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# MIXED CONNECTIVE TISSUE DISEASE: A FOLLOW-UP STUDY OF 12 PATIENTS WITH SPECIAL REFERENCE TO COLD SENSITIVITY AND SKIN MANIFESTATIONS

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*Abstract.* A great variety of skin manifestations and the frequent occurrence of cold sensitivity, vascular symptoms with peripheral painful ulcerations, headache of migraine type, and muscle and joint symptoms are described in a series of 12 patients with an overlap syndrome compatible with mixed connective tissue disease (MCTD). The patients have been followed up for an average of 7 years. The peripheral symptoms on the extremities in particular were exacerbated by exposure to cold and caused much inconvenience and early disability for the patients. In addition to the symptoms generally connected with MCTD, some of the patients presented signs of other diseases of autoimmune type as well. For instance 2 of the patients presented autoimmune thyroiditis and one patient developed myasthenia gravis and cold agglutinin syndrome.

*Key words:* MCTD; Histopathology of the skin; Cold sensitivity; Headache

In typical cases the diagnosis of systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) does not present any difficulties, and well established diagnostic criteria are available (3, 4, 18). However, clinicians come across many patients with autoimmune types of disease, which do not fulfil these criteria; among them are the so-called overlap syndromes. The classification of these syndromes varies from institution to institution and no definite criteria have been established (5, 14, 16, 21). An overlap syndrome with clinical features from SLE, RA, progressive systemic sclerosis (PSS) and dermatomyositis has been defined by Sharp et al. (21, 22) as a distinct entitiy, viz. the mixed connective tissue syndrome (MCTD). A speckled pattern of antinuclear antibody fluoresence and antinuclear ribonucleic acid protein (anti-nRNP) antibodies have been considered typical for MCTD (5, 7, 21, 22). However, these antibodies are also found in patients with SLE and they have been connected with a low prevalence of renal disease (5, 15, 17, 20). On the other hand a clinical picture compatible with MCTD has been noted in a series of patients in whose sera these antibodies have not been found (5, 14, 21).

In the series of MCTD patients reported, Raynaud's phenomenon has been a common finding but otherwise little attention has been paid to the symptoms caused or aggravated by cold. In a group of patients with MCTD, followed up for an average of 7 years, we found sensitivity to cold to be a common complaint.

In this report special attention is paid to cold sensitivity, peripheral vascular symptoms and various symptoms aggravated by cold. The histopathological and immunohistological findings of various skin manifestations are reported.

## PATIENTS AND METHODS

The patients included in this study were taken from a series of 86 patients hospitalized at the Department of Dermatology, University Central Hospital, Helsinki in 1970–75, because of suspected or definite autoimmune disease. Those patients with clinical symptoms compatible with progressive systemic sclerosis, dermatomyositis and fulfilling the diagnostic criteria for SLE and RA were excluded (3, 4, 18). Fourteen of the remaining patients were diagnosed as having MCTD, on the basis of clinical and laboratory findings presenting overlapping features of SLE, RA, PSS and dermatomyositis. We were able to re-examine 12 of these patients during the period of September 1978 – April 1980. The mean age of the patients was 29.9 (range 17 to 41) years at the time of onset of the disease; 8 patients were women. Routine laboratory tests and the following serological examinations were serially performed; antinuclear antibodies were determined by the indirect immunofluorescence technique using mouse liver cryostat sections as antigen and FITC-conjugated antihuman globulin (total ANA titre) anti-IgG, (IgG ANA) and anti-IgM sera (IgM ANA). Serum immunoglobulin and $C_3$, $C_4$ levels were measured by

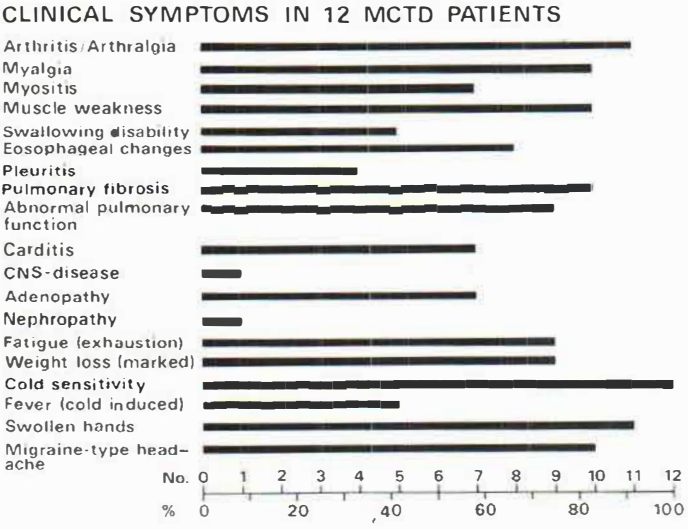


*Fig. 1.* Clinical manifestations in the 12 patients with MCTD.

