DIRECT IMMUNOFLUORESCENCE OF CLINICALLY NORMAL SKIN MAY INDICATE SOME TYPES OF SYSTEMIC DISEASE

Z.Vlasin, M.D., DSc.,Prof.
H. Jedlickova, M.D.
I. Hlubinka, Ph.D.
J. Rulcova, M.D., PhD.
Masaryk University Hospital, Brno - Bohunice, Czech Republic

Abstract :

Healthy skin from the sun-protected part of the forearm was examined by direct immunofluorescence in the following groups of patients : SLE(60), serious systemic diseases(87) - partly patients with complaints resembling SLE but not fulfilling the American Rheumatism Association criteria, further patients with systemic sclerosis, chronic liver disease, diabetes mellitus, glomerulonephritis and diseases of the gut - and healthy volunteers (19). The dermal-epidermal junction zone, the vessel walls and the eccrine sweat glands with their secretory and ductal parts were evaluated. The subepidermal deposits of IgG and IgM were detected in 52% and C3 in 27% of SLE patients. In the SLE Group IgG was detected in the basement membrane zone of sweat glands in 47% and the vessels showed dilatation with IgG deposits in their walls in 18%. Fibrin deposits indicated vessel impairment (50%). As the immune complex deposition is an integral part of tissue damage in serious diseases, it was not surprising that the group of non-SLE systemic diseases patients showed remarkably similar DIF patterns of immunoglobulin deposition, but their prevalence and intensity were diminished/e.g. the sub epidermal IgG deposits were detected in 31% of this group. In healthy volunteers IgM was detected in the subepidermal area and in the vessel walls in 10%. It is important to take the biopsy from the sun-protected part of the forearm and examine the sweat glands area too.

The dilated vessels with IgG and fibrin deposits in clinically normal skin, commonly described in sun exposed skin in porphyria cutanea tarda, may indicate either SLE or chronic liver impairment and severe diebetes mellitus.

Introduction :

Direct immunofluorescence (DIF) of involved as well as of uninvolved skin is a useful diagnostic tool in systemic lupus erythematosus (SLE). The dermal- epidermal junction zone (DEJZ) is routinely examined and the deposits of IgG, other immunoglobulins and complement are well known as the "lupus band (LB)[1,2,3,4,5,6,7,8 et al.] The lupus band was described also in some other diseases [9,10,11] and even in the skin of healthy persons [12,13,14]. The DIF findings in the uninvolved skin are mostly due to the pathologic activity of immune complexes (IC) in tissues. Therefore the typical DIF changes could be detected first of all in SLE and its variants. The LB presence in nonlesional skin of SLE patients suggests mostly the concomitant renal involvement[15,16,17].

In patients with glomerulonephritis the seemingly uninvolved forearm skin may show considerable immunoglobulin deposits when examined by DIF[18]. The sun radiation provokes the IC deposition too and the immunoglobulin deposits in the sun exposed skin of healthy persons are a relatively common finding [13,14]. Therefore the skin not exposed to the sun is to be preferred for DIF examination. We suppose that any serious systemic disease with elevated circulating IC levels can simulate to some extent the well known subepidermal deposits or other DIF changes typical for SLE. In order to complete the relatively scanty data in this field we have studied the DIF findings in the clinically normal skin in groups of patients with SLE [19], with other (non-SLE) systemic diseases [11] and with diseases of the gut [20].

This paper summarizes and evaluates all these previously obtained data with some further clinical implications. We evaluated not only the findings in the basement membrane zone (BMZ), but also the changes in the vessel walls and in the eccrine sweat glands (ESG) area.

Patients and methods :

In 147 patients with SLE and with other systemic diseases and in 19 healthy volunteers the clinically normal skin from the sun-protected part of the forearm was examined by DIF. The skin samples were obtained by biopsy and divided in two parts - for DIF and for routine histopathology. The latter had only control value and is not discussed in this paper. The sample for DIF was snap frozen in liquid nitrogen and processed in a routine way. Sections were tested for the presence of IgG, IgA,IgM, C3 and fibrin. At the same time the following criteria were analyzed : history of the disease, clinical examination/skin changes, occurrence of the American Rheumatism Association (ARA) criteria (21). The blood

serum samples were tested for the presence of anti-nuclear antibodies (Hep-2 cells), anti DNP,anti ss DNA, anti ds DNA, anti ENA, anti Ro (SSA), anti La(SSB), anti cardiolipin and anti VDRL antigen <IgG,IgM,IgA> antibodies (ELISA). Proteinuria/ 24h was measured in SLE patients. After analysis of all obtainable clinical data the DIF results were evaluated in the following groups of patients :

1) SLE and its variants (ARA criteria positive)-60 patients
2) other serious systemic diseases - 87 patients. This rather unhomogenous group consisted of 39 patients with complaints resembling rheumatic disease/e.g.arthralgias, fever, malaise/but not fulfilling ARA criteria, 15 patients with systemic sclerosis, 11 patients with colitis ulcerosa, 2 patients with Crohn disease, 9 patients with chronic liver disease, 6 patients with insulin dependent diabetes mellitus and 5 patients with IC glomerulonephritis with proteinuria.
3) healthy volunteers - 19 persons The mean age was 43,6 years in SLE patients, 49, 1 years in systemic diseasepatients, and 41,2 years in healthy controls.

Results and discussion :

The overview of the results is given in Table 1. The presence of the deposits of immunoglobulins, C3 and fibrin in the BMZ, in the vessel walls and in the BMZ of ESG difffers in all three groups. The difference between the findings in the skin of healthy volunteers and other examined groups is remarkable (Graph 1,2,3).

SLE patients :

The deposits of IgG and IgM at the BMZ were the most common finding/both in 52%/. The subepidermal band had mostly granular pattern (Fig 1). Detection of IgM alone is less important than the presence of IgG and/or the simultaneous proof of more immunoreactants.

In approximately 10% of SLE patients the dilated vessels with homogenous deposits of IgG and fibrin in their walls were detected.(Fig.2,3,4 and 5). This type of vessel change is common in sun-exposed skin of porphyria cutanea tarda [22,23,24,25].

