Letters

RESEARCH LETTER

Efficacy of Autologous Melanocyte Transplantation on Amniotic Membrane in Patients With Stable Leukoderma: A Randomized Clinical Trial

Vitiligo is a disfiguring disease with no definitive treatment options that significantly affects patients' quality of life. We aimed to compare, for what we believe to be the first time, the repigmentation efficacy of cultured epidermal cell suspension (CES) and amniotic membrane (AM)–cultured epidermal cell grafting (CEG) in the treatment of stable vitiligo.

Methods | A 2½-year (December 15, 2010, to June 5, 2013), randomized, double-blind, intraindividually placebo-controlled clinical trial with a 6-month posttreatment follow-up period (last follow-up, November 26, 2012) was carried out in the dermatology department of the University Clinic of Navarra, Spain. The study was approved by the local institutional review board (Comité Ético de Investigación Clínica de Navarra, Health Department, Government of Navarra, Spain). Written informed consent was received from all patients. The participants did not receive financial compensation.

Of 30 eligible patients with stable leukoderma, 24 individuals (15 women; age range, 18-57 years) were included in the final analyses. Dermatologic examination was performed on each patient to select one large vitiligo lesion (≥90 cm^2) or several smaller vitiligo lesions (up to 5 lesions, ≥90 cm^2 in total) per patient.

Amniotic membranes were obtained during elective cesarean delivery as described.[1] Melanocyte growth medium M2 (M2; PromoCell) was used for the culture. A superficial shave biopsy (0.5 cm^2) was taken from pigmented buttock skin under local anesthesia. Epidermal cells were obtained (Dispase II neutral protease, grade II; Roche; and TrypLE Select enzyme; Gibco-Life Technologies) as described.[1] Cells were subcultured in two 75-cm^2 culture flasks. When 70% to 80% confluence was reached, cells from one of the flasks were harvested with TrypLE Select enzyme and the cell suspension was replated onto the basement membrane side of AM prepared, as described above, at a density of 5 to 25 × 10^3 cells/cm^2. Cells were stained with monoclonal mouse antihuman melanosome antibody (clone HMB45; Dako), according to the manufacturer's instructions. After laser carbon dioxide ablation (5.5-7 W with 0.2-second pulse; Sharplan 1030) of the areas of vitiligo, 3 different skin areas (≥30 cm^2 per treated area) in each patient were randomly assigned to receive CES, AM-CEG, or no epidermal cell transplantation. A nonblinded investigator (P.R.) applied the different treatments. In summer, natural sun exposure was recommended during the following 2 months. In winter, UV-A irradiation (3-6 J/cm^2) twice per week for approximately 2 months was indicated.

The primary outcome was the percentage of skin repigmentation in each of the 3 intraindividual randomized areas at the 3- and 6-month evaluations. The secondary outcome was the patients' perception of pigmentation improvement. Statistical analysis was conducted using the Friedman test and the Wilcoxon matched-pairs signed-rank test.

Results | The study results are summarized in the **Table** and **Figure**. The highest percentage points of repigmentation were observed in the skin area receiving CES. Both CES and AM-CEG treatments at the 3- and 6-month evaluations appeared to have a greater repigmentation effect compared with the control intervention. The CES and AM-CEG areas presented similar mean values of the score that evaluated the patients' perception of pigmentation improvement at the 3- and 6-month visits. According to the patients' perception, the pigmentation improvement for the CES and AM-CEG areas was greater than in the control areas at both visits, although these differences did not reach statistical significance.

All adverse events were classified as mild and occurred in 3 patients. These events included inflammation in a verruca