fluoronephemetry (11) Waaler-Rose and RF-Latex test, cryoglobulins, and cold agglutinins were determined with standard techniques.

*Anti-RNP antibodies*

Antibodies against extractable nuclear antigens (ENA) were demonstrated by double immunodiffusion and by passive hemagglutination (2). The antigen preparation was obtained from rabbit thymus acetone powder (Pel-Freez Biologicals, Rogers, Arkansas) and has a protein concentration of 12 mg/ml (13).

Immunodiffusion was performed in 1% agarose by using heat-inactivated sera and the antigen preparation as such. The sera giving a precipitin line were subsequently retested in two-fold serial dilutions against ENA and ribonuclease-A (RNase, Sigma Chem. Co., St. Louis, Missouri) -treated ENA. The specificity of the antibodies was further established by using reference sera known to contain anti-RNP and anti-SM antibodies.

For the passive hemagglutination, human O Rh-erythrocytes were sensitized with the ENA preparation (2). Ribonuclease digestion of the cells was achieved by incubating a 10% suspension of the sensitized cells with an equal volume of ribonuclease-A; 1 mg/ml for 30 min at 37°C. The heat-inactivated sera were assayed in two-fold serial dilutions against ENA-coated and RNase-treated sensitized cells. Unsensitized tanned erythrocytes were used as controls. Titres above 40 were considered positive, and a four-fold or greater reduction in the titre obtained with RNase-digested cells as compared with the titre seen without RNase digestion was considered to indicate the presence of anti-RNP antibodies. Antibodies against native DNA were demonstrated by the *Crithidia luciliae* immunofluorescence test (1) and class-specific antibodies against denatured DNA were determined by the ELISA method (8).

The X-ray studies included radiography of the chest, esophagus and the entire gastrointestinal tract. Pulmonary function studies and pulmonary transfer factor estimations were performed.

Nineteen biopsies from skin lesions were obtained from 11 patients and deltoid muscle biopsies from 5 patients. The specimens were processed in paraffin wax, cut and stained with hematoxylin-eosin, toluidine blue and Alcian blue. Specimens for direct immunofluorescence (IF) examination were obtained from uninvolved, sun-protected skin in all cases and from skin lesions in 10 cases.

## RESULTS

The clinical manifestations observed during the observation period of an average of 7 years are presented in Fig. 1.

*Reaction to cold*

Raynaud's phenomenon was found in each patient and 8 of them presented this phenomenon as the first clinical manifestation of the disease process. All of the patients complained of cold sensitivity, arthralgia and/or myalgia which, especially during Finland's cold winter months, caused considerable disability. Five patients had attacks of fever after exposure to cold and 2 patients suspected that their attacks of headache were due to cold. Three of the patients were especially exposed to cold in their occupation (a diver, a florist and a carpenter) and shortly after the onset of their illness the became completely unable to continue their work. They had troublesome peripheral vasospasms and aches (in cold), stiffness and tenderness of the joints, especially of the hands. Gradually small painful ulcerations developed on the fingertips. One female with severe finger ulcerations developed gangrene in her right I and II finger, which had to be ampu-

