Subclinical Disease Activity in Systemic Lupus Erythematosus: Immunoinflammatory Markers Do Not Normalize in Clinical Remission

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ABSTRACT. ***Objective.*** To correlate various laboratory measures in systemic lupus erythematosus (SLE) with the British Isles Lupus Assessment Group (BILAG) disease activity index, to search for organ-specific laboratory patterns and to compare with a control population.

Methods. A cohort of 57 Caucasian outpatients fulfilling the American College of Rheumatology criteria for SLE and a control population of 17 patients admitted for coronarography were examined. Disease activity was assessed with BILAG index. Plasma samples were investigated for sCD44, interleukin 6 (IL-6), IL-10, IL-12, tumor necrosis factor-α (TNF-α), soluble TNF receptor-55 (sTNFR-55), sTNFR-75, IL-1-receptor antagonist, soluble intercellular adhesion molecule (sICAM), soluble vascular cell adhesion molecule (sVCAM), E-selectin, and neopterin as well as for C3, C4, dsDNA, and other conventional indicators.

Results. Thirty-nine patients had inactive disease (total BILAG score ≤ 5), 18 patients had active SLE. Surprisingly, except for C-reactive protein ($p < 0.001$), no statistically significant difference of the laboratory indicators was found between patients with active and those with inactive SLE. However, there was a significant difference between SLE patients and controls for sTNFR-75 ($p < 0.008$). We found significant correlations between laboratory markers and some BILAG organ system scores, such as between IL-1ra and the musculoskeletal score ($p < 0.003$) and between sTNFR-55/sTNFR-75 and renal BILAG ($p < 0.001$, $p < 0.004$, respectively). Significant nonparametric correlations were revealed between C3 and C4 ($p < 0.0001$), and between sTNFR-75 and dsDNA, neopterin, sVCAM, sICAM and sTNFR-55 concentrations ($p < 0.0001$ for all), and between sTNFR-75 and IL-1ra ($p < 0.006$).

Conclusion. Patients with SLE in clinical remission show ongoing systemic immunoinflammatory activity measured with a variety of cytokines, adhesion molecules, and other inflammatory markers. This indicates that laboratory measures may provide qualitatively different additional information to validated disease activity indexes such as the BILAG. Different laboratory markers correlate with disease activity in different organ systems. This suggests differences in pathogenic mechanisms in SLE depending on the organ system involved. (J Rheumatol 2003;30:2133–9)

Key Indexing Terms:

CYTOKINES	CYTOKINE ANTAGONISTS	DISEASE ACTIVITY
SYSTEMIC LUPUS ERYTHEMATOSUS	BILAG	ADHESION MOLECULES

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder of unknown etiology affecting different organ systems and showing a fluctuating disease course. Several genetic factors appear to contribute to disease susceptibility and disease expression. Immunologically, the disease is characterized by a polyclonal B cell stimulation, autoantibody production, and formation of immune complexes. In addition, T cell dysfunctions have been characterized. Regarding cytokine profiles, the disorder shows the characteristics of a Th2–dominated immune response. A characteristic feature of SLE is that the pattern of organ involvement differs from one patient to another. This suggests differences in pathogenic mechanisms in different organs, a hypothesis supported by the differences in autoantibody profiles correlating with involvement of different organs.

In recent years, more than 60 clinical scoring systems have been developed to assess disease activity. Currently in most widespread use are the SLE Disease Activity Index, the Systemic Lupus Activity Measure, the Lupus Activity Index, the British Isles Lupus Assessment Group (BILAG) index, and the European Consensus Lupus Activity Measure[1,2]. We chose the BILAG because it gives an accurate organ-specific measurement of disease activity[3-5], as this would allow correlation of immunoinflammatory

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markers with disease activity in the different organs involved.

In addition to the clinical evaluation, laboratory markers may help to assess disease activity. The most frequently used are the complement components and the double stranded-DNA antibody titer. Further, it is known that the C–reactive protein (CRP) parallels disease activity, although to a much lesser degree than seen in other systemic rheumatic diseases[6,7]. To date, no single test has proven to be sufficiently sensitive and predictive to guide therapy[8-14].