According to our experience the detection of these vessel changes in normal skin may indicate e.g. the diagnosis of SLE, hepatic disease, severe diabetes mellitus or some other serious disease (Fig.6). Presence of IgA in vessel walls correlates with IgA nepropathy [26].At the BMZ of ESG the most conspicuous change was the BM thickening, sometimes with their distortion and deposits of IgG/mostly of homogenous type/(Fig.3) This can be parallel to the BM thickening and Ig deposits in glomerulus[18]. These changes are caused not only by the glomerular damage in SLE but also by other factors e.g.the aging process which is accompanied by accumulation of collagen fibres [27]. The ESG basement mem-

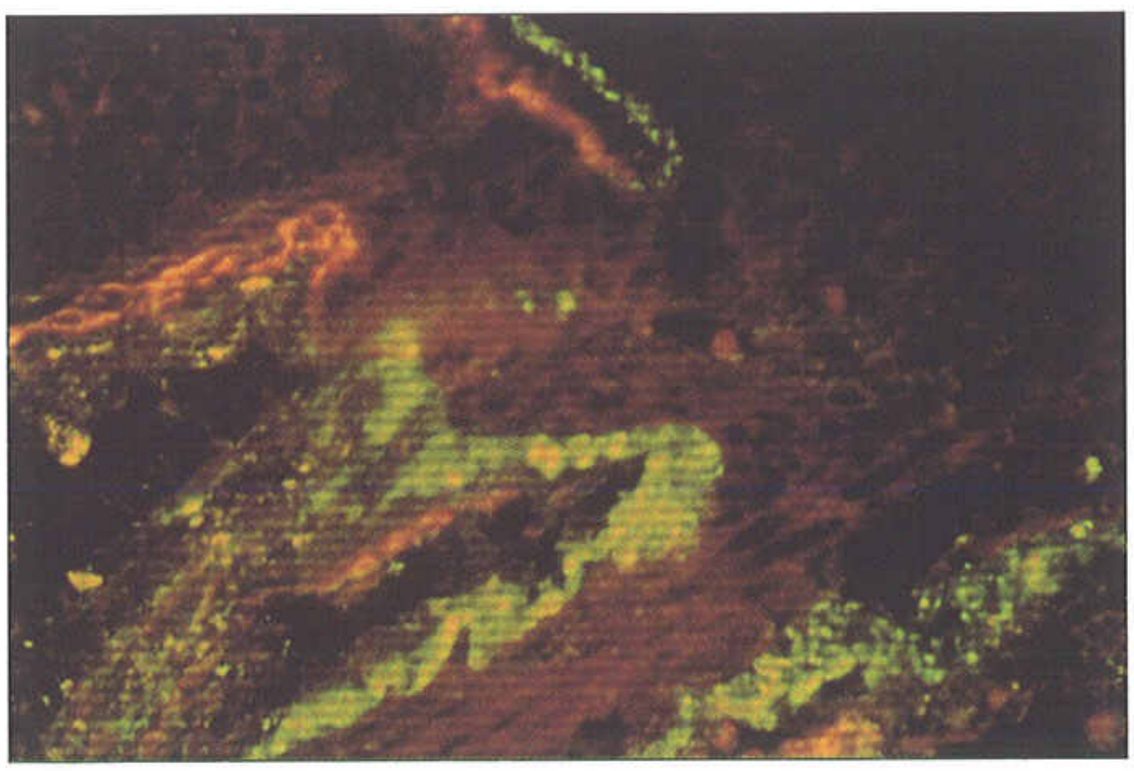


Fig. 1. Granular IgG deposits in the BMZ of healthy skin in a SLE patient. Lupus band of depicted intensity is more characteristic for lesional skin DIF 400x.

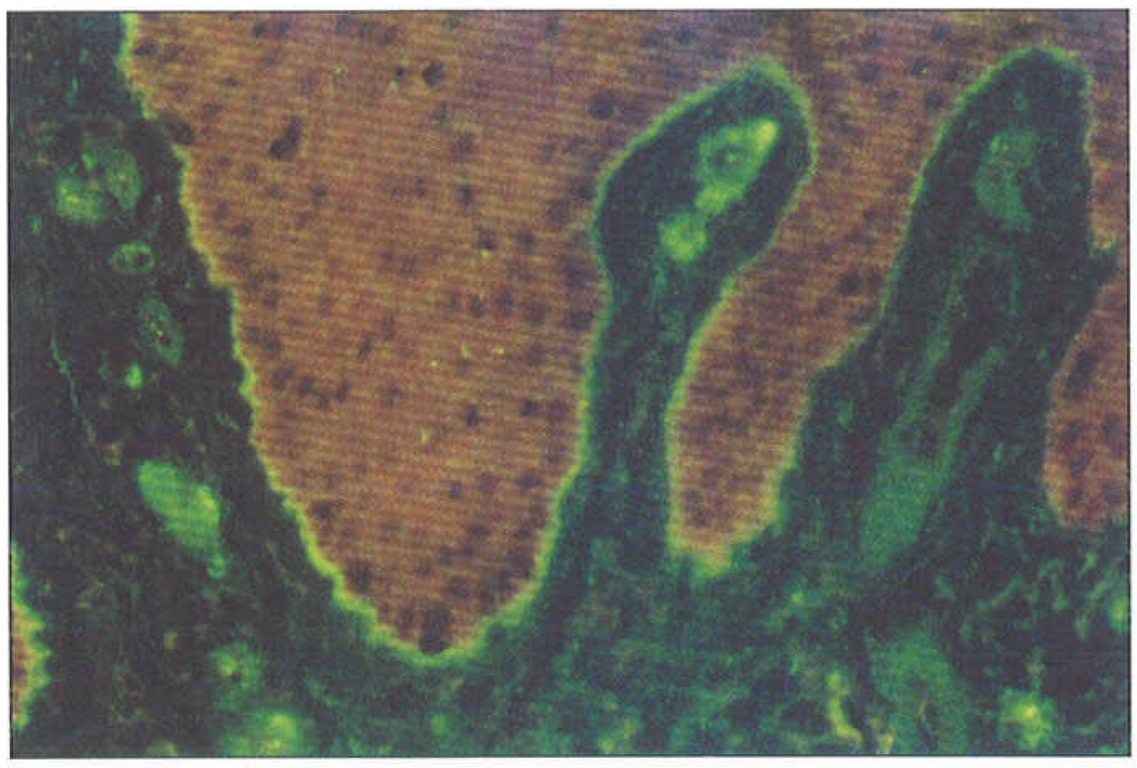


Fig. 2. Dilated papillary vessels with homogeneous IgG deposits. Traces of homogeneous LB in DEJZ. SLE patient. 200x.

