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Vitiligo management: combination of surgical treatment and phototherapy under reflectance confocal microscopy monitoring

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Abstract. – OBJECTIVE: Vitiligo is a chronic acquired pigmentary skin disorder characterized by well-defined asymptomatic white macule as a result of loss of functional melanocytes in the epidermis. The psychological burden experienced by patients is of great interest and consequently research of the best medical approach is constantly developing. This review focuses on surgical approach and the combination of surgery and phototherapy. In addition, reflectance confocal microscopy (RCM) could be useful to discriminate between stable or active vitiligo and to evaluate efficacy of therapy.

MATERIALS AND METHODS: We searched PubMed with the following keywords: (vitiligo[Title/Abstract]) AND therapy[Title/Abstract]) AND surgery[Title/Abstract]) AND phototherapy[Title/Abstract]) AND reflectance confocal microscopy[Title/Abstract]).

RESULTS: To date, surgery is an effective therapeutic approach in stable vitiligo. Phototherapy, which is the most effective medical option, can improve the results obtained with surgery if performed in combination. Preliminary data show that RCM help physician in evaluating stability of vitiligo and is also useful to monitor clinical response.

CONCLUSIONS: Vitiligo is a psychosocially debilitating disease requiring a multidisciplinary approach. Even if a standard management could not be stated, combination of surgery and phototherapy in stable vitiligo could lead to great improvement than monotherapy. RCM is a modern tool which should be used in order to perform surgery and phototherapy properly and to subsequently evaluate efficacy on a microscopic level.

Key Words:
Vitiligo, Therapy, Surgery, Confocal microscopy.

Introduction

Vitiligo is a multifactorial disorder in which affected individuals are increasingly prone to melanin loss hence to depigmented skin, mucous membrane and hair bulbs. The prevalence is up to 2% worldwide with two main types of vitiligo. Non segmental vitiligo (NSV) is a chronic and progressive loss of melanin characterized by well-defined white patches in the midst of normal pigmented skin, symmetrical and which increase in size overtime. Common locations include hands, wrists, underarms, eyelids, philtrum, lips, ears, hips, ano-genital area, knees, ankles and feet. At early stage, lesions can be localized just to one or few areas (localized disease) till white patches spread in different areas (generalized vitiligo) at rate of progression that can be unpredictable. Segmental vitiligo (SV) appears as one or more areas of depigmented skin usually only on one side of the body. Lesions can occur on the face, neck, trunks, arms or legs often during childhood and usually stabilize in few years[1]. Patients with vitiligo often show a lot of comorbidities, such as thyroid disease, alopecia areata, diabetes mellitus, pernicious anemia, systemic lupus erythematosus, rheumatoid arthritis, Addison's disease, inflammatory bowel disease, Sjögren's syndrome, dermatomyositis, scleroderma, ocular and audiological abnormalities, psoriasis, and atopic dermatitis. So in the same patient, behind skin color alteration, immunological and systemic disorder are often present. As a consequence, a general medical history is essential to get a complete picture of the patient especially because patients often follow a lot of medical therapies to treat their disorders[2].

Concerning age of onset, vitiligo can be divided in early vitiligo (before 20-30 years of age, and late vitiligo (after 30 years of age). This is an important parameter to be determined since clinical presentation and response to treatment are very different. Specifically, patients given a

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diagnosis of vitiligo at younger ages tend to have more extensive and progressive disease[3]. Lesions began on the face most commonly in children and adolescents and the elderly and on hands in adults. Segmental vitiligo is prevalent in children and adolescents; and vitiligo with stable evolution is more frequent in childhood and adolescents than in adults and elderly[4,5].

Different pathogenic mechanisms have been put forward in order to explain the loss of melanocytes, such as disorder related to melanocytes, disorders related to cellular and humoral immunity, release of epidermal cytokines toxic for the melanocytes, self-destruction (melanocythorragia), release of cathecolamines and oxidative stress[5]. The autoimmune theory is the leading hypothesis because (1) vitiligo is often associated with autoimmune diseases; (2) most vitiligo susceptibility loci identified through genome-wide association studies encode immunomodulatory proteins; and (3) prominent immune cell infiltrates are found in the perilesional margin of actively depigmenting skin. Recent progress in the understanding of immune pathomechanisms opens interesting perspectives for innovative treatment strategies[6].