Among recently detected molecules that might qualify as disease activity markers are cytokines and adhesion molecules. Interleukin 6 (IL-6) is a pleiotropic cytokine with a profound effect on the terminal differentiation of B lymphocytes into plasma cells[15]. As expected, high systemic levels of this cytokine were found in patients with SLE[16,17]. However, the initial finding of a positive correlation between IL-6 and disease activity could not be confirmed by other investigators[18]. IL-10, a typical Th2 cytokine, has also been found at elevated concentrations in SLE, showing a positive correlation to disease activity in cross-sectional studies[19,20]. IL-12 counterbalances many of the effects of IL-10[21-23]. It can be found systemically elevated in disorders with a Th1-dominant immune response such as Behçet's disease[24]. In contrast to conflicting results about systemic levels of tumor necrosis factor-α (TNF-α)[25,26], several studies have documented a positive correlation between concentrations of soluble TNF receptors (sTNFR-55 and sTNFR-75)[25-29] as well as IL-1 receptor antagonist (IL-1ra)[30] and disease activity in SLE. Interestingly, IL-1ra levels were increased in extrarenal SLE, but decreased in renal lupus[31]. So far, this is the only study that might suggest that cytokine levels and/or patterns could differ if different organs are involved.

Recent studies have measured normal concentrations of soluble intercellular adhesion molecule (sICAM) and reduced concentrations of E-selectin in sera of SLE patients in comparison to healthy controls[32]. As SLE is an independent risk factor for coronary artery disease (CAD) and sICAM levels have been found elevated in CAD, this finding is surprising. In contrast to sICAM and E-selectin, soluble vascular cell adhesion molecule (sVCAM) was found to be elevated and showed a positive correlation with disease activity[33,34]. CD30 is a surface marker of Th2 lymphocytes. As expected for a Th2-dominated disease, sera of SLE patients contained elevated levels of soluble CD30, and again a positive correlation to disease activity could be calculated[35]. CD44 acts as an adhesion molecule, binds hyaluronic acid, and is expressed by antigen-activated T lymphocytes[36]. A soluble form has been found in sera of patients with neoplastic diseases that correlates with tumor burden[37]. In SLE, an increase in the numbers of CD44+ T cells was described; however, systemic levels of its soluble form have not been analyzed so far. Neopterin is considered to be a marker of monocyte/macrophage activation. It is very sensitive and has been found to correlate with activity of a variety of immunoinflammatory conditions[38]. Interestingly, neopterin was also found as a marker for disease activity in urine of patients with SLE[39].

In summary the aims of this study were (1) to investigate whether immunoinflammatory variables might depict "subclinical" disease activity; (2) to compare the different variables with conventional activity measures and with the BILAG score; and (3) to investigate whether different variables correlate with any type of organ involvement as measured with the "sub-BILAG."

MATERIALS AND METHODS

A convenience sample of German-speaking Caucasian Swiss patients with SLE was recruited via local rheumatologists, nephrologists, dermatologists, and general practitioners. In addition a letter was sent to each member of the regional SLE patients' organization asking for participation in the study. To be eligible, patients had to fulfill the American College of Rheumatology (ACR) classification criteria[40]. In total, 78 patients were assessed. In 60 cases the criteria for SLE were fulfilled; in 8 patients SLE was diagnosed, but without fulfilling the criteria. The remaining 10 patients had other connective tissue diseases.

Disease activity was assessed by the BILAG index. This score system measures SLE–related disease activity in 8 organ-based components (general, mucocutaneous, neurological, musculoskeletal, cardiovascular/respiratory, vasculitis, renal, and hematological) and is based upon the principle of the physician's intention to treat. To obtain a global score, BILAG component scores can be assigned numerical values: A = 9 (most active disease), B = 3 (intermediate activity), C = 1 (mild and stable disease activity), D = 0 (inactive disease), and E = 0 (no activity ever), resulting in a potential global score ranging from 0 to 72. This numerical score as well as the total BILAG score have been shown to be valid[3].