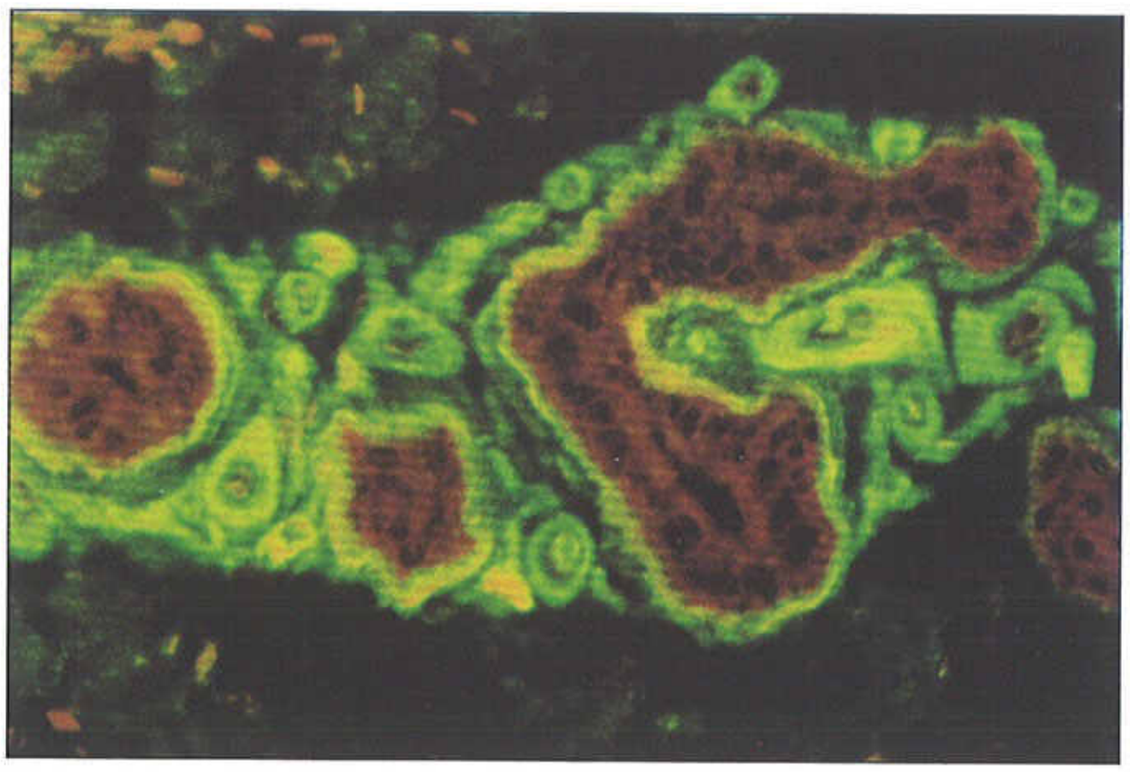


Fig. 3. ESG show homogenous IgG deposits in the BMZ of both secretory and ductal parts and in the vessel walls. Healthy skin in a patient with diabetes mellitus and chronic hepatitis DIF 400x.

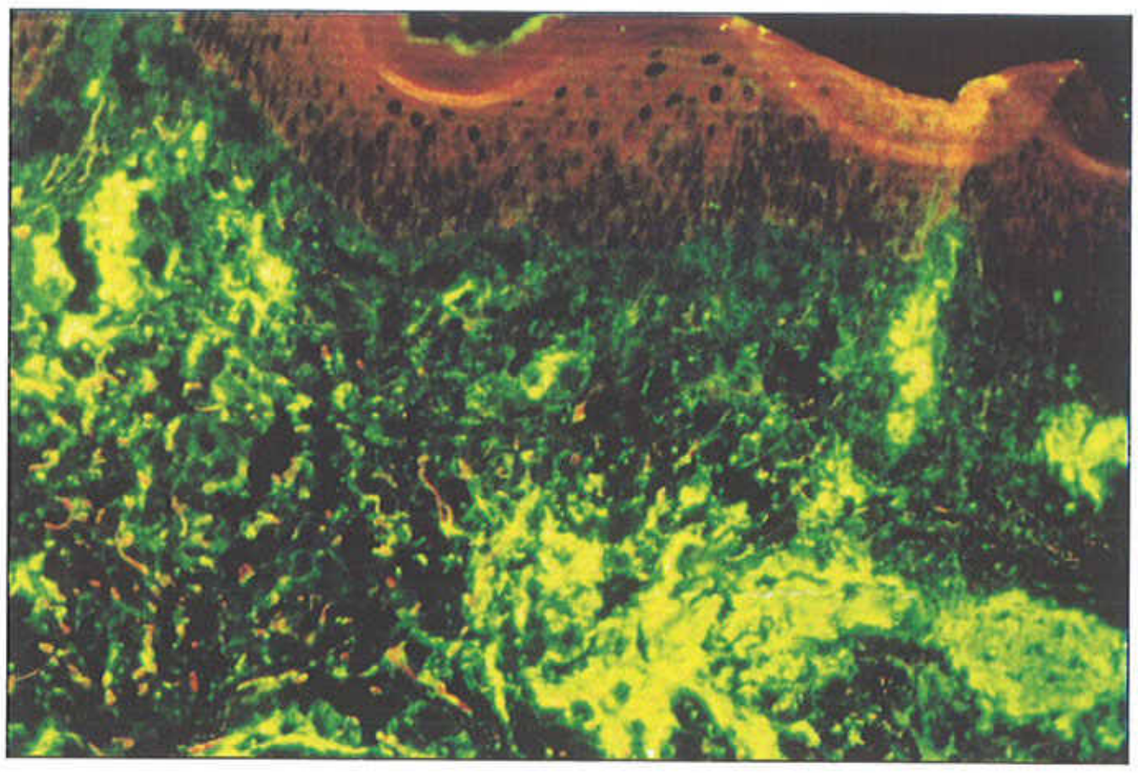


Fig. 4. Fibrin deposits around dermal vessels of healthy skin indicate systemic type of vascular damage in drug induced SLE DIF 200x.