Medical therapy is considered as first choice approach in vitiligo: topical corticosteroids or calcineurin inhibitors for localized vitiligo; phototherapy mainly NB-UVB for more extensive subset affecting more than 10-20% of cutaneous surface[7]. Improvement in phototherapy is noted in 70-80% of patients, but complete repigmentation is seen only in 20% of them since relapse can and often occur after treatment. NB-UVB can lead to faster repigmentation rates than any other medical method. In the current viewpoint, the target is to optimize and personalize therapy to find the best management for that patient[8]. Although a standardized cure has not been established yet, the main goal of current therapies is at first to halt the disease and to stimulate repigmentation. Considering that vitiligo can be socially and psychologically devastating, it is mandatory to approach surgical techniques if repigmentation is unsatisfactory, especially for those areas with high cosmetic impact as face, neck and extremities. In addition, the combination of surgery and phototherapy is here discussed according to recent data.

Eligibility for Surgical Therapy

Surgical interventions are indicated for all types of stable vitiligo, including segmental, generalized and acrofacial forms unresponsive to medical therapies. The commonly accepted guidelines for diagnosis and identification of inclusion ad exclusion criteria is the paper of Taieb et al[1]. Definition of disease stability is no new lesions, existing lesions should not become bigger, absence of koebner phenomenom and absence of spontaneous repigmentation[1,9]. Clinical and experimental studies have tried to assess the stability of a lesion on the basis of biochemical and immunological parameters. For example, cluster of differentiation CD8+ T-cell counts has been shown to be associated with the stability of the disease process. The presence of lesional and perilesional CD8+ T-cell count seems to have a positive correlation with disease activity in vitiligo[10]. Again, confocal microscopy has also been reported to be useful in predicting or establishing lesional stability in vitiligo. In lesional skin, the bright rings normally seen at the dermoepidermal junction on confocal microscopy are usually lost. Also, non-lesional skin in vitiligo cases shows some abnormalities of the dermoepidermal junction on confocal microscopy[11]. Biochemical markers, such as the catecholamine levels in urine or plasma have been reported in active vitiligo patients[12] and the antioxidant status can also be correlated with the stability of the disease process[13]. However, since monitoring biochemical markers and using such rarely device is not possible in routine practice, stability of the disease is assessed mostly on the basis of clinical parameters by physicians. Data from literature show that the period for which clinical criteria are applied is extremely various ranging from 3 months to 4 years, depending mostly on physicians' experience[1,9]. We suggest that one year of disease inactivity could be the cut-off period for stability. An absolute exclusion criteria is the predisposition to keloid formation and hypertrophic scars.

Surgical Therapy

In the last decades the surgical options for vitiligo underwent to a lot of advances.

The main basic methods for melanocytes transplantation are essentially five: punch grafting; suction blister grafting, thin dermo-epidermal grafts, non-cultured epidermal suspensions and *in vitro* cultured epidermis with melanocytes or pure melanocytes suspensions[1,14-19]. The efficacy of the treatments depends on surgeon skills, quality of special instruments (high quality dermatome for thin dermo-epidermal grafts or vac-

uum devices for suction epidermal grafting); area to treat, age and phototype (young patients with Fitzpatrick's skin types from III to VI reach better results). In details, it has been estimated that the minimum number of melanocytes required for a good result in term of repigmentation is approximately 210-250/mm^2.

Each surgical option has its own pro and cons, timing, efficacy, cost and preferred targeted area. Local anesthesia is preferred although for larger areas to graft general anesthesia may be required.

– **Punch grafting (PG):** it is considered as one of the most used techniques, it does not require special instruments, it is cost effective, easy to perform and it has a short duration timing process (45 min for 50 cm^2). It may be useful for small or medium sized lesions especially for face, neck, hands and nipples. PG involves biopsy punches both for donor and recipient site. The ideal size for PG should be of 1 mm for facial areas and maximum 1.2 mm for other regions. No significant differences in the total pigmented surface between different punch depth are found. In general, deep grafts create more erythema compared to superficial grafts. Once each minigraft is trasplanted at distance of 4-5 mm apart from the others, repigmentation spreads peripherally from the graft in the nearby areas till complete coalescence (satellite repigmentation).

The most common reported complication for punch grafting is "cobblestoning" appearence of recipient site especially for punches larger than 1.5 mm.

The effectiveness is of 90%-100% of repigmentation in 74% of patients with a depigmentation rate of 2.4 % only.

– **Suction blister grafting (SBG):** this technique creates, by applying a cup or syringe under constant negative pressure, a subepidermal bulla at the donor site while on the recipient site blistering of the achromic areas is induced using liquid nitrogen two days before the transplantation. Other methods to prepare recipient site are ablative lasers or dermoabrasion. The blistering process at the donor site takes from 30 min to 3 hours and it is followed by the surgical removal of the subepidermal bulla which is grafted in the recipient area. The preferred donor site is the flexor aspect of the forearm. Most common complications are temporary hyperpigmentation or color mismatching.