Table 1. *Skin manifestations observed during the observation period in the 12 MCTD patients*

| Cutaneous manifestation | No. positive |
|---|---|
| Cutaneous lupus erythematosus | |
| chronic discoid | 3 |
| subacute LE | 3 |
| acute malar rash | 5 |
| Scaling erythematous extensor eruption | 6 |
| Rash over eyelids | 3 |
| Swollen hands and/or sclerodactyly | 11 |
| Finger ulcerations | 9 |
| Peripheral erythemas | 9 |
| Bird face | 4 |
| Scleroderma | |
| widespread | 3 |
| local | 2 |
| Teleangiectasiae | |
| facial | 6 |
| periungual | 9 |
| Hyperpigmentations (reticular) | 4 |
| Poikiloderma | 2 |
| Cold urticaria | 2 |
| Photosensitivity | 2 |
| Mucous membrane ulcerations | 2 |
| Alopecia | |
| diffuse | 2 |
| scarring | 1 |
| Subcutaneous nodules | 0 |
| Calcinosis cutis | 0 |

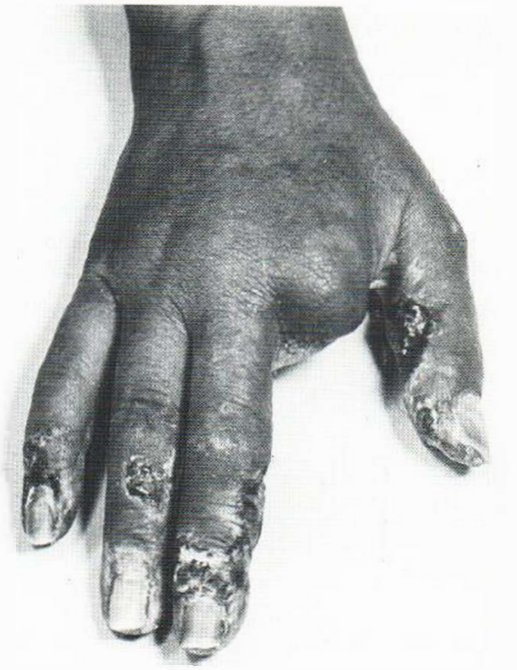

*Fig. 2.* In 4 years the peripheral vasospasms caused gangrene of the fingers.

tated (Fig. 2). Arteriography revealed only marked peripheral vasospasm.

*Myositis*

Muscle tenderness or weakness developed in all but 2 patients during the disease process. An electromyogram was performed on 7 patients and pathological findings were recorded in all but one case. All of the 4 male patients suffered from severe muscular weakness. After careful neurological examinations myasthenia gravis was diagnosed in one of them. In addition, a high titre of non-syphilitic antilipoidal antibodies and cold agglutinins were found in his sera. Corticosteroid and mestinone treatment was initiated. In the other patient with muscle atrophy of the upper extremities and thighs, myositis was confirmed by biopsy. He had normal serum creatine phosphokinase and aldolase values but the lactic dehydrogenase values were considerably elevated. The urinary creatine values were normal, while the acid mucopolysaccharide values were elevated. The third patient with muscle atrophy had severe myalgis aggravated by cold. Electromyography of the muscle disclosed increased fibrillation and fasciculation potentials and altered neuron potentials, especially in the lower extremities. In some muscles there was a clearly demonstrable denervation pattern. A muscle biopsy revealed only a few lymphocytes.

*Laboratory findings*

A speckled pattern of antinuclear antibodies was seen in 10 of the 12 patients, whereas one patient, the one with myasthenia gravis and cold hemagglutinin syndrome had a nucleolar and the remaining patient had a homogeneous fluorescence pattern. Anti-ribonucleoprotein (RNP) antibodies were demonstrated in 6 and anti-Sm antibodies in one patient. Two of the patients had anti-DNA antibodies as determined with the immunofluorescence method employing *Crithidia luciliae* as a substrate. Anti-ssDNA antibodies were found in 4 patients; IgG anti-ssDNA in 4 and IgM anti-ssDNA in 2 patients. Cryoglobulins in the serum of the patients were repeatedly sought but only in one patient was a positive findings encountered in the beginning of the disease process. Cold agglutinins in a high titre were found in one patient. The mean complement level of the patients was elevated as compared with

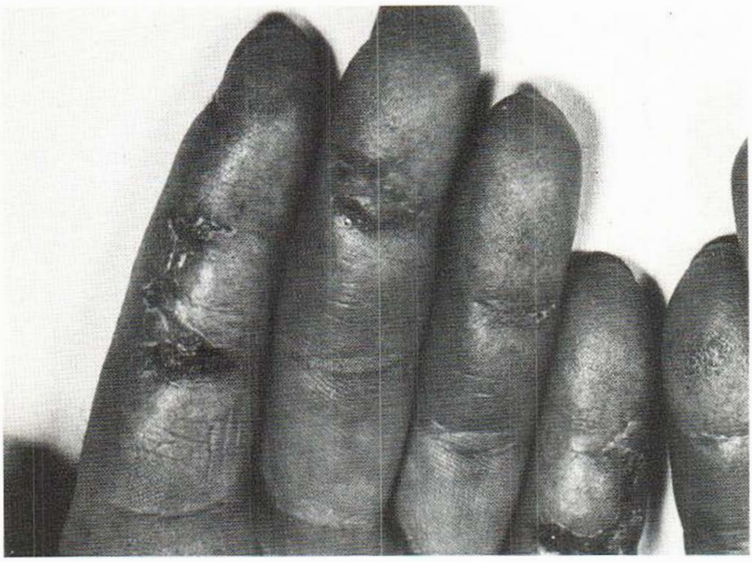

*Fig. 3.* Swollen fingers with ulcers.

the standard level of $C_3$. The $C_4$ level was slightly lowered in 2 cases.

Two female patients had thyroiditis with high titres of thyroid antibodies (antithyreoglobulin and antimicrosomal antibodies) in their sera. A thyroid biopsy disclosed chronic lymphocytic thyroiditis.

*Skin manifestations*

The skin manifestations of the 12 patients occurring during the observation period are summarized in Table I. Eleven of the 12 patients had swollen hands or some grade of scleordactyly. The hands—and especially the fingers—were swollen, giving a tapered or sausage-like appearance (Fig. 3). Small areas of necrosis and painful ulcerations developed on the fingertips. In 3 patients ulcerations were seen on the sclerodermatous skin, leaving round atrophic scars. Four patients developed bird face' and 2 had more widespread scleroderma-like involvement of the skin. Skin lesions compatible with lupus erythematosus included malar rash, discoid lesions (Fig. 4) and non-scarring diffuse erythematous lesions. Scaling erythematous or violaceous plaques over the extensor surfaces—especially over

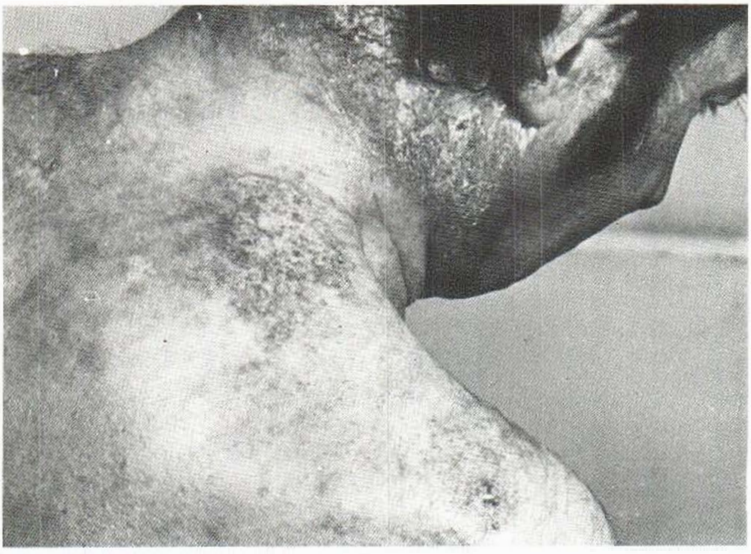

*Fig. 4.* Red scaling rash with small ulcerations and poikiloderma on the shoulders.

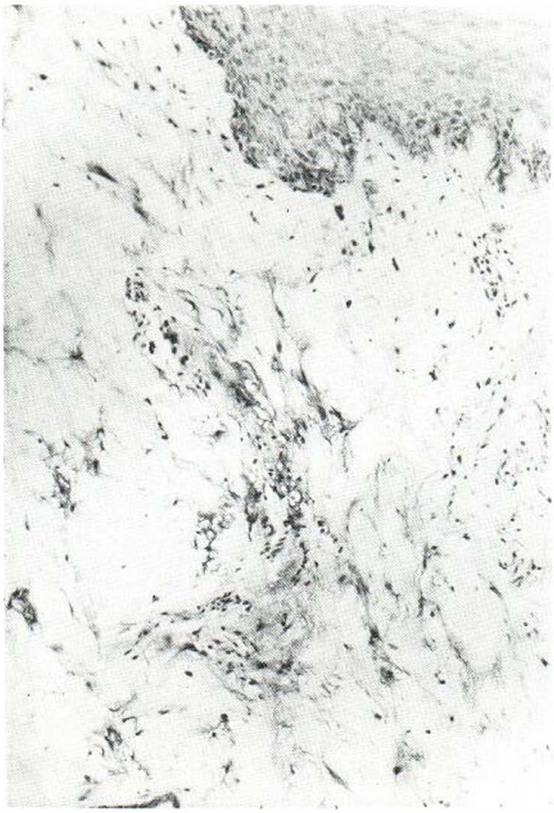

*Fig. 5.* Toluidine blue staining revealed a large amount of acid mucopolysaccharides as thin threads in the upper third of the dermis (Tol., 12.5×10).

the hand joints—were found in 6 of the patients, and 3 patients had the violaceous rash also over the eyelids, similar to that seen in dermatomyositis. One patient had scarring alopecia.

### *Histological findings of the skin*

Histopathological findings compatible with discoid lupus erythematosus were found in 3 of the patients. In 2 of these, in the active stage of the disease the lesions were ulcerative on the shoulders. A biopsy of one such lesion revealed massive amounts of mucopolysaccharides in the dermis (Fig. 5). In later biopsies of subsiding lesions these deposits had depleted but the typical features of erythematodes remained. In one of these 3 patients another biopsy taken from poikilodermatous skin revealed a liquefaction of basal cells in the atrophic epidermis and the upper dermis dilated capillaries were surrounded by melanin pigment and lymphocytes. In 2 patients biopsies taken from erythematous plaques clinically of subacute LE type showed capillary destruction and vasculitis of the dermal vessel walls, with neurotrophilic infiltrates consistent with SLE. Accumulations of acid mucopolysaccharides were found between collagen bundles.

In 4 patients biopsies were taken from sclerodermatous or acrosclerotic skin with ulcerations. These biopsies revealed homogenization of col-

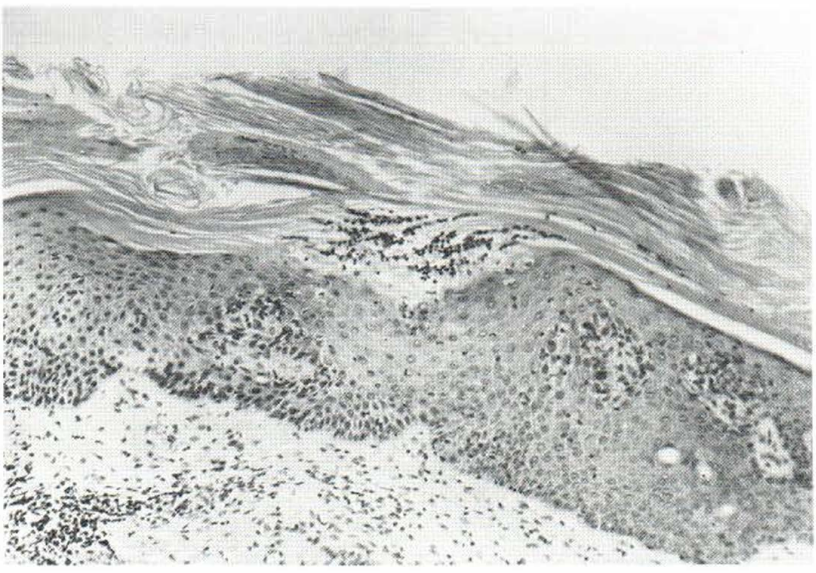

*Fig. 6.* A biopsy of extensor erythema showing 12 parakeratotic layers and accumulations of the nuclei of inflammatory cells in the subcorneal space resembling psoriasis (HE, 12.5×10).

lagen, mucoid degeneration of endothelial cells of the blood vessels, progressing to a complete destruction of the vessel wall. In some of these specimens the epidermis was thick and the basal cell layer was loaded with melanin pigment. In one of these patients a biopsy taken from an ulceration on the sclerodermatous skin of the leg revealed at all levels of dermal tissue, islands of multiple proliferating capillaries with hemosiderin deposits. In 2 of the male patients biopsies taken from extensor erythema showed a psoriform inflammatory reaction with parakeratotic epidermis, Kokoj's microabscesses, elongated rete ridges and prolonged dermal papillae (Fig. 6).

The immunofluorescent band test (IgM) was positive in 3 patients when the test was performed on uninvolved sun-protected skin. The lesional skin of 6 patients was examined and at the basement membrane immunoglobulin M was found in all these cases, and in addition immunoglobulin G in one case. Nuclear staining in epidermis was found in five specimens.

## DISCUSSION

The clinical symptoms of our 12 patients with an overlap syndrome corresponded to those previously described in the mixed connective tissue disease (5, 12, 14, 19, 21), with certain new features added.

The serological findings were compatible with those presented in previous reports (5, 7, 14, 19, 21). Anti-RNP antibodies were found in 7 of the cases, which is probably due to the fact that the patients with a negative finding were examined once only. They had been under corticosteroid therapy for several years at the time of the examination, and were clinically in remission.

As far as we know there have been no previous reports of an association of myasthenia gravis and MCTD and the occurrence of autoimmune thyroiditis as well as the 'cold hemagglutinin syndrome' (23) are also exceptional. Even though Raynaud's phenomenon is a well known and a common symptom in the MCTD syndrome, its association with cold and cool weather with its effect on the prognosis, have not been emphasized in other series reported from countries where the winter is not so long and cold as it is in Scandinavia. In our series Raynaud's phenomenon, stiffness and tenderness of the peripheral joints aggravated by cold, and cold-induced attacks of fever were in many cases the initial symptoms. The most prominent skin manifestation was swelling of the hands, especially of the fingers, leading to tapered or sausage appearance, and small painful necrotic areas and ulcerations on the fingertips. In 3 patients ulcerations were also seen on the sclerodermatous skin.

So far, histological studies on the skin in MCTD have not been done consistently enough to define the typical changes; in our series the conspicuous feature in sclerodermatous skin was the peculiar mucoid degeneration of endothelial cells in dermal capillaries and small vessels, without acid mucopolysaccharides in the vessel wall. In some of our cases these peripheral vascular manifestations developed to extreme stages such as finger ulcerations, gangrene and even amputation, and in every case they led to ultimate disability. Erythematodes were found in 3 patients, and in 2 of these cases deposits of acid mucopolysaccharides were present in the dermal tissue, 2 findings typical of dermatomyositis (9, 24). Proliferating capillaritis found in one of our patients has also been reported in connection with the peripheral vascular syndrome associated with circulating anticoagulants and nonsyphilitic antilipoidal antibodies (10). Both poikiloderma and extensor erythema usually occurring in dermatomyositis, were also found in our series. Evidently there is no outstanding skin marker typical of the MCTD syndrome.

The prognosis of patients with MCTD has been considered better than that of patients with classic SLE, and is not connected with severe renal disease (5, 9, 14, 20). However, contradictory opinions have also been expressed and there are some studies indicating that the mortality is about the same in these diseases (6). Corticosteroid treatment has been considered of great benefit. The scleroderma-like skin changes, Raynaud's phenomenon, esophageal and pulmonary changes have been reported to disappear or at least improve markedly after steroid therapy (20, 21). In our series we found it difficult to estimate the value of corticosteroid treatment for several reasons. First of all the symptoms of MCTD varied, as did the serverity and course of the disease. In many cases the general condition of the patient improved but, in contrast to previous findings, it had very little effect on the scleroderma-like skin changes or other skin manifestations. Corticosteroids had hardly any effect on Raynaud's phenomenon or on the other symptoms induced or aggravated by cold. The esophageal and

pulmonary changes were probably too advanced to respond. Altogether 5 of the patients became early disabled because of sensitivity to cold and peripheral symptoms.

There is still controversy over whether MCTD should be considered a distinct entity. The diagnosis is based mainly on clinical symptoms, excluding SLE and RA in the first place. The etiopathogenesis of MCTD is not known and on the basis of this study no far-reaching speculations can be made. However, the unusual cold sensitivity observed in these patients is an interesting feature. In northern countries MCTD must be considered as a serious illness and the patients ought to be observed closely.

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