pathways in HS described by Kelly and Prens (12), therapies could be divided into two groups. The first one would include those options that act in the subclinical stage, prior to visible active HS lesions. Anti-inflammatory and antimicrobial agents could fit into this first group. The second group would encompass those therapies that act in the clinically visible stage of the disease. Regarding that the diagnosis of HS is usually

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made when clinical lesions are already established, the best therapeutic options are those that act at both levels (subclinical and clinical). Taking this into account, photodynamic therapy (PDT) has been successfully attempted in localized lesions of HS, as it produces an immune-regulating, an antibacterial and a cicatrizing effect (16,19). Most regimes have included the use of topical or intra-lesional methyl-amino levulinate (MAL), or topic amino-levulinic acid (ALA) activated by a blue light source, a red light source or a laser diode. Methylene blue (MB) has also been used as a photosensitizer with intense pulsed light (IPL) with a 630 nm filter, or 635 nm-light emitting diode lamps as photo-activators (20-35).

Encouraged by the preliminary results of PDT in HS, we have performed this therapeutic option in seven of our HS patients, with variable success, and we herein report our results.

Experimental

It was a retrospective longitudinal cohort study, with a total of 8 patients treated in the Photodynamic Therapy Unit of the Hospital San Jorge of Huesca and Hospital Parc Taulí of Sabadell (Spain) over a period of 8 months. Written informed consent was obtained from all patients. Inclusion criteria were having a Hurley stage II or III, involvement of at least one active anatomical site at the moment of the inclusion and failure of other treatment options (such as antibiotic therapy, retinoic acid derivatives, sulfone or corticosteroid infiltration). Exclusion criteria were pregnancy, breast-feeding, and history of photosensitivity. In all cases, the treated lesions were abscesses, ultrasonographically defined as anechoic or hypoechoic fluid deposits in the dermis and/or hypodermis, connected to the base of widened hair follicles (37-44).

Initially, 8 patients were included, but 1 patient was excluded because of lost for follow-up after the first photodynamic session. Iconography, visual analog scale for pain, dermatological life quality index (DLQI), physician's global assessment (PGA) scale score, Sartorius index and ultrasonographic assessments were tracked for each patient at the initial visit and at every follow-up visit (transverse and longitudinal diameter of the lesion, dermal thickness, Doppler). The treatment procedure was as follows. The photosensitizer used was intralesional methylene blue (MB) 1% solution, injected into the abscess with ultrasonography guidance until the lesion became dark blue. After an incubation period of 15 minutes, lesions were illuminated using a 635 nm red light-emitting diode (LED) lamp (Aktilite, Photocure ASA, Oslo, Norway and BF-Rhodo LED, Biofrontera Pharma GmbH, Leerkusen, Germany as they are the lamps available in our Departments) at 37 J/cm2 to each lesion (8 minutes of irradiation time in average) (Fig 1). Two patients received only one session; all other patients underwent two PDT sessions with a separation of a 15-day period. Patients were followed up at 1, 2, 4 and 6 months. Efficacy of treatment was assessed according to the improvement of the lesions (measured by ultrasonographical transverse diameter reduction of the lesions): good (improvement greater than 75%), bad (improvement between 75-50%), no response (improvement lesser than 50%).

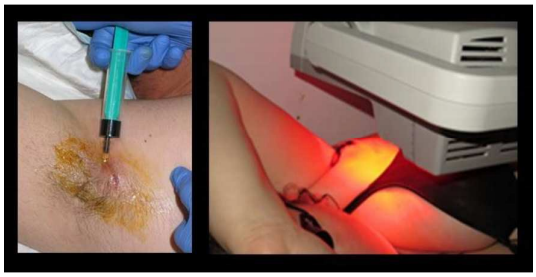


Figure 1: Images showing the procedure. Intralesional administration of methylene blue followed by irradiation with 635 nm light-emitting diode lamp.

Results and discussion

Four patients were male (57%) and three were female (43%). Their clinical characteristics, treated areas, comorbidities, and outcomes can be seen in Table I. Pain related to the procedure was low (NRS 1-3) in one patient (14%), and moderate (NRS 4-6) in 6 patients (86%), although this was not a handicap to complete the treatment. Mild adverse events, such as slight swelling and erythema, which resolved within a week, were present in 6 patients (86%). One patient presented a severe adverse event: cellulitis at the treated site, which was attributed to self-manipulation after the therapy session. She underwent a 10-day antibiotic therapy with Cefuroxime with excellent response. At 1-month follow-up good response was achieved in 6 patients (86%) (Fig 2 and Fig 3). The patient who did not respond was the woman affected by cellulitis. At 2-months follow up, patient number 1, who had a medical history of PASH syndrome (38) and who had initially responded, presented relapse of symptoms so he started oral cyclosporine and intravenous infliximab. Meanwhile, all other 6 patients, including the woman diagnosed with cellulitis after antibiotic treatment, remained with good response (86%). At 4-months of follow-up another patient presented relapse of symptoms (29%), and all other 5 patients (71%) remained with good response at 6-months follow-up (Fig 4). Most notably, the patients with Hurley stage II responded better than those with Hurley stage III (99% versus 87% improvement respectively) and the groin region yielded the best results.