In addition to calculations of correlations between laboratory measures and disease activity as assessed by the BILAG system, comparisons between patient groups were performed. For this purpose SLE patients were arbitrarily categorized into one group with active disease (BILAG score > 5) and a second group with inactive disease (BILAG ≤ 5). A total BILAG score > 5 usually consists of at least one organ score = 3 (which signifies one organ system with a minor flare). According to recent reports, authors have chosen 3, 5, or even 8 as a cutoff for active disease, thus a score of 5 corresponds approximately to the mean of this range.

Blood was drawn at the time of clinical examination. Plasma was aliquoted and stored immediately at –20°C until analysis. Routine hematological, biochemical, and immunological blood tests were performed in the hospital laboratories (Kantonsspital, St. Gallen, Switzerland). They included measurements of CRP, C3 and C4 serum levels, and quantification of dsDNA antibody titers (using an enzyme immunoassay as well as the *Crithidia* assay). IL-6 and TNF-α were quantified by an immunoassay (Innogenetics NV, Gand, Belgium). Neopterin was measured with an ELISA (IBL, Hamburg, Germany). sCD44, sVCAM-1, sICAM-1, sE-selectin, IL-10, IL-1ra, and IL-12 were quantified with an immunoassay (R&D Systems, Abingdon, UK). sCD30 was measured with an ELISA (Dako, Glostrup, Denmark). Quantification of sTNFR-55 and sTNFR-75 was performed using an ELIBA (Hoffmann La-Roche, Basel, Switzerland).

Blood of the control patients was drawn at the time of coronarography and plasma was stored at –20°C. The samples of the SLE cohort and controls were analyzed together at the same time to avoid methodological errors.

Statistics. Statistical analysis was performed with SPSS for Windows, release 9.0, at the Department of Psychosocial Medicine, University Hospital, Zürich, Switzerland. Correlations between laboratory measures

and disease activity were assessed by Spearman rank correlations. To account for the effect of multiple comparisons of this exploratory analysis, only differences of $p < 0.01$ were considered significant[3]. Comparisons between groups were performed with t tests and one-way analyses of variance followed by Scheffé tests.

RESULTS

Plasma samples of 57 of the 60 patients (fulfilling the American Rheumatism Association, now ACR criteria) were available for analysis. Of these 57 patients, 52 were female, 5 male. The mean age at study entry was 45.7 years (range 22–77) and mean disease duration was 10.5 years (range 0–36). The mean age at onset of symptoms was 30.2 years in women and 37.9 in men. Thirty-nine patients were treated with glucocorticoids at the time of the study, 19 with azathioprine, 15 with antimalarials, 4 with cyclophosphamide, and 2 with methotrexate. Analysis of correlations between drug use (glucocorticoids, azathioprine, antimalarials) and clinical and laboratory measures did not yield significant results, with the exception of a lipid-lowering effect of antimalarials (whether used as a single drug or in combination with glucocorticoids).

The control population consisted of 17 outpatients admitted for coronarography; 9 were male and 8 female. Their mean age was 61.8 years (range 50–78). In 5 cases no coronary heart disease was found; 6 patients had stable and 6 unstable angina pectoris.

Clinical assessment. Table 1 shows organ system involvement and disease activity as assessed by BILAG. It is noteworthy that large variations in disease activity were found. As in other studies[41], more activity was registered in mucocutaneous, hematological, and musculoskeletal systems as compared to neurological and cardiovascular systems. On average, 32% of the cases showed no activity ever in at least one of the organ systems (category E).

Using the BILAG cutoff of > 5 to separate active from inactive SLE, 39 patients were categorized as having inactive and 18 as having active disease at the time of investigation.