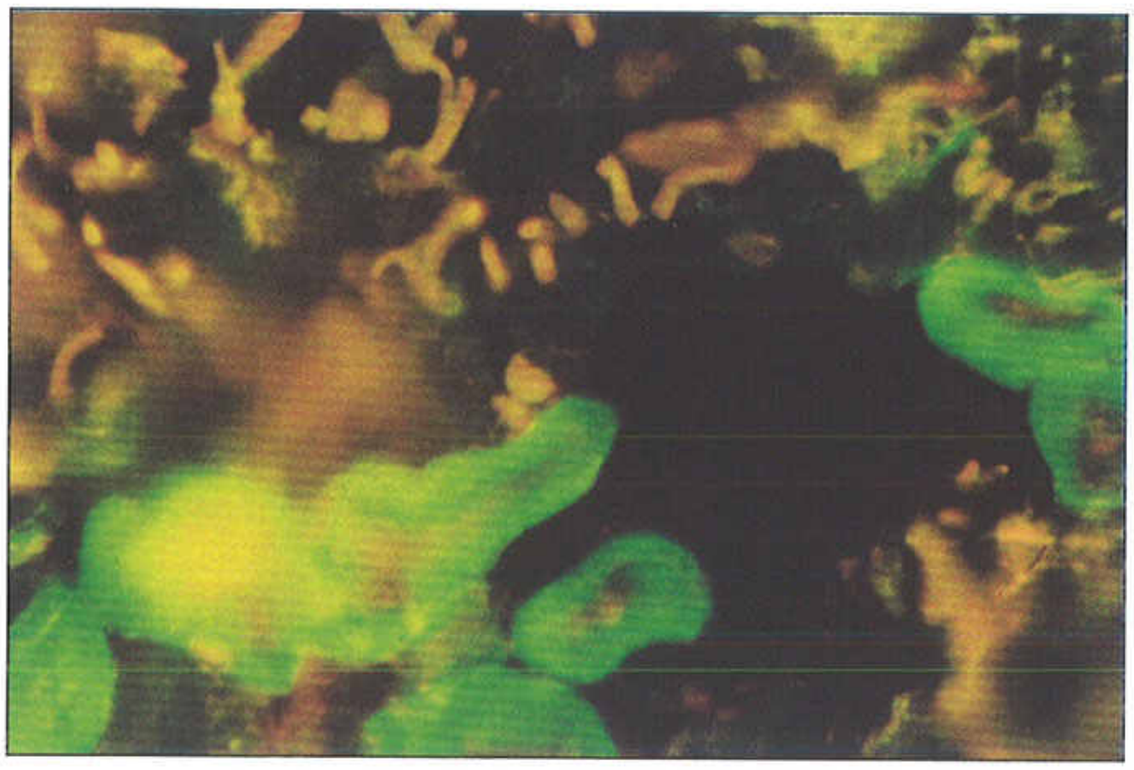


Fig. 5. Thickening of vessel walls with homogenous IgG deposits in SLE. Similar changes can be seen in chronic liver disease and diabetes mellitus DIF 400x.

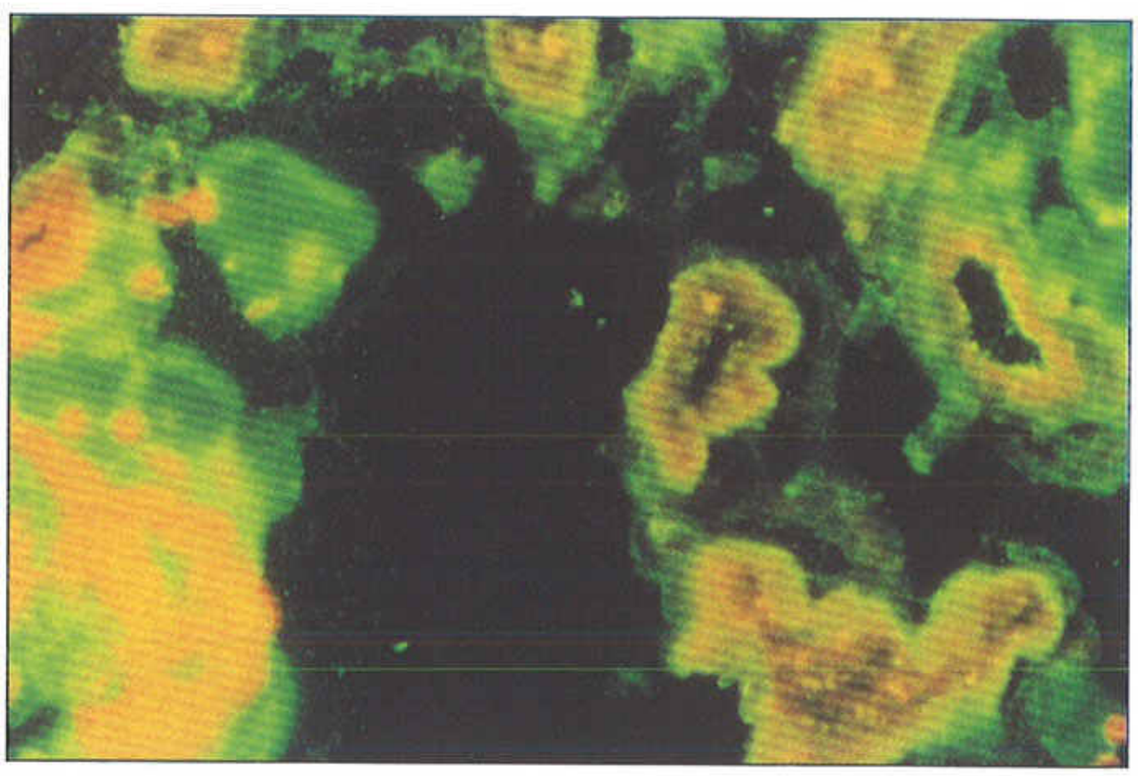


Fig. 6. Thickening, distortion and deposits of IgG in BMZ of ESG. Healthy skin of the forearm in an ulcerative colitis patient DIF 200x.

brane thickening with deposits of immunoreactants is quite common (unimportant). DIF finding in very old persons, too.

The group of non-SLE systemic diseases :

In this group there were found the subepidermal IgG deposits in 31% and the deposits of immunoreactants were less intensive. In spite of this the difference in comparison with the group of healthy volunteers is striking. The group was rather unhomogenous but some details are to be mentioned: The subepidermal Ig G deposits were detected in 25% of 11 patients with ulcerture colites, 40% of 15, patients with systemic sclerosis, 66% of 9 patients with chronic hepatic diseose and in 33% of 6 patients with severe diatbetes mellitus. The results in the group of systemic diseases were influenced by the fact that only the most serious cases, usually resembling SLE were examined. The subepidermal IgG deposits in systemic sclerosis are not common, but the presence of IgM and C3 was described [28]. IgG presence in our systemic sclerosis patients could be explained by the fact that 5 of them suffered from renal disease with proteinuria.

In ulcerative colitis and Crohn disease the band like depositions of IgG in DEJZ were detected fre-

quently and in addition the morphological changes of sweat glands with IgG deposits were observed[20] (Fig.6). Surprisingly, in 54,5% of patients with ulcerative colitis the positive pemphigus-like intercellular IgG/occasionally with C3/in epidermis was detected (fig.7). Our explanation is that this phenomenon can be drug induced.

The vessel walls showed nearly similar results as in SLE group. The frequent deposition of fibrin in vessels is remarkable and it should be remembered that it indicates some types of tissue damage. A homogenous type of IgG or fibrin deposits in dilated vessels similar to changes in SLE (Fig.5) and porphyria was characteristic also for chronic liver disease and diabetes mellitus.