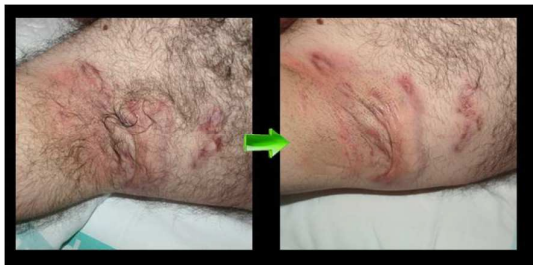


Figure 2: Effect of PDT on axillar lesions in patient number 1, one month after treatment.

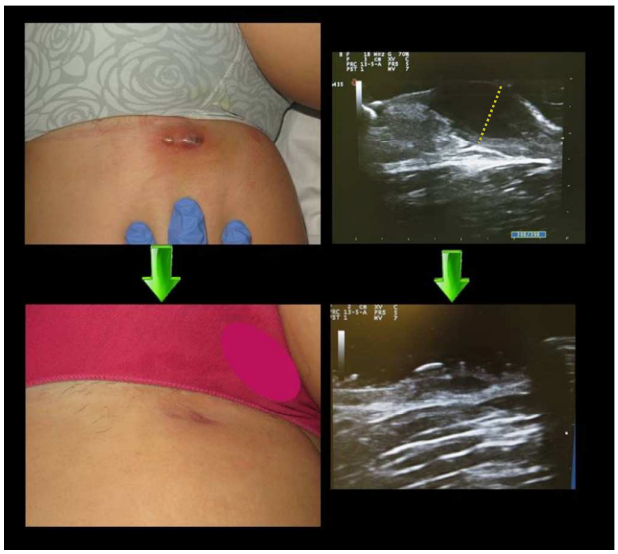


Figure 3: Effect of PDT over lesion located in right groin in patient 5. Clinical and ultrasonographical resolution 1 month after treatment. Note the change in transverse diameter.

PDT is highly regarded and widely used for the treatment of cutaneous tumours. Its current indications in inflammatory and infectious processes are more controversial and less protocolised. PDT is known to exert an antimicrobial, immunomodulatory and anti-inflammatory effect on the irradiated skin, and it could be successful for the treatment of HS by a triple effect: breakage of biofilms, direct cytotoxic effect and induction of an immunomodulatory response (16,36). The mechanism of action is as follows: precursors of protoporphyrins (for example: 5-nanosomated ALA and MAL) and some non-porphyrinic sensitizers (for example: MB) accumulate in the sebaceous glands, the follicular epithelium and the bacterial wall (16). The interaction between the photosensitizer (PS) with the light and the oxygen, results in the formation of reactive oxygen species (ROS), which induce cell death. Due to this non-specific action on multiple cellular targets (bacterial cells, epithelial cells and sebaceous glands), the development of resistance to PDT is not thought to occur (3). On the other hand, it is known that PDT acts selectively on the hypertrophic follicular epithelium that produces follicular plugging, without affecting healthy cells (29).

As of January 2016, 9 articles endorse PDT for HS (20-23,25-27,31,33,), whereas 4 articles describe failure or no response (24,29,34,35). Only in one of the four articles that report failure, blue light instead of red light was used (35). In all of them, the PS used was ALA, with different incubation times. Of the 9 articles favouring PDT, ALA was used in 6 of them (23,25-27,31,33), MAL in 2 of them (20,21) and MB in only 1 (22). The results of these articles are summarized in Table II. No randomized studies have been performed to assess the superiority of MAL or ALA, and to compare these results to the ones achieved with MB. Although direct intralesional injection of MB has not been used in any of these studies, Fadel et al used niosome-loaded MB gel (22).

Two main factors determine the efficacy of PDT: the photosensitizer itself and the type of light used for irradiation.

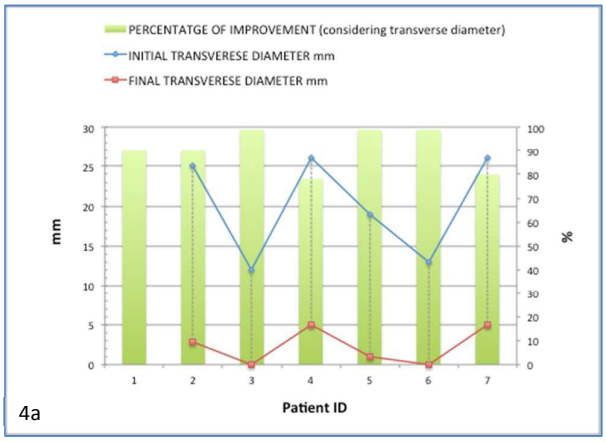
The ideal PS should be highly selective, penetrate the bacterial wall, spread homogenously in the whole lesion, be non-toxic, cheap and easy to administer.