Conventional laboratory measures. Percentages of patients with elevated CRP and dsDNA antibody titers and levels of C3 and C4 are shown in Table 2. As expected, CRP levels were significantly different between inactive and active SLE ($p < 0.001$). However, no difference was found between patients with inactive SLE and the control population. This latter finding is not surprising, as the control population consisted of patients admitted for coronarography, with 12/17 of the cases with proven CAD. As expected, controls did not have dsDNA autoantibodies in their sera. The percentage of dsDNA positive patients was comparable in the 2 SLE groups (56.4% versus 55.6%) No differences were found for serum levels of the complement components C3 and C4 in those with inactive versus active SLE.

Cytokines and other laboratory variables. Circulating concentrations of the cytokines IL-6, IL-10, IL-12, and TNF-α were measured. In contrast to others' results, the number of samples containing cytokines in quantities above background level was low in our population. Only 18 samples of the active SLE group, 22 of the inactive SLE group, and 7 of the control group showed elevated concentrations of one or several cytokines. Because of the small number of findings of elevated IL-6, IL-10, IL-12, and TNF-α it was not appropriate to perform any statistical analysis on these variables. In contrast, sCD30, a surface marker of Th2 lymphocytes, was elevated above the titration curve in 11 plasma samples. This precluded statistical analysis of this variable.

Analysis of the 3 patient groups. The results for neopterin, soluble adhesion molecules, IL-1ra, sCD44, sTNFR-55, and sTNFR-75 are summarized in Table 3: sTNFR-75 was significantly elevated in active SLE compared to control patients. Because we corrected for multiple comparisons, other correlations did not reach statistical significance. However, levels of neopterin ($p = 0.031$), sICAM-1 ($p = 0.022$), sCD44 ($p = 0.04$), and sTNFR-55 ($p = 0.029$) were also higher in active and inactive SLE compared to controls. Remarkably, and in contrast to sTNFR, circulating levels of IL-1ra were not statistically different between the patient groups. Neopterin, sICAM-1, sCD44, sTNFR–55, and sTNFR–75 were significantly different if we combined the 2 SLE groups and compared all SLE patients with the control group (data not shown).

Because sex hormones may modulate immunological processes and influence disease activity, female patients with SLE were categorized as pre- or postmenopausal. Statistical analysis showed no significant difference for any of the variables analyzed, except for C3, which was higher in postmenopausal patients. These findings argue against a role of age and hormonal situation.

To address the question of subclinical activity of immunoinflammatory processes, we compared the group of

Table 1. Disease activity in various organ systems according to BILAG scores in 57 patients with SLE.

Disease Activity	General	Mucocutaneous	Nervous System	Musculoskeletal	Cardiovascular/Respiratory	Vasculitis	Renal	Hematological
Strong (A)	0	1	0	0	0	0	0	0
Moderate (B)	0	20	0	9	0	0	9	3
Low (C)	5	14	7	27	7	18	9	47
None presently (D)	31	18	9	21	20	16	13	6
None ever (E)	21	4	41	0	30	23	26	1

Table 2. Laboratory measurements in 18 active SLE patients (total BILAG > 5), 39 inactive, and 17 controls admitted for coronarography.

Laboratory Measurement	Active SLE	Inactive SLE	Control
CRP, > 5 mg/l, %	61.1	15.8	17.6
dsDNA, > 1:40, %	56.4	55.6	0
C3, g/l, mean	0.876	0.815	ND
C4, g/l, mean	0.153	0.145	ND

ND: not done.

Table 3. Mean values of biological markers in 18 active SLE patients (total BILAG > 5), 39 inactive, and 17 controls.

Biological Marker	Active SLE	Inactive SLE	Control	p*
Neopterin, nmol/l	12.2	9.8	6.4	0.031
sVCAM-1, ng/ml	1008	1071	789	0.117
sE-selectin, ng/ml	49	57.6	37.9	0.432
sICAM1, ng/ml	447.7	368.6	313.1	0.022
IL-1ra, pg/ml	2073	1597	1414	0.112
sCD44, ng/ml	388.9	371.2	235.6	0.04
sTNFR-55, ng/ml	2.42	1.72	1.61	0.029
sTNFR-75, ng/ml	9.28	6.87	4.75	0.008