The IgG deposits in the BMZ of ESG were relatively common (37%), C3 and fibrin were detected both in 18%. The BM was often thickened. Morphological changes of ESG with deposits of IgG were conspicuous in patients with ulcerative colitis and Crohn disease.

In our former study [18] we examined by DIF the renal biopsy specimens and the healthy skin in patients with immune complex glomerulonephritis (GN). We had found the deposits of immunoglobulins and/or C3 and fibrin in glomerular area in 83,7%.

The parallel DIF of the healthy skin of the forearm revealed the presence of LB in 18,4%. 63,3% of GN patients showed deposits of at least one immunoreactant.

The mentioned results together with other reports indicate that the DIF changes in healthy skin can be found in any IC disease, even without visible skin lesions (Fig 8,9,10).

In conclusion the DIF examination of the healthy skin of the forearm is a valuable complementary method in diagnostics of SLE. However, other serious systemic diseases may show similar reaction pattern as in SLE. It can be explained by the depositions of IC present in any serious systemic disease. According to our experience it is advisable to focus not only on the DEJZ but also on the vessel walls and the BMZ of adnexal structures, preferably the sweat glands. Although in healthy controls, no considerable DIF findings in ESG were detected, the morphologic changes of the BM can be often expected with increasing age and with serious diseases. The results have to be interpreted with caution, but if an extensive damage of ESG is found, the impairment of renal function and glomerular damage is probable and should always be excluded. The homogenous deposits of IgG and fibrin in the walls of dilated vessels in clinically normal skin may indicate not only SLE but also chronic hepatic disease and diabetes mellitus.

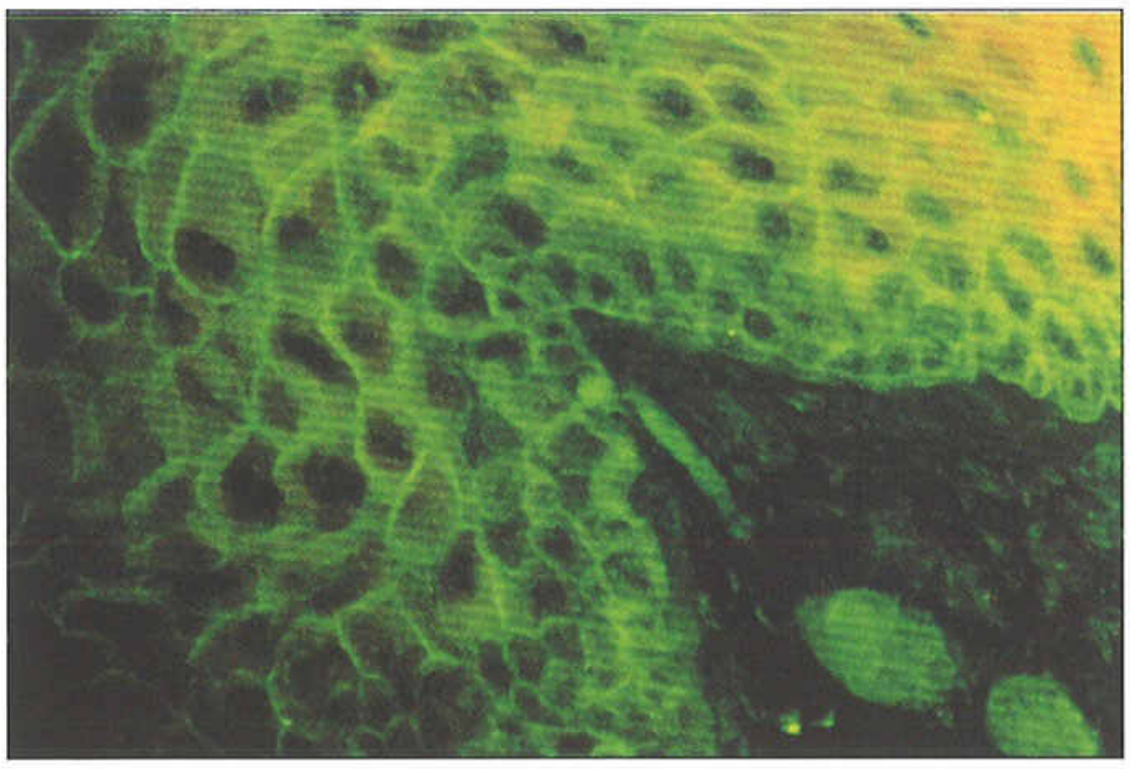


Fig. 7. Deposits of IgG in intercellular spaces similar to findings in pemphigus vulgaris. Healthy forearm skin of a patient with ulcerative colitis DIF 400x.