Gram positive and Gram negative bacteria, fungi and protozoa all have proved to be susceptible in vitro to photosensitizers (47,48,49). For instance, Gad et al (50) showed that PDT could inhibit the growth phase and extracellular matrix of gram-positive pathogenic bacteria. Caffarel-Salvador et al, (36) also carried out an in vitro study reporting dark toxicity of MB (with concentracions between 0.1 and 2.5 mg/mL) against *S aureus*, *E coli* and *C albicans*. This is in keeping with other findings in the literature. (51,52). Their data suggest that cationic materials can chemically overcome the obstacles resulting form the slime production and stationary phase of gram-positive bacteria. The mechanism of action may be associated with guanylate cyclase inhibition and oxidation of the coenzyme nicotinamide adenine dinucleotide (53).

To resume, because of the bacterial wall's characteristics, whereas cationic, anionic or even neutral PS can inactivate Gram-positive bacteria, only cationic PS can inactivate Gram-negative bacteria. MB

ID	Age	Gender	Comorbidities	HURLEY /PGA	Previous treatments	Affected regions	Sessions	DLQI (before-after) treatment	VAS-NRS pain	Adverse effects	Results	Evolution
1	49	M	PASH, Pilonidal sinus	3/6	Rifampicin 300mg/12hr + Clindamycin 300mg/12hr x 16 weeks	Right axilla	2	6	2	Slight swelling and erythema	Good response (90%)	Relapse at week 7. Starts Cyclosporine + Infliximab with good response at 6-months follow-up.
2	41	M	Type II DM, Limb amputation	3/6	Oral antibiotics	Both axillae	2	5-1	4	Slight swelling and erythema	Good response (90%).	Relapse at 3 months. Starts Infliximab
3	24	M	-	2/2	Rifampicin 300mg/12hr + Clindamycin 300mg/12hr x 10 weeks	Right axilla	2	12-0	5	Slight swelling and erythema	Good response (99%).	6-months follow-up
4	24	F	-	3/3	Oral antibiotics	Axilla	2	8-0	5	Slight swelling and erythema	Good response (78%)	6-months follow-up. Remitted to reconstructive surgery Dpt.
5	33	F	Gestational diabetes	2/1	Rifampicin 300mg/12hr + Clindamycin 300mg/12hr x 10 weeks	Right groin	2	13-3	6	Slight swelling and erythema	Good response (99%)	6-months follow-up
6	26	F	-	3/1	Oral antibiotics	Left groin	1	7-5	4	Cellulitis (self-manipulation)	Good after antibiotic (99%)	Good response (99%) at 6-months follow-up.
7	29	M	HIV psoriasis	3/2	Acitretin, surgery, oral antibiotics	Left axilla	1	15-4	6	Slight swelling and erythema	Good response (80%)	Starts Rifampicin 300mg/12hr + Clindamycin 300mg/12hr x 10 weeks for relapse at other anatomical sites.

Table 1: Patients characteristics' and outcomes. VAS-NRS: Visual analogue scale - numerical rating scale at the time of the procedure.



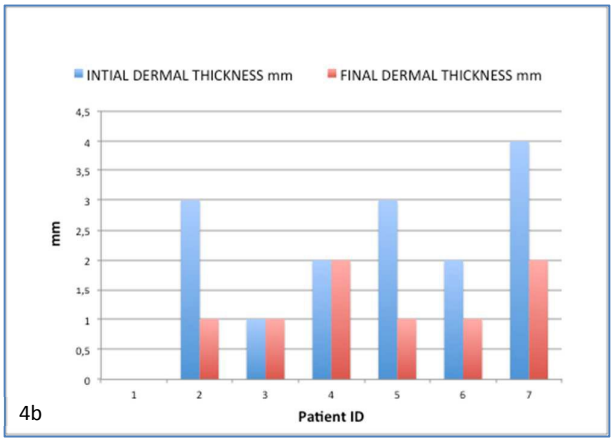


Figure 4. Variation in Ultrasound parameters before and after treatment. a) See a superposed graphic showing reduction of the transverse diameter of the treated lesions measured by ultrasound and percentage of improvement regarding the reduction of the transverse axis. Horizontal axis: patient ID. Primary vertical axis: diameter of the lesion in millimetres. Secondary vertical axis: percentage of improvement. b) See a graphic representing dermal thickness before (in blue) and after (in red) treatment.

(3, 7-bis (Dimethylamino-phenothiazin-5-ium chloride) is a cationic phenothiazinium salt, which is easily available in pharmaceutical grade. Although most studies use either ALA or MAL, the molecular profile of MB, better matches the criteria of the ideal PS for PDT (36). Moreover, MB has proofed to be non-toxic in humans (54, 55). Apart from this, concerning that biofilms play a major role in HS pathogenesis, it is important to know that as the biofilm expands, the central bacteria lose some access to nutrients and oxygen, which are more abundant in the periphery of the biofilm (56). The net result is a slower metabolism and thus lower efficacy of antibiotics, which work on rapidly dividing cells (57). Sbarra (58) proved that the reduction in the number of biofilms when exposed to a cationic porphyrin (tetra-substituted N-methyl-pyridylporphine) and visible light was greater in young biofilms than in mature ones. To overcome this problem, it is reasonable to assume that, if a predetermined optimum concentration of PS is achieved in the lesion containing the biofilms (for instance, MB concentrations grater than 0.1mg/mL (46), these can be eradicated. For example, Soria-Lozano et al proofed that MB, when used in optimal concentrations, is the most efficient PS in killing different types of bacterial biofilms in vitro (59). Moreover, in vitro photoinactivation tests of *Sporothrix schenckii* complexes comparing MB with MAL, revealed phenotiazinium salts to be more effective than MAL (60). Additionally, biofilms treated with a combination of PDT and either antibiotics or host defenses are eradicated at a higher percentage (58, 61).

On the other hand, the drug delivery device should minimize the time between application and irradiation, conform to the shape of the wound whilst maintaining structural integrity and overcome the barrier of necrotic tissue (52). MB is non-toxic, cheap and widely available in the hospital pharmacy. Its incubation period (5-20mins) is much lower than ALA or MAL (30mins-3hrs) and the fact of being in a liquid state allows proper distribution inside the lesion. Some authors have tried to overcome these barriers in vitro (63). Fadel et al (22) employed niosomated MB-gel in comparison to freeMB-gel and achieved better results with the first one, as penetration into the wounds was greater. Except for Valladares et al. (23) who used intralesional 5-ALA in saline at a concentration of 1% and us, no other authors have tried to administer the PS intralesionaly. The advantage is that whereas the PS can't penetrate in fibrotic lesions and fistulas after topical administration, the PS directly reaches the lesions when injected.

As mentioned above, apart from the PS, light source is also a decisive factor in ensuring noted efficacy. Blue and red lights are the most common light sources in PDT. Light absorption by endogenous PS is to be expected, thus leading to a penetration of 1-2 mm for blue light and 8-10 mm for red light. Abscesses characteristic of HS are deep-settled lesions. When the PS is injected, only red light (not blue light) is capable of penetrating deep enough to activate the PS (64,65). Moreover, when comparing MAL and ALA (both precursors of PpIX) with MB, the later has a much higher molar absorption coefficient in the red spectrum than PpIX, and would give higher ROS production for the same fluence (60).

Taking into consideration that our inclusion criteria were patients with fluid collections (no-matter whether these were confined to the dermis or also involved hypodermis), lesions always responded at a considerable percentage. This can be explained because MB was directly injected into the cavities and the capacity of penetration of red light was enough to excite the MB inside the lesions. On the other hand, we couldn't have expected achieving the same results, had we treated dermic or hypodermic non-inflamed long-standing lesions.

It is logical to think that direct illumination of the lesions can be ever more effective. We have read with interest the papers published by Valladares et al (23). They report Intralesional PDT using a diode laser attached to an optical cable inserted into the lesions, in order to enhance the penetration of the light. The treatment was well tolerated, effective, cheaper than systemic therapy and less invasive than surgery. On the other hand, it has been proofed that light itself promotes wound healing via different mechanisms. At a low-energy laser it exerts a stimulating effect on cells and at a high-energy radiation it has an inhibitory effect. Moreover, macrophages exposed to 660nm low-level wavelengths release cytokines that stimulate fibroblast proliferation and the production of growth factors, thus influencing the inflammatory process and healing (20,66).

The main limitation of our study is that treatment of HS lesions with PDT has not been protocolled yet. Key points such as incubation period, dosage, illumination time or when to withhold treatment have been extrapolated from other standardized procedures with photodynamic therapy. Besides, the dermatologist that performs the treatment must be trained and expertise in this field. Therefore, to ensure a therapeutic benefit, its use should be limited to inflamed and accessible lesions (unless a diode laser attached to an optical cable is available).

Table II: See a summarizing table with the key points of original articles published as of January 2016 using PDT for the treatment of HS patients. References 24,29,34,35 belong to those articles that reported either failure or no response. VAS: visual analogue pain during procedure. IL: intralesional.

Ref	n	PS	Route of administration of the PS	Incubation time	Light source	Lamp	Distance between lesion and light source	Power of the lamp	Time of irradiation	Pain (VAS)	Number of sessions	Time between sessions	Adverse effects	Final results	Follow-up
20	1	MAL	Topical	3hr in occlusion	Red (570-670nm)	-	5cm	37J/cm²	8min	5/10	9	15 days	Erythema	Improvement 80%	6m
21	1	MAL	Topical	-	-	-	-	-	-	-	3	-	-	Improvement 90%	-
22	10	MB niosomated (AMN) vs. MB in free gel (FMB)	Topical	30 min without occlusion	IPL with a 630nm filter	EPI-C PLUS	-	25J/cm²	-	0/10	12 max	15 days	-	AMN: improvement95-60% FMB: improvement 72-18%	2/10 pats: relapse at 3m. 1/10 pats: axilla relapse at 6m
23	27	5-ALA 1%	IL	3hr	630nm	Diode laser (optic fibre)	IL	1 W/cm³	3 min	1pat: 10/10 4pats: 4/10	1-3	-	Pain and erythema. 1pat: Fever and influenza-like symptoms	Improvement >75%: 37%pats 75-50%: 41%pats 50-25%: 19%pats	6m
24	5	5-ALA	Topical	3hr in occlusion	Red (570-670nm)	Waldmann PDT	-	20J/cm²	-	2/10	4	15 days	5/5: Erythema 2/5: blistering and swelling	Sartorius (mean): from 18.8 to 17.2. DLQI improvement6.4%	2m
25	1	5-ALA	Topical	3hr in occlusion	Red (570-670nm)	Aktilite	8cm	37J/cm²	8min	5/10	2	10 days	Erythema	Improvement 90%	Relapse at 12m
26	5	5-ALA, solution 20%	Topical	1,5hr	Red (635nm)	PDT 1200L Waldman System	-	37J/cm²	-	2pats: 5/10	4	7-14 days	-	At 2m: Severity improvement: from 35+-5 to 18+-8 Mean DLQI improvement: 21pt	2m
29	4	5-ALA occlusion	Topical	4hr in occlusion	3 pats: diode laser (633nm) 1 pat: Red (570-670nm)	Ceramoptec CureLight	-	-	Dose: 15J/cm²	1pat 10/10 (abandon)	3	7 days	Pain and burning 4/5	1pat: worsens (Sartorius 22 to 38) 2pats: very mild response	2m
31	3	5-ALA solution + bupivacaine 0,5%	IL (Except pat 1: one previous session with topical ALA).	3hr in occlusion	Diode laser (630m)	-	IL	1W/cm³	3 min	EVA 0/10		-	-	Improvement (100%)	9,14,7 m respectively
33	12	ALA	Topical		Blue (630nm) IPL				-	Better tolerance to treatment with blue light	4	7 days		1m: mean improvement 50,8% (100% in 3 pats) DLQI improvement 27,2%	2m: mean improvement 29,9% DLQI improvement 19,3%
27	4	ALA	Topical	15-30min	Blue (630nm)	-	Topical	-	18min	-	3-4	7-15 days	-	Improvement 75-100%	3 pats, good response at 3-years 1 pat relapses every 6 m
35	2	ALA	Topical	-	Blue and Beam laser	BLU-U Blue Light (DUSA Pharmaceuticals) and Candela V-beam laser	Topical	Blue light: 6-10 J/cm² Laser: 4-5J/cm²	Pat 1. 10min 12min 12min Pat 2. 18min 18min 18min	-	3	15 days	-	Pat 1. No improvement. Pat 2. Improvement (degree not reported)	-
34	4	ALA	-	-	PDL-PDT	-	Topical	-	-	EVA 9/10	-	-	-	Mild improvement at 1m	Relapse at 3m

Conclusions

PDT with intralesional MB is a therapeutic option with good results to be taken into account in those patients with prior failure of conventional antibiotic therapy. It is more efficient in Hurley II than Hurley III patients'. Randomized controlled studies should ensure the efficacy of such treatment and increase its level of evidence.

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References

1 Gill L, Williams M, Hamzavi I. Update on HS: connecting the tracts. F1000prime Reports 2014; 6: 112-22
2 Sellheyer K, Krahl D. "Hidradenitis suppurativa" is acne inversa! An appeal to (finally) abandon a misnomer. Int J of Dermatol 2005; 44: 535–40.
3 Prens EP, Deckers I. Pathophysiology of hidradenitis suppurativa: an update. Suppl J Am Acad Dermatol 2015; 73: S8-S11.
4 Kelly G, Sweeney C.M, Tobin AM, Kirby B. Hidradenitis supurativa: the role of immune dysregulation. Int J Dermatol 2014; 53, 1186-96.
5 Ingram JR. The genetics of hidradenitis suppurativa. Dermatol Clin 2016; 34: 23-8.
6 Gao M, Wang PG, Cui Y, Yang S, Zhang YH, Lin D, Zhang KY, et al. Inversa Acne (Hidradenitis Suppurativa): A Case Report and Identification of the Locus at Chromosome 1p21.1–1q25.3. J. of Inv Dermatol 2006; 126, 1302–06.
7 Ring HC, Emtestam L. The microbiology of hidradenitis supurativa. Dermatolclin 2016; 34: 29-35
8 Sandeep K, Lasko LA, Stoodley P. Considering hidradenitis suppurativa as a bacterial biofilm disease. FEMS Immunol Med Microbiol 2012; 65: 385-89.
9 Jahns AC, Killasli H, Nosek D, Lundskog B, Lenngren A, Muratova Z, Emtestam L, Alexeyev OA. Microbiology of hidradenitis supurativa (acne inversa): a histological study of 27 patients. APMIS. 2014; 122: 804-9.
10 Matusiak L, Bieniek A, Szepietowski JC. Bacteriology of hidradenitis suppurativa-which antibiotics are the treatment of choice? Acta Derm Venereol 2014;94: 699–702.
11 Nikolakis G, Join-Lambert O, Karagiannidis I, Guet-Revillet H, Zouboulis CC, Nassif A. Bacteriology of hidradenitis suppurativa/acne inversa: a review. Suppl J Am Acad Dermatol 2015; 73: S12-8.
12 Kelly G, Prens EP. Inflammatory mechanisms in hidradenitis suppurativa. DermatolClin 2016; 34: 51-8.
13 Boer J, Nazary M, Theut Riis P. The role of mechanichal stress in hidradenitis suppurativa. Dermatol Clin 2016; 34: 37-43.
14 Miller IM, Rytgaard H, Mogensen UB, Miller E, Ring HC, Ellervik C, Jemec GB. Body composition and basal metabolic rate in Hidradenitis suppurativa: a danish population-based and hospital-based cross-sectional study. J Europ Acad Dermatol and Venererol 2015;Epub ahead of print.
15 Ingram JR, Woo PN, Chua SL, Ormerod AD, Desai N, Kai AC, Hood K, Burton T, Kerdel F, Garner SE, Piguet V. Interventions for hidradenitis suppurativa. Cochrane Database Syst Rev. 2015; 7:10.
16 Hamblin, M.R.; Hasan, T. Photodynamic therapy: A new antimicrobial approach to infectious disease? Photochem. Photobiol. Sci. 2004; 3, 436–50.
17 Buggiani G, Trolano M, Rossi R, Lotti T. Photodynamic therapy: off-label and alternative use in dermatological practice. Photodiag and Photodynam Ther. 2008; 5: 134-8.
18 Calzavara-Pinton PG, Rossi MT, Aronson E, Sala R. A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MAL-PDT) in 20 Italian dermatology departments. Part 1: inflammatory and aesthetic indications. Photochem PhotobiolSci. 2013; 12:148-57
19 Calzavara-Pinton PG, Rossi MT, Aronson E, Sala R. A retrospective analysis of real-life practice of off-label photodynamic therapy using methyl aminolevulinate (MAL-PDT) in 20 Italian dermatology departments. Part 2: oncologic and infectious indications. Photochem Photobiol Sci. 2013; 12:158-65.
20 Saraceno R, Teoli M, Casciello C, Chimenti S. Methyl aminolaevulinate photodynamic therapy for the treatment of hidradenitis suppurativa and pilonidal cysts. Photodermatol, Photoimmunol & Photomed 2009; 25: 164–165.
21 Silva-Feistnera M, Salomonea C, Silvaay S, Vial-Letelier V. Hidradenitis supurativa prepuberal: presentación de 2 casos clínicos. Rev Chil Pediatr 2015; 21. epubahead of print.
22 Fadel MA, Tawfik AA. New topical PDT for treatment of HS using methylene blue niosomal gel: a single-blind, randomized, comparative study. Clin and Experim Dermatol 2015; 40: 116–22.
23 Valladares-Narganes LM, Rodríguez-Prieto MA, Blanco-Suárez MD, Rodríguez-Lage, García-Doval I. Treatment of hidradenitis suppurativa with intralesional photodynamic therapy using a laser diode attached to an optical cable: a promising new approach. Br J of Dermatol 2015; 172: 1136–1139.
24 Sotiriou E, Apalla Z, Maliamani F, Ioannides D. Treatment of recalcitrant hidradenitis suppurativa with photodynamic therapy: report of five cases. Clin and Experim Dermatol 2009; 34: e235–e236.
25 Guglielmetti A, Bedoya J, Acuna M, Leiva V, Gonzalez S. Successful aminolevulinic acid photodynamic therapy for recalcitrant severe hidradenitis suppurativa. Photodermato photoimmunol and photomed 2010; 26: 110-11
26 Andino-Navarrete R, Hasson-Nisis A, Parra-Cares J. Effectiveness of 5-aminolevulinic acid photodynamic therapy in the treatment of hidradenitis suppurativa: a report of 5 cases. Actas dermosifilogr 2014; 105: 614-617.
27 Gold MH, Bridges TM, Bradshow VL, Boring M. ALA-PDT and blue light therapy for hidradenitis suppurativa. J Drugs Dermatol. 2004; 3: S32-35.
28 Gold MH. Aminolevulinic Acid Photodynamic Therapy for Hidradenitis Suppurativa. Dermatol Clin 2007; 25: 67–73
29 Strauss RM, Pollock B, Stables G I, Gouldeen V, WJ Cunliffe. Photodynamic therapy using aminolaevulinic acid does not lead to clinical improvement in hidradenitis suppurativa. Br J of Dermatol 2005; 152: 803–4.

30 Rose RF, Stables GI. Topical photodynamic therapy in the treatment of hidradenitis suppurativa. Photodiagnosis and Photodynamic Therapy 2008; 5: 171-5.
31 Rodríguez-Prieto MA, Valladares-Narganes LM, Gonzalez- Sixto B, Noguerol-Cal M. Efficacy of intralesional photodynamic therapy for the treatment of hidradenitis suppurativa. J Am Acad Dermatol 2013; 69: 873-75.
32 Scheinfeld N. The use of photodynamic therapy to treat hidradenitis suppurativa a review and critical analysis. Dermatol Online J 2015; 21: 1. LI. doj-25433.
33 Schweiger ES, Riddle CC, Aires DJ. Treatment of hidradenitis suppurativa by photodynamic therapy with aminolevulinic acid: preliminary results. J Drugs Dermatol 2011; 10: 381-6.
34 Passeron T, Khemis A, Ortonne JP. Pulsed dye laser-mediated photodynamic therapy for acne inversa is not successful: a pilot study on four cases. J DermatologTreat. 2009; 20: 297-8.
35 Rivard J, Ozog D.Henry Ford Hospital dermatology experience with LevulanKerastick and blue light photodynamic therapy.J. Drugs Dermatol. 2006; 5:556-61.
36 Caffarel-Salvador E, Kearney MC, Mairs R, Gallo L, Stewart SA, Brady AJ, Donnelly RF. Methylene Blue-Loaded Dissolving Microneedles: Potential use in Photodynamic antimicrobial chemotherapy of infected wounds. Pharmaceutics 2015; 7: 397-412.
37 Wortsman X. Imaging of hidradenitis suppurativa. Dermatol Clin. 2016; 34: 59–68.
38 Martorell A, Segura Palacios JM. Ultrasound examination of hidradenitis suppurativa. Actas Dermosifiliogr. 2015; 106 S1:49-59
39 Wortsman X, Wortsman J. Ultrasound Detection of Retained Hair Tracts in Hidradenitis Suppurativa. Dermatol Surg. 2015 ;41: 867-9
40 Zarchi K, Yazdanyar N, Yazdanyar S, Wortsman X, Jemec GB. Pain and inflammation in hidradenitis suppurativa correspond to morphological changes identified by high-frequency ultrasound. J Eur Acad Dermatol Venereol. 2015;29:527-32
41 Wortsman X, Moreno C, Soto R, Arellano J, Pezo C, Wortsman J. Ultrasound in-depth characterization and staging of hidradenitis suppurativa. Dermatol Surg. 2013;39:1835-42
42 Wortsman X, Jemec G. A 3D ultrasound study of sinus tract formation in hidradenitis suppurativa. Dermatol Online J. 2013;19:18564
43 Kelekis NL, Efstathopoulos E, Balanika A, Spyridopoulos TN, Pelekanou A, Kanni, T, Savva A, Brountzos E, Giamarellos-Bourboulis EJ. Ultrasound aids in diagnosis and severity assessment of hidradenitis supurativa. Br J Dermatol 2010; 162: 1400-2
44 Wortsman X, Jemec GB. Real-time compound imaging ultrasound of hidradenitis suppurativa. Dermatol Surg. 2007;33:1340-2
45 Braun-Falco M, Kovnerystyy O, Lohse P. Pyodermagangrenosum, acne and suppurativehidradenitis (PASH)- a new antiinflammatory syndrome distinct from PAPA syndrome. J Am Acad Dermatol 2012; 66: 409-15.
46 Donnelly, R.F.; McCarron, P.A.; Tunney, M.M.; Woolfson, D.A. Potential of photodynamic therapy in treatment of fungal infections of the mouth. Design and characterisation of a mucoadhesive patch containing toluidine blue O. J. Photochem. Photobiol. B 2007; 86, 59–69.
47 Cassidy, C.M.; Tunney, M.M.; McCarron, P.A.; Donnelly, R.F. Drug delivery strategies for photodynamic antimicrobial chemotherapy: From benchtop to clinical practice. J. Photochem.Photobiol. B 2009; 95, 71–80.
48 Donnelly, R.F.; McCarron, P.A.; Tunney, M.M. Antifungal photodynamic therapy. Microbiol.Res. 2008; 163, 1–12.
49 Zolfaghari, P.S.; Packer, S.; Singer, M.; Nair, S.P.; Bennett, J.; Street, C.; Wilson, M. In vivo killing of Staphylococcus aureus using a light-activated antimicrobial agent. BMC Microbiol. 2009; 9, 27.
50 Gad F, Zahra T, Hasan T, Hamblin MR. Effects of growth phase and extracellular slime on photodynamic inactivation of gram-positive pathogenic bacteria. Antimicrob Agents Chemother. 2004;48:2173-8.
51 Usacheva, M.N.; Teichert, M.C.; Biel, M.A. Comparison of the methylene blue and toluidine blue photobactericidal efficacy against gram-positive and gram-negative microorganisms. Lasers Surg. Med. 2001, 29, 165–173.
52 Donnelly, R.F.; Cassidy, C.M.; Loughlin, R.G.; Brown, A.; Tunney, M.M.; Jenkins, M.G. et al. Delivery of methylene blue and meso-tetra (N-methyl-4-pyridyl) porphine tetra tosylate from cross-linked poly(vinyl alcohol) hydrogels: A potential means of photodynamic therapy of infected wounds. J. Photochem. Photobiol. 2009; 96, 223–231.
53 Khanal, A.; Bui, M-P.N.; Seo, S.S. Microgel-encapsulated methylene blue for the treatment of breast cancer cells by photodynamic therapy. J. Breast Cancer 2014; 17: 18–24.
54 Pasin L, Umbrello M, Greco T, et al: Methylene blue as a vasopressor: A meta-analysis of randomised trials. Crit Care Resusc 15: 42-48, 2013 12.
55 Weiner MM, Lin HM, Danforth D, et al: Methylene blue is associated with poor outcomes in vasoplegic shock. J. Cardiothorac Vasc Anesth 2013; 27:1233-38
56 Lasa I, del Pozo JL, Penadés JR, Leiva F. Bacterialbiofilms and infection. An Sist Sanit Navar 2005; 28: 163-175.
57 Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002, 15:167-93.
58 Sbarra MS, Arciola CR, Di Poto A, Saino E, Rohde H, Speziale P, Visai L. The photodynamic effect of tetra-substituted Nmethyl- pyridyl-porphine combined with the action of vancomycin or host defense mechanisms disrupts Staphylococcus epidermidis bio-films. Int J Artif Organs. 2009;32:574-83.
59 Soria-Lozano P, Gilaberte Y, Paz-Cristobal MP, Perez-Artiaga L, Lampaya-Perez V, Aporta J, et al. In vitro effect photodynamic therapy with different photosensitizers on cariogenic microorganisms. BMC Microbiol. 2015; 15:187.
60 Gilaberte Y, Aspiroz C, Alejandre C et al. Cutaneous sporothrichosis treated with photodynamic therapy: an in vitro and in vivo study. Photomed Laser Surg 2014; 32: 54-57.
61 Cassidy, C.M.; Donnelly, R.F.; Elborn, J.S.; Magee, N.D.; Tunney, M.M. Photodynamic Antimicrobial Chemotherapy (PACT) in combination with antibiotics for treatment of Burkholderiacepacia complex infection. J. Photochem Photobiol. 2012;106: 95–100.
62 Vilela, S.F.; Junqueira, J.C.; Barbosa, J.O.; Majewski, M.; Munin, E.; Jorge, A.O. Photodynamic inactivation of Staphylococcus aureus and Escherichia coli biofilms by malachite green and phenothiazine dyes: An in vitro study. Arch. Oral Biol. 2012; 57: 704–710.

63 Kearney, M-C; Brown, S.; McCrudden, M.T.C.; Brady, A.J.; Donnelly, R.F. Potential of microneedles in enhancing delivery of photosensitising agents for photodynamic therapy. Photodiagn. Photodyn. Ther. 2014; 11: 459–466.
64 Steven L Jacques. Optical properties biological tissues: a review. Phys. Med. Biol. 2013; 45: R37–R61.
65 Grossweiner LL; Messina JW. Investigation of power absorption by methylene blue in a light scattering medium with an internal photodynamic actinometer. Photochem Phtobiol. 1987; 45: 671-24.
66 Jori, G.; Fabris, C.; Soncin, M.; Ferro, S.; Coppellotti, O.; Dei, D.; Fantetti, L.; Chiti, G.; Roncucci, G. Photodynamic therapy in the treatment of microbial infections: Basic principles and perspective applications. Lasers Surg. Med. 2006, 38, 468–481.