* p denotes the difference between active SLE patients and controls; $p < 0.01$ = statistically significant difference.

patients with "inactive" SLE with the control population. There were no statistically significant differences in the variables shown in Table 3 between the inactive SLE group and the control group. As mentioned, the cutoff of 5 to separate active from inactive SLE is arbitrary, and other groups have used 3, 5, or even 8 to split active from inactive disease. Therefore, we were interested to know at which cutoff score the differences would become statistically significant. At a cutoff of > 7, significant differences were found between inactive SLE and the control cohort for the following variables: neopterin ($p < 0.0001$), sICAM-1 ($p < 0.01$), and sTNFR-75 ($p < 0.002$).

Correlations between immunoinflammatory markers and the BILAG score. In addition to the comparison of the 3 patient groups, correlations were calculated for laboratory variables and the BILAG score as a global index as well as the different organ systems constituting the BILAG score. Correlations between BILAG score as a global index and laboratory measures are shown in Table 4. Nearly significant correlations were found between BILAG and CRP (Spearman rank correlation coefficient = 0.328, p = 0.014), but not between BILAG and dsDNA titers or levels of the complement components (C3 and C4). Interestingly, sTNFR-55 showed a nearly positive correlation with the total BILAG score as well (Spearman rank correlation coefficient = 0.318, p = 0.016).

Calculations of correlations between serological markers and disease activity of different BILAG organ systems gave remarkable results. We chose the 3 organ systems where the number of patients with a BILAG score > 3 was big enough (n > 9) for a statistical determination (i.e., mucocutaneous, musculoskeletal, and renal involvement). A positive correlation was found between renal disease activity and the sTNFR-55 and –75 ($p < 0.001$ and $p < 0.004$, respectively); between IL-1ra and musculoskeletal disease activity ($p < 0.003$), however, no significant correlation was found for mucocutaneous disease.

Table 4. Correlations between biological and biochemical markers and the total BILAG score in 57 SLE patients.

Marker	Correlation Coefficient	p
Neopterin	0.234	0.08
sVCAM-1	–0.5	0.71
sE-selectin	–0.09	0.95
sICAM-1	0.235	0.08
IL-1ra	0.202	0.14
sCD44	–0.62	0.65
sTNFR-55	0.318	0.016
sTNFR-75	0.204	0.128
CRP	0.328	0.014
dsDNA	0.01	0.942
C3	0	1
C4	–0.45	0.74

$p < 0.01$ = statistically significant difference.

Correlations between different immunoinflammatory markers. Finally, we investigated correlations between conventional laboratory measures of disease activity such as CRP concentrations, complement components, and dsDNA antibody titers, and compared these with concentrations of the other immunoinflammatory markers (Table 5). Significant positive correlations were found between C3 and C4. CRP correlated positively with IL-1ra and sTNFR-55. Highly significant correlations were found between sTNFR-75 and dsDNA, neopterin, sVCAM, sICAM, and sTNFR-55 levels (p < 0.0001 for all); moreover, sTNFR-75 was significantly correlated with IL-1ra, sCD44, and even antinuclear antibodies (p < 0.006, p < 0.003, p < 0.001, respectively). These results show an unexpectedly high interdependence of different immunoinflammatory markers at the systemic level. This contrasts with the discrete patterns of correlations between some of these markers and active disease as measured with the global BILAG score. On the other hand, there were even more significant correlations between immunoinflammatory measures and BILAG organ/system scores, reinforcing previous findings[3] that using only total BILAG and neglecting organ/system BILAG scores leads to a loss of important information.

DISCUSSION

With the measurements of a set of biological molecules such as cytokines, cytokine inhibitors, adhesion molecules, and soluble markers of immunocompetent cells in a well characterized cohort of SLE patients, we addressed 3 questions: Do some of these molecules qualify for detection of subclinical disease activity? How are these markers correlated with the more conventional markers used in clinical practice, and what is their association to global disease activity? Can we identify discrete patterns of biological molecules to correlate with disease activity in different organs?