Blister grafting method shows the highest effectiveness rate (complete repigmentation in 90% of patients) but it is time consuming and requires special suctions instruments.

– **Split tickness skin grafting (STSG):** this technique requires the use of dermatome only and in terms of timing approximately allows to harvest 200 cm^2 of grafts in 120 minutes. Grafts are harvested from hidden areas with a uniform tickness (0.1-0.3 mm). The major advantage of this method is the ability to cover large braded surfaces of depigmented skin in one single procedure. Most common complications are scar or keloid formation in the donor site and partial loss of grafts or tick margins of the grafts in the recipient area.

Along with the blister grafts this procedure shows the most effective results in 78-91% of patients with 90% of repigmentation.

1. **Non cultured epidermal suspension (cellular grafts):** The skin obtained from donor site through PG, BG, STSG or curettage is processed for enzymatic separation creating an epidermal cellular suspension, a mix of melanocytes and keratinocytes, ready to be inoculated into blisters created with liquid nitrogen in the recipient area. The intact roof of these blisters acts as a natural dressing allowing the cells transplantation. It is suggested to do not separate melanocytes from keratinocytes since growth factors released for the second ones can boost melanocytes 'growth.

The procedure is more sophisticated than the others above mentioned, time consuming (18 hours immersion in trypsin 0.25% and 1-2 days for blisters creation) and cost effective since it requires laboratory facilities or however special expensive kits for enzymatic separation.

Epidermal suspensions are less effective when compared to the other methods reported in literature: only 56% of patients with NSV obtained more than 90% of repigmentation; on the other side, in contrast to the poor results given from the previously described methods for refractory areas as hands, ankles and feet, epidermal suspensions claim better results in these areas and especially in patients with SV (84% of patients).

2. ***In vitro* cultured epidermis with melanocytes or pure melanocytes suspensions:** Cells culturing is a technique used to expand the number of cells suitable for transplant harvesting donor tissue with a minor sacrifice. This is possible adding M2 melanocytes culture medium to the cellular suspension. The cultured sheet obtained with a petrolatum gauze as support is applied then to the dermo abraded area. Pure cultured melanocytes expanded *in vitro* can treat achromic patches up to 500 cm^2. The procedure is cost effective, time consuming (up to 8 weeks of culturing period) and requires laboratory facilities used by specialized staff.

In summary, the aim of surgical options for vitiligo is to provide complete or almost complete repigmentation to be satisfactory for the patient. The most effective, simple and easy to perform methods are blister grafting and split thickness grafting while cultured and non-cultured epidermal suspensions are sophisticated and time consuming. The highest rate of complications occurs with punch grafting (cobblestone effect) and split thickness grafts (poor scarring or keloids on the donor site). Color mismatching can occur with all techniques: hypopigmentation of the grafts can be related to reactivation of the disease and hyperpigmentation can be related to an hyperactivation of melanocytes during the re-epithelialization process.

Phototherapy

Phototherapy NB-UVB is useful in rapidly spreading vitiligo or extensive generalized vitiligo involving >5% to 10% of body surface area. Its effectiveness is due to immunosuppressive effect, able to induce differentiation of melanocytes and production of melanin[20]. A standard starting regimen of 0.1/0.2 J/cm2 twice or at maximum thrice weekly, can be performed in all phototypes avoiding the possibility of phototoxic reaction[21]. Subsequently, the dose should be escalated in 10% to 20% until endpoint of erythema is reached. Patients should not sunbathe the same day of phototherapy to avoid phototoxicity. If it occurs, or if the last dose of NB-UVB was too high, the next dose should be reduced or skipped, and a local corticosteroid should be applied on the affected area for few days.

In patients with localized vitiligo involving few areas (<10%) or in early, segmental disease, targeted phototherapy (excimer lasers and excimer lamps) should be preferred in order to avoid the generalized effect induced by NB-UVB. In this case, though, a disease stabilization could not be reached because the unaffected skin is not treated.

Guidelines of the Vitiligo Working Group concerning NB suggest that maximal acceptable delivered NB-UVB dose is 1500 mJ/cm^2 for the face and 3000 mJ/cm^2 for the body[21] with differences among phototypes: in skin phototypes I-III, a NB-UVB dosing upper limit is the above mentioned; in skin phototypes IV-VI an upper limit of NB-UVB dosing is not defined.

Compared to PUVA, NB-UVB phototherapy is much easier to perform (lack of photosensitizer) with fewer adverse effects and is more effective with a lower cumulative dose[22]. It is safe in children and pregnant or lactating women. So, in the years, NB-UVB has become the most common used type of phototherapy. PUVA should be a treatment option in those patients not responding to NB-UVB or in V-VI skin phototypes, if needed.

Effectiveness of Combination Treatment: Surgery and Phototherapy

Since surgery and phototherapy have shown in the last decades good results in vitiligo management as monotherapy, Authors tried to combine surgical treatment and UV exposure in order to obtain quicker and better results in stable vitiligo. The treatment efficacy varies with duration, distribution of disease and type of vitiligo.

It seems that the therapeutic effect depends mostly from vitiligo location: face and neck usually show the maximum response in a short period, while proximal extremities and trunk show slowly and less repigmentation; acral zone are the most difficult-to-treat area with poor response rate. To assess the therapeutic success of a specific repigmentation method (in monotherapy and in combination), most authors use a percentage scale of improvement from 0% to 100% evaluating the clinical aspect of vitiligo. Data show that combination therapy increases the chances of repigmentation and lead to quicker results than surgery alone when phototherapy is performed post-surgery and pre-surgery[23]. The best timing for NB-UVB is not yet assessed but we could say that phototherapy before surgery could generate an immunosuppression of the recipient site and activation of melanocytes in donor site. These two effects could together create the best

condition to reach clinical improvement after surgery. Hypothetically, NB-UVB performed after surgery, could maintain the results obtained by combination therapy and also continue to improve repigmentation. No consensus is available yet. The main points not yet established are frequency of phototherapy, duration, dose, timing, cumulative dose since data from literature are extremely different. A common consensus was reached in the recommendation of NB-UVB than PUVA since it is safer and easier to perform according to recent international guidelines[1]. In our point of view, based on our experience, NB-UVB twice weekly for 2 months could be a reasonable preparatory treatment period followed by surgery and a subsequent phototherapy cycle twice weekly for at least other 2-3 months could improve and stabilize results. However, refractory patients could need longer exposure period, more than 2-3 months. Exposure should be prolonged until a satisfactory and stable repigmentation is reached. In addition, we suggest that patients following this novel combination therapy should not be treated simultaneously with other medical therapies for vitiligo which could act as confusing elements, at least in this preliminary phase in which data are not validated yet.

The Role of In Vivo Reflectance Confocal Microscopy (RCM)

RCM is a non-invasive, repetitive imaging tool that provides real-time images at a nearly cellular histological resolution[24]. In the last decades it has been used in both hyperpigmentary and hypopigmentary disorders since melanin is the strongest endogenous contrast in human skin[25]. In vitiligo can be useful to identify the form of stable vitiligo which may consequently underwent surgical treatment; to assess clinical response in repigmentation with different pattern after therapy and to evaluate persistence of clinical success or initial regression. In detail, active phase vitiligo can show a complete loss of melanin in lesional skin; disappearance of part of the bright dermal papillary rings at the dermo-epidermal junction; loss of integrity by part of the rings with decrease of melanin's content; presence of highly refractile inflammatory cells in the papillary dermis both at the lesional and adjacent normal skin. Interestingly, also perilesional skin shows loss of integrity of dermal papillary rings leading to ill-define border[11]. On the contrary, stable vitiligo shows complete loss of melanin in lesional skin and clear border, no changes in the content of melanin nor in the dermal papillary rings at the adjacent normal skin. Repigmentation instead could be detected and monitored with the identification of dendritic and highly refractile melanocytes which can lead to 3 different repigmentation. These patterns are (1) marginal repigmentation with highly refractile melanocytes moving from adjacent normal skin to lesional area; (2 perifollicular repigmentation with melanocytes surrounding hair follicle and (3) diffuse repigmentation with melanocytes distributed in lesional skin[11,26]. Notably, there is no difference with RCM between the distant normal skin of vitiligo patient and skin of healthy patients. This clue is important because suggests that surgery in vitiligo, which implies the aid of distal normal-appearing skin, can lead to improvement like it was healthy skin. These preliminary data should be implemented and supported by more clinical studies.

Conclusions

Vitiligo is a psychosocially debilitating disease in which an effective and multidisciplinary approach is mandatory. We suggest that phototherapy should be performed in addition to surgery since data have shown better clinical improvement than monotherapy. More data are needed to establish the best timing protocol. In this paper we highlighted the new and promising role of RCM in discriminate between active or stable vitiligo, assess repigmentation and monitor clinical results. Since melanin is the strongest endogenous contrast of the skin, RCM can play a role in the study of hypo-/hyper-pigmented disorder. Combination of phototherapy and surgery under RCM monitoring permit to reach better and quicker results. However, literature shows only preliminary data of the use of RCM in vitiligo, and no validated combination therapy protocols, therefore more studies are needed.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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