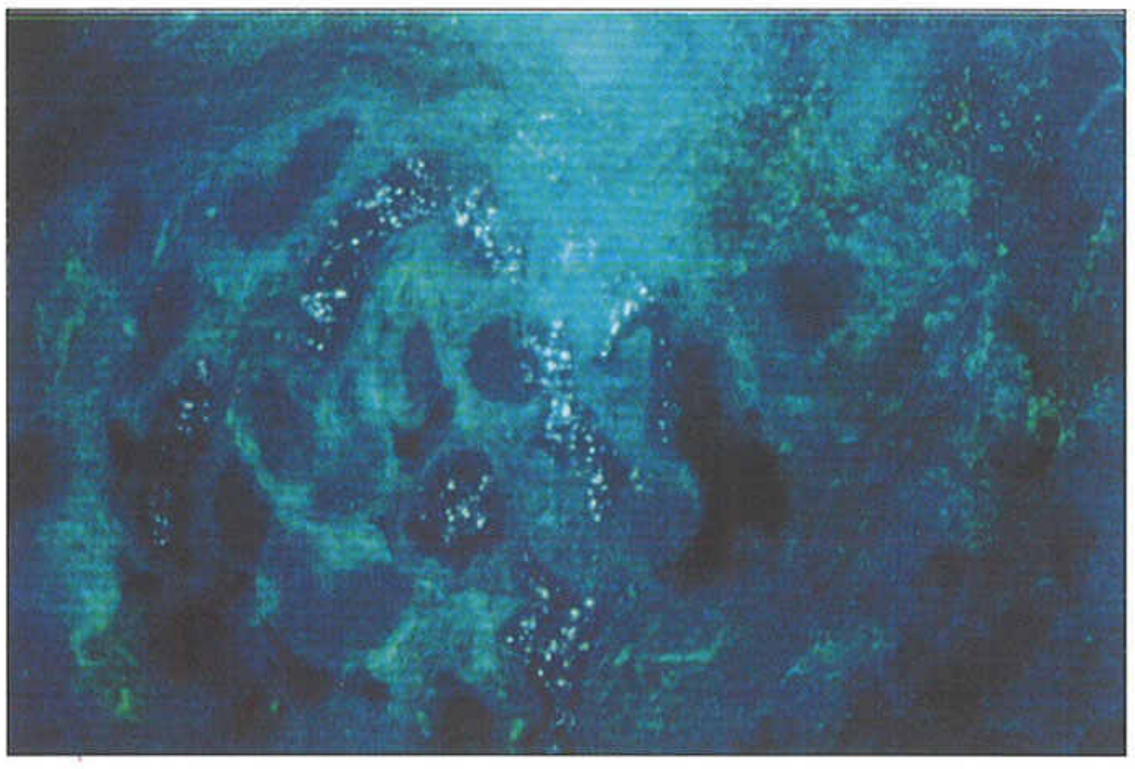


Fig. 8.- ESG from healthy forearm skin of a patient with severe glomerulonephritis/end stage kidney/. Both secretory and ductal parts of ESG are compressed by rich protein deposits (without specific fluorescence) DIF 160 x (from previous study(17).).

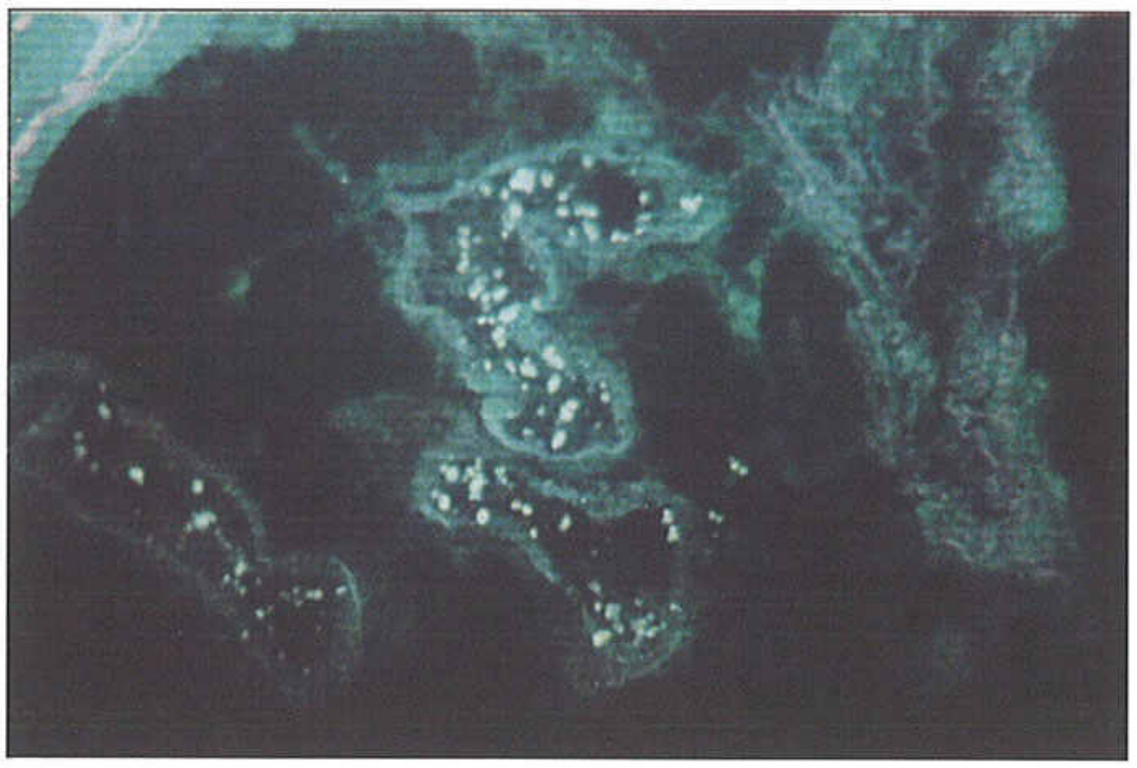


Fig. 9.- Eccrine sweat gland of a healthy person in fluorescence microscope/no conjugate was added/. The BM is not thickened, the non specific fluorescence of lipoid granules in secretory parts of ESG is a constant finding DIF 200x.

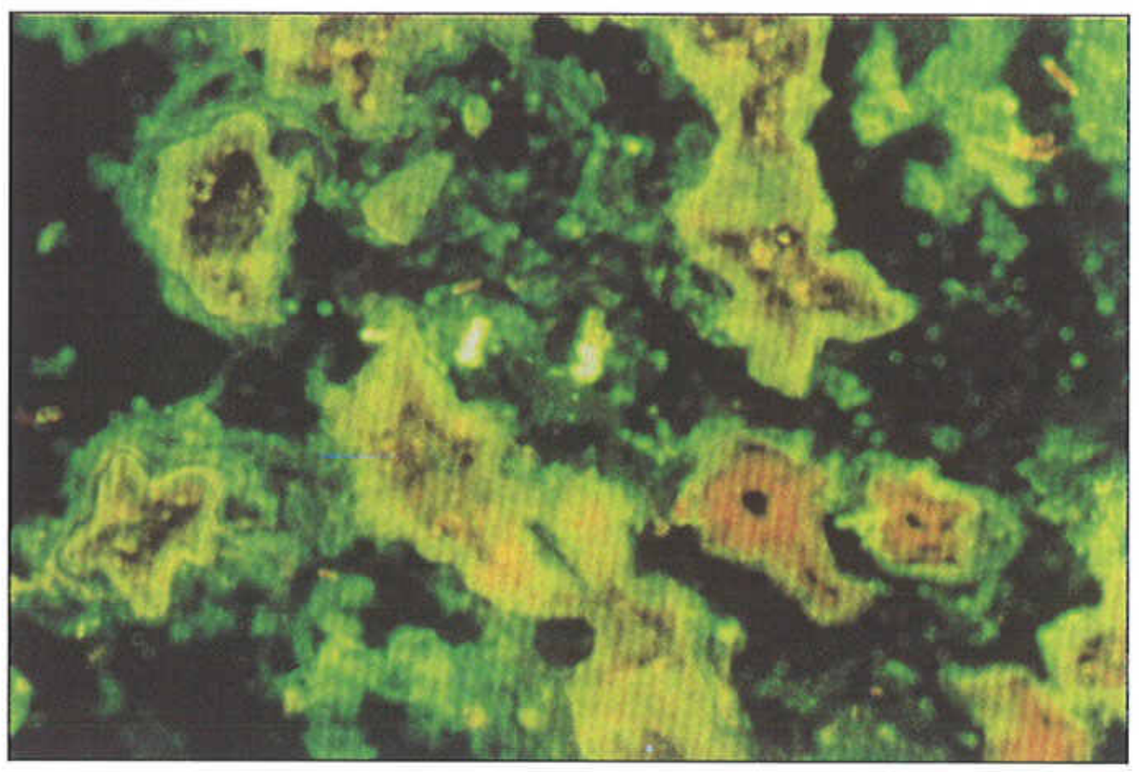
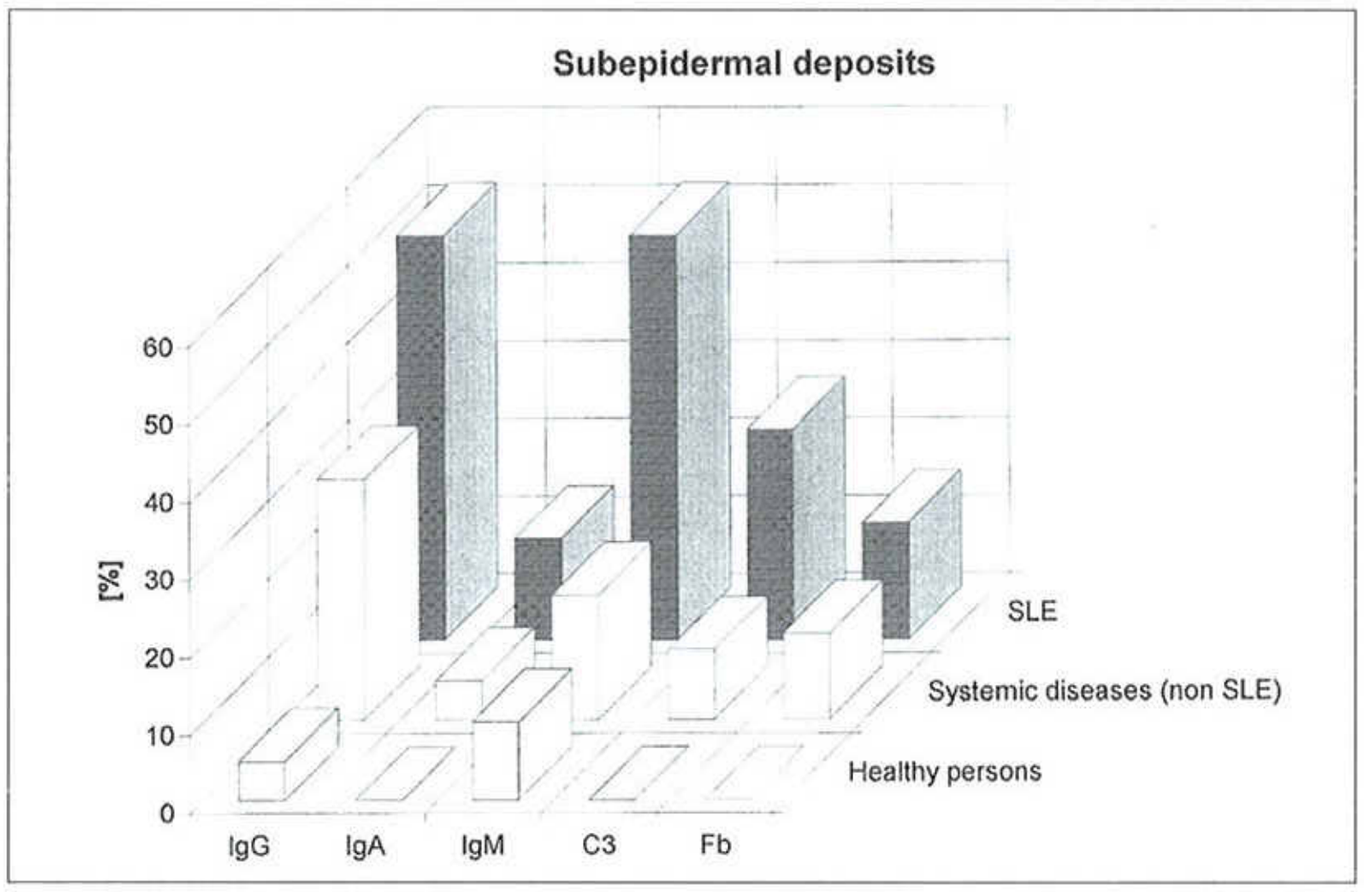


Fig. 10. IgG deposits in the BMZ of ESG in a patient with systemic scleroris, glomerulopathy and proteinuria DIF 200x.

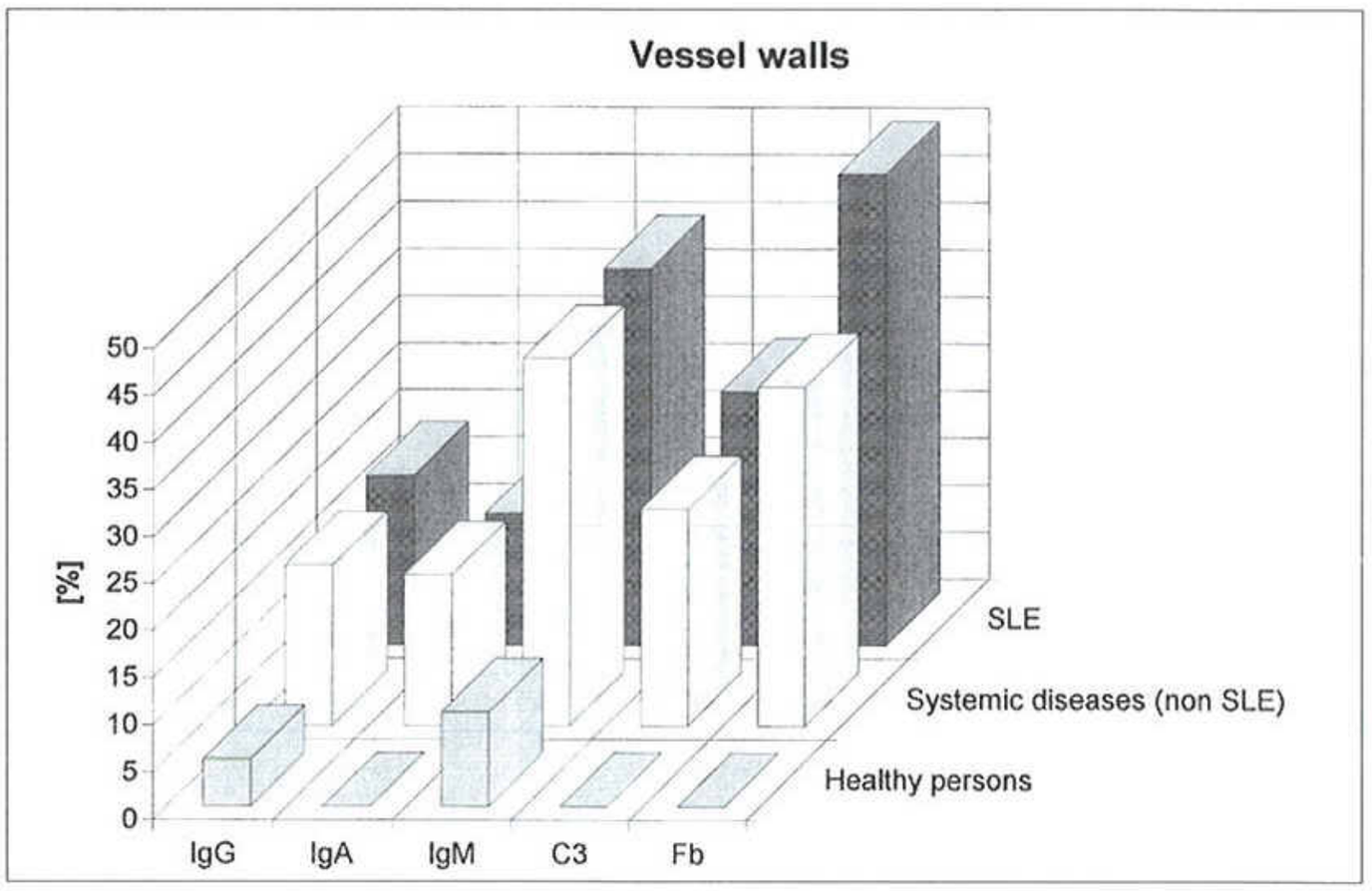
Deposits detected in examined areas (in %)

Diagnosis		Subepidermal					Vessel walls					Sweat glands				
	No.	IgG	IgA	IgM	C3	Fb	IgG	IgA	IgM	C3	Fb	IgG	IgA	IgM	C3	Fb
SLE	60	52	13	52	27	15	18	14	40	27	50	47	13	13	18	31
Systemic disease (non SLE)	87	31	5	16	9	11	17	16	39	23	36	37	3	15	18	18
Healthy volunteers	19	5	0	10	0	0	5	0	1	0	0	0	0	0	0	0

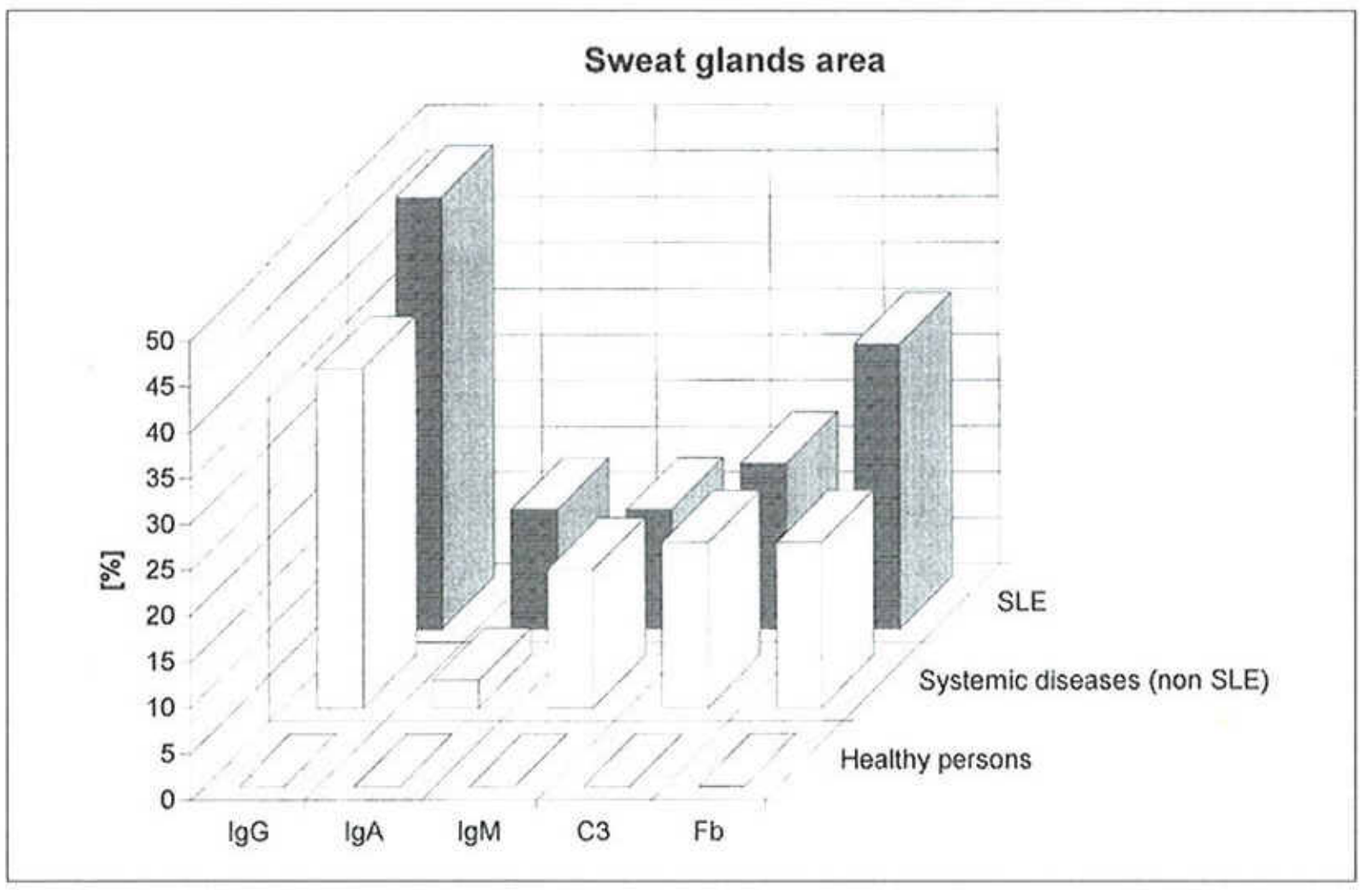
Table No. 1 : Direct limmuno fluorescence of clinically normal skin may imdicate some types of systemic disease.



Graph 1



Graph 2



Graph 3

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