Compared to several other cohorts, our SLE patients showed a rather low overall disease activity. This may partly be because we collected patients from private practice (not merely referral centers). On the other hand, our cohort was comparable in its disease activity to other samples known to

be representative of outpatients, such as the Bloomsbury SLE patient cohort[3] (mean of total BILAG index 4.8 and 5.2, respectively). Indeed, the distribution pattern of disease activities in our study appeared ideal to address the first question, namely, whether immunoinflammatory indicators normalize in clinical remission. Patients with active and with inactive SLE showed higher systemic levels of a variety of biological molecules than controls, with a continuous rise from the control population (lowest levels) to active disease (highest levels). These findings are reinforced by the fact that our control cohort consisted of patients admitted for coronarography. Only 5 of 17 control patients proved to be healthy — 6 had unstable angina pectoris, a condition with well characterized inflammatory changes[41]. Thus the difference between clinically inactive SLE and healthy controls would likely have been even more pronounced than in these results. It can be argued that sex hormones might account for the differences found in the patient and control populations[42]. However, statistical analysis did not show significant differences except for C3, which proved to be higher in postmenopausal women. Thus, age and hormonal situation do not appear to have influenced our results. Our findings suggest that sTNFR-75, sICAM-1, and neopterin are candidates for monitoring subclinical disease activity. Prospective studies are needed to confirm this.

In contrast to the biological markers, classical laboratory markers for disease activity in SLE such as C3, C4, and dsDNA antibodies did not differentiate between active and inactive disease in our cohort. It is noteworthy that the values of the complement components did not correlate with the BILAG scores. Of all the immunoinflammatory variables we investigated, CRP and sTNFR-55 correlated best with disease activity measured with the total BILAG. This is in agreement with other studies[6,7].

Only a few plasma samples of our cohort contained detectable amounts of TNF-α, IL-6, IL-10, or IL-12. This contrasts with other publications, which found elevated systemic concentrations for IL-6 and IL-10 and suggested that these 2 cytokines might qualify as markers of disease

Table 5. Significance of correlations of biological and biochemical markers measured in 57 SLE patients.

Marker	ANA	dsDNA	C4	Neopterin	sVCAM-1	sICAM-1	sE-selectin	IL-1ra	sCD44	sTNFR-55
CRP								0.001		0.006
C3			0.0001							
dsDNA	0.001			0.001					0.001	0.003
sCD44	0.0001	0.001		0.004						
Neopterin	0.0001	0.001								
sVCAM-1				0.001						
IL-1ra										
sTNFR-55		0.003		0.002	0.001	0.001	0.009			
sTNFR-75	0.001	0.0001		0.0001	0.0001	0.0001		0.006	0.003	0.0001

p < 0.01 = statistically significant difference.

activity[16,17,19,20,43]. That other biological markers were detectable at expected values in the same plasma samples argues against methodological problems. However, as recently shown, different assays may produce rather different results[44,45]. Further, our studies with other patient populations and studies from other groups describe similar findings in Behçet's disease[24].

The finding of elevated concentrations of sICAM-1 in our cohort is of interest. First, it is noteworthy that sICAM-1 was higher in SLE patients than in the controls, although 2/3 of the control subjects had CAD, a disease with elevated systemic levels of this adhesion molecule[41]. Second, it is remarkable that sICAM-1 correlated strongly with the sTNF receptors. Considering the current hypothesis of an inflammatory component in the pathogenesis of arteriosclerosis, our results suggest a biological basis for the the recent epidemiological finding that SLE is an independent risk factor for CAD and that this risk increases with active disease[46].

The third question was whether involvement of different organ systems was correlated with different patterns of immunoinflammatory indicators. This might appear likely, as it is well known that ds-DNA antibodies are correlated with renal disease, but not with musculoskeletal involvement. Further, SLE differs from several other systemic autoimmune diseases because there is no typical and unique pattern of organ involvement. There are patients with predominant renal disease and other individuals with long-lasting mucocutaneous and musculoskeletal problems. Again, this indicates that different pathogenetic mechanