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Autologous hematopoietic stem cell transplantation reverses skin fibrosis but does not change skin vessel density in patients with systemic sclerosis

La greffe de cellules souches hématopoïétiques autologues améliore la fibrose cutanée, non la densité des vaisseaux cutanés chez les patients atteints de sclérodermie systémique

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ABSTRACT

Hematopoetic stem cell transplantation (HSCT) improves survival in patients with severe systemic sclerosis (SSc) by resetting the immune system. We studied how HSCT acts on the key SSc skin pathology findings (fibrosis and vascularization). In mean, 3 skin punch biopsies per patient (range 2–6) were analyzed from 13 patients (5 females) with severe diffuse SSc before and up to 96 months after HSCT. Fibrosis of the four skin layers was graded semi-quantitatively and an overall fibrosis score was then calculated. Vessel numbers and calibers were assessed in the superficial and deeper dermis after immune-staining for endothelial antigens (CD31, VE-cadherin and vWF). The median age of patients at HSCT was 47 (24–64) years. The overall median modified Rodnan skin score decreased from 24 to 10 ($P = 0.003$) at first follow-up within a median of 9 (6–36) months after HSCT as did the histological skin score ($P = 0.03$). The modified Rodnan skin score and the fibrosis score correlated positively ($r = 0.589$, $P < 0.001$). The vessels density did not significantly change after HSCT nor did the expression of the tested endothelial markers. Although improving skin fibrosis in patients with SSc, HSCT does not alter vessel density within skin biopsies.

Mots clés :
Sclérodermie systémique
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RÉSUMÉ

L'autogreffe de cellules souches hématopoïétiques (HSCT) améliore la survie des patients atteints de sclérodermie systémique (SSc) sévère par la réinitialisation de la réponse immune. Nous avons étudié comment l'autogreffe agit au niveau de la fibrose et la vascularisation cutanée au cours de la SSc. En moyenne, 3 biopsies cutanées par patient (extrêmes 2–6) ont été analysées, chez 13 patients (dont 5 femmes) atteints de SSc sévère, avant et jusqu'à 96 mois après autogreffe. La fibrose des quatre couches cutanées a été évaluée de manière semi-quantitative et un score global de fibrose a été calculé. Le nombre et le calibre des vaisseaux a été évalué dans le derme superficiel et profond après immunocoloration pour les antigènes endothéliaux (CD31, VE-cadherin et vWF). L'âge moyen des patients au moment de l'autogreffe était de 47 (24–64) ans. La moyenne globale du score de Rodnan modifié (mRSS) diminuait de 24 à 10 ($p = 0{,}003$) lors de la première évaluation, à une moyenne de 9 (6–36) mois après l'autogreffe, ainsi que le score global de fibrose ($p = 0{,}03$). Il existe une corrélation positive entre le score de Rodnan modifié et le score global de fibrose ($r = 0{,}589$,

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$p < 0{,}001$). La densité vasculaire n'a pas changé de façon significative après l'autogreffe, ni l'expression des marqueurs endothéliaux testés. Malgré l'amélioration de la fibrose cutanée chez les patients porteurs de SSc, l'HSCT ne modifie pas la densité vasculaire au niveau des biopsies cutanées.

1. Introduction

Systemic sclerosis (SSc) is a multisystem disease in which vasculopathy, autoimmunity and fibrosis contribute to the pathology and hence to organ dysfunction. Cutaneous manifestations of the disease often represent the first clinical symptoms leading to diagnosis. Although the exact pathogenesis of SSc is still unrevealed, skin symptoms are related to infiltration and increased synthesis of extracellular matrix by fibroblasts [1].

Vasculopathy manifests early in the disease and capillaries deteriorate and regress. Factors contributing to the failure of vascular regeneration include an imbalance between proangiogenic and antiangiogenic mediators, intrinsic abnormal properties of the cellular components of the vessels, and active suppression of angiogenesis by the immune system [2].

Among patients with diffuse SSc, the subset of patients with early and rapidly progressive disease course and internal organ involvement is notably associated with an increased mortality [3]. These patients with a poor prognosis have been treated by autologous hematopoetic stem cell transplantation (HSCT). The results of the phase III ASTIS trial [4] demonstrated a survival benefit of HSCT over cyclophosphamide pulse therapy. A decrease of skin thickness after HSCT measured by the modified Rodnan skin score (mRSS) has been reported after HSCT [4]. In addition we and others have previously assessed on a small number of patients the evolution of skin as measured semi-quantitatively on histology and compared to clinical evaluation by mRSS before and after HSCT; both groups proposed that clinical and histological skin fibrosis may be reversible after HSCT [5,6].

Capillaroscopy of the nailfolds (NFC) is considered as the gold standard for the assessment of vasculopathy in SSc. NFC findings in patients with SSc are rarefication of capillaries, the appearance of giant capillaries and bleeding. In SSc patients, the pathology of the nailfold capillaries usually progresses over time and has been considered irreversible [7]. To this end, we had originally studied NFC pattern before and after HSCT and firstly reported a rapid and sustained improvement of the numbers and the morphology of capillary patterns after HSCT in two patients [8].

However, technically, in a considerable number of SSc patients with very severe disease and extended skin involvement with handgrip retraction, it may be impossible to perform NFC. Moreover, collapsed capillaries are in fact difficult to distinguish from absent vessels.

Alternatively, capillaries can be analyzed and quantified in skin biopsies of patients with SSc. It has previously been shown that the number of capillaries in the affected skin of SSc patients is decreased compared to healthy controls and that 5 years after HSCT the density (numbers) of skin capillaries have improved in five out of seven tested patients [9]. We therefore designed the present study to test the hypothesis that autologous HSCT induces tissue remodeling including restoration of skin vasculature in patients with progressive diffuse SSc. To that aim, we longitudinally assessed the evolution of skin histology for fibrosis and vessel density in patients with SSc before and after autologous HSCT.

2. Patients and Methods

All included patients gave written informed consent and the study was approved by the St-Louis hospital (Paris, France) ethical committee. Patients have been treated within the ASTIS trial [4] at St-Louis Hospital. Only patients that have received at least one skin biopsy before and one after HSCT were included and analyzed.

Thirteen patients (5 females, 8 males), with a median age of 47 years (range 24–64 years) at HSCT, were studied for longitudinal outcome of skin pathologies. The treatment procedure for HSCT consisted of mobilization of peripheral blood stem cells using cyclophosphamide (4 g/m2 total dose and G CSF) followed by intensification using high-dose cyclophosphamide (200 mg/kg total dose), with Antithymocyte globulins (ATG) (7,5 mg/kg bw) and selection for CD34+ cells of the graft. The extent of skin involvement was evaluated by repeated measures of the mRSS [10], which were performed by the same local clinical investigator blinded to the results of histology.

All patients underwent a punch skin biopsy (4 mm in diameter). The biopsy was sampled at the site clinically judged to be most involved before HSCT (except the face) and during follow-up visits thereafter within the proximity of the previously affected skin area in the most affected site at each time point. In median, 3 biopsies per patient (range 2–6) were taken before ($n = 1$ for each patient) and up to 96 months after HSCT (Table 1). Biopsies were obtained between 03/2001 and 09/2009.

2.1. Histological quantification of skin fibrosis

Consecutive serial sections from paraffin embedded tissue, stained with haematoxylin–eosin–safran on Masson's trichrome and Sirius red were analyzed using standard light microscopy. The presence and distribution of fibrosis was assessed by 3 investigators blinded to clinical data within the four layers of the dermis: papillary dermis (PD), superficial reticular dermis (SRD), medial reticular dermis (MRD) and deep reticular dermis (DRD). Three increasing grades of fibrosis were then defined in comparison with normal controls as previously described [8]. The overall calculated histological fibrosis score was then established for each biopsy as: grade 1: weak fibrosis; grade 2: moderate fibrosis; grade 3: severe fibrosis.

2.2. Staining and assessment of dermal capillaries

The technique has been described elsewhere in detail [11]. For each patient, three microscopic slides containing a representative paraffin section from the skin

Table 1
Timing and site of skin biopsy for all SSc patients treated by HSCT before (O) and after (in months) the procedure.

Patient (number)	Biopsy (months after HSCT)	Region of biopsy at the different time points
1	0, 12	Abdomen, right forearm
2	0, 12	Abdomen, right tight
3	0, 12	Right tight, left tight
4	0, 9, 18, 24, 36, 48	Right forearm × 3, right tight, right forearm, right tight
5	0, 6, 12, 30, 54	Left tight, right forearm, left forearm × 2, right tight
6	0, 9, 30	Right forearm, left forearm, right forearm
7	0, 6, 9, 48	Right forearm, left forearm, right forearm × 2
8	0, 9, 24	Abdomen, right tight × 2
9	0, 9	Abdomen × 2
10	0, 15	Abdomen × 2
11	0, 36, 48, 60, 96	Right tight × 2, left tight, right tight × 2
12	0, 9, 30, 66	Right tight, left tight × 2, right tight
13	0, 6, 15	Right tight × 3