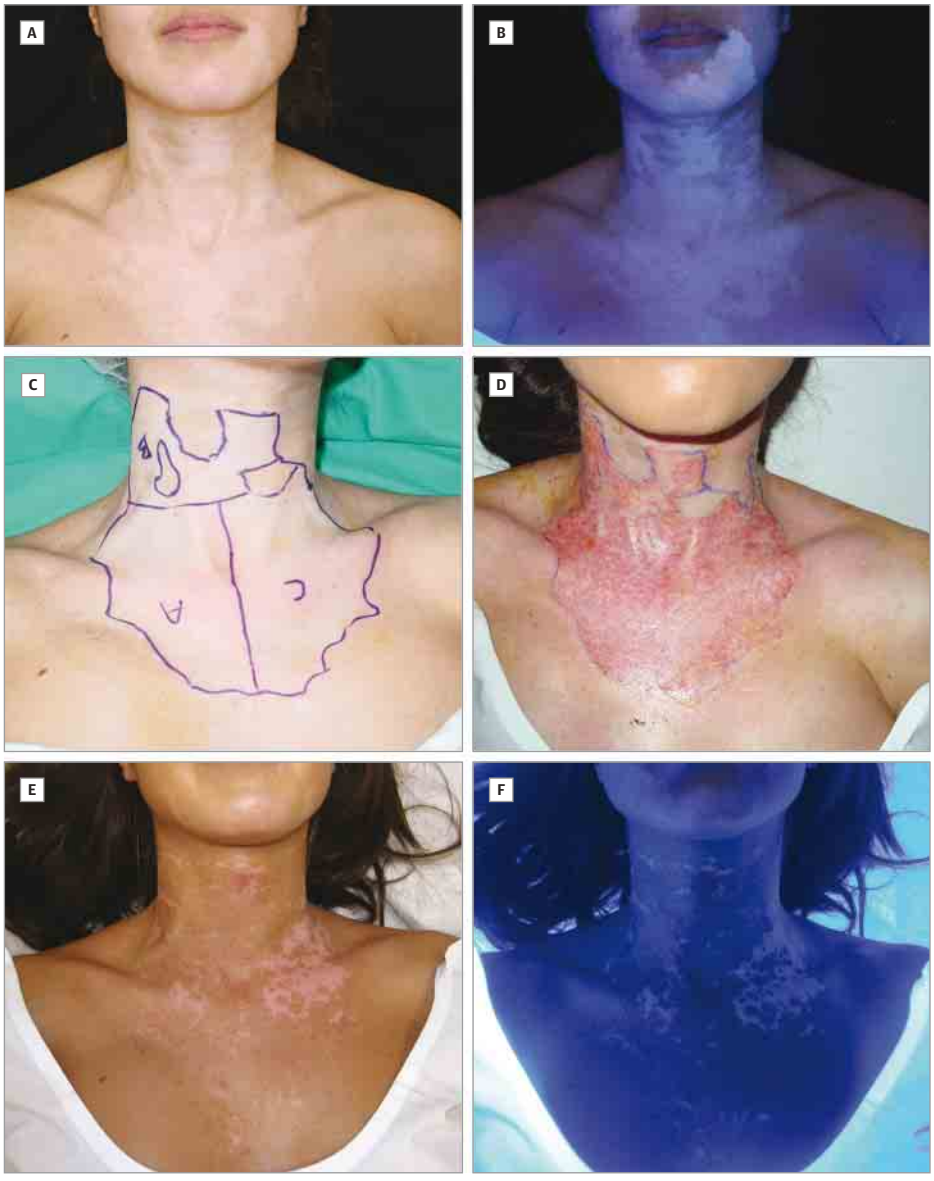
Table. Effects of the Experimental Intervention on Pigmentation

	Repigmentation at Follow-up, Percentage Points						Patients' Perception of Improvement at Follow-up, Score[a]					
	3 mo (n = 23)			6 mo (n = 19)			3 mo (n = 24)			6 mo (n = 18)		
Intervention	Mean (SD)	Median (25th-75th Percentile)	*P* Value	Mean (SD)	Median (25th-75th Percentile)	*P* Value	Mean (SD)	Median (25th-75th Percentile)	*P* Value	Mean (SD)	Median (25th-75th Percentile)	*P* Value
Control	23.5 (32.7)	5 (0-40)	.08	27.9 (34.5)	10 (5-50)	.81	1.5 (1.1)	2 (1-2)	.27	1.5 (1.2)	1 (0.75-2.25)	.33
CES	37.8 (37.8)	20 (5-80)		43.4 (38.3)	25 (10-85)		1.9 (1.7)	2 (1-3)		2.3 (1.1)	2.5 (1-3)	
AM-CEG	30.2 (35.7)	10 (0-70)		38.9 (38.7)	20 (0-80)		1.9 (1.4)	2 (1-3)		2.0 (1.5)	1.5 (1-3.3)	

Abbreviations: AM-CEG, amniotic membrane–cultured epidermal cell grafting; CES, cultured epidermal cell suspension.

[a] The patients' perception of pigmentation improvement in each of the 3 intraindividual randomized areas was evaluated at the 3- and 6-month visits using an 11-point self-reported repigmentation scale (from 0 [no changes since treatment started] to −5 [vitiligo worsened intensely since treatment started and incapacitated the patient for everyday activities] or 5 [vitiligo disappeared since treatment started]).

Figure. A Woman With Vitiligo



A, A woman in her 30s with vitiligo on the neck and trunk. B, The Wood light examination clearly reveals sites of vitiligo. C, Three areas were randomized to receive: amniotic membrane–cultured epidermal cell grafting (area A), cultured epidermal cell suspension (area B), or placebo (area C). The randomization process was designed and executed by a distance centralized randomization service formed by staff with no clinical involvement in the trial. A computer-generated, permuted, block-randomization scheme was used to allocate interventions. Patients, data analysts, and physicians involved in the recruitment, as well as those delivering the intervention or measuring outcomes, were blinded to allocation. D, The achromic epidermis was removed using carbon dioxide laser, with a similar clinical appearance 96 hours later. E, Results 6 months after transplantation. F, Wood's light examination following intervention.

vulgaris lesion in the first patient, minimal scarring in the second patient, and mild hypertrophy and delayed wound healing in the third individual.

Discussion | This study showed differences in the repigmentation efficacy of CES and AM-CEG in the treatment of stable leukoderma, although they were not statistically significant. Two previous studies[1,2] demonstrated the efficacy of AM as a scaffold for the implantation of autologous melanocytes in patients with stable vitiligo.

Factors that may explain the variable response to cellular implants include the anatomic site of the treated area and the history of the vitiligo regardless of the minimal period that vitiligo was stable and the type of vitiligo (segmental or nonsegmental).[3-5] These factors were fairly well controlled in the present study. The main limitations of the study were the small number of patients and the fact that follow-up could not be completed in all patients. The percentage of repigmentation achieved in the placebo area may be the result of epidermal trauma stimulated by UV-A irradiation, which could be a melanocyte-stimulating trigger to a reservoir of melanocytes.[6] In conclusion, this study suggests greater efficacy of the transplantation techniques compared with placebo, being slightly more evident with CES compared with AM-CEG.

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Biopsy Use in Skin Cancer Diagnosis: Comparing Dermatology Physicians and Advanced Practice Professionals

Histopathologic evaluation is the criterion standard for diagnosis of skin cancer. Underuse of biopsies may promote misdiagnosis, and overuse will increase cost and morbidity. There is no benchmark with which to quantitatively compare health care professionals' diagnostic accuracy and biopsy use. Prior studies suggest wide variability in biopsy use among practice settings and health care professionals.[1-5] We conducted a retrospective review on the number of skin biopsies needed per malignant neoplasm in our department. The recent article by Coldiron and Ratnarathorn[6] documents that, in 2012, midlevel health care professionals independently billed approximately 2.6 million dermatologic procedures, most of which required clinical distinction between benign and malignant lesions. To our knowledge, our study is the first to compare the number needed to biopsy (NNB) per malignant neoplasm between dermatology physicians and advanced practice professionals (APPs).

Table 1. Patient Demographics by Type of Health Care Professional

Characteristic	No. (%)			*P* Value[b]
	Physician (n = 458)	Advanced Practice Professional (n = 240)	Both (n = 45)[a]	
Age, y				
<18	13 (2.8)	0	0	<.001
18-64	277 (60.5)	211 (87.9)	28 (62.2)	
≥65	168 (36.7)	29 (12.1)	17 (37.8)	
Sex				
Male	238 (52.0)	88 (36.7)	14 (31.1)	<.001
Female	220 (48.0)	152 (63.3)	31 (68.9)	
Ethnicity				
Hispanic or Latino	3 (0.7)	1 (0.4)	0	.03
Non-Hispanic	445 (97.2)	235 (97.9)	45 (100)	
Unknown	10 (2.2)	4 (1.7)	0	
Race				
Black or African American	4 (0.9)	0	0	<.001
Asian	6 (1.3)	1 (0.4)	0	
White	435 (95.0)	234 (97.5)	43 (95.6)	
American Indian and Native Alaskan	3 (0.7)	1 (0.4)	2 (4.4)	
Native Hawaiian or other Pacific Islander	1 (0.2)	0	0	
Unknown	9 (2.0)	4 (1.7)	0	
Skin Type				
I-II	282 (61.6)	102 (42.5)	30 (66.7)	<.001
III-IV	80 (17.5)	66 (27.5)	12 (26.7)	
V-VI	2 (0.4)	0	0	
Unknown	94 (20.5)	72 (30.0)	3 (6.7)	

[a] Patients with more than 1 biopsy from 2 different types of health care professionals within the data collection period.

[b] *P* value obtained via χ^2 test or Fisher exact test.

Methods | We performed a retrospective study of all biopsies submitted to our laboratory by 13 dermatology physicians (5 men and 8 women) and 5 APPs (1 physician assistant and 4 nurse practitioners, all women) between January 1 and February 15, 2010. The study was approved by the University of Wisconsin Institutional Review Board. We reviewed requisition forms and clinical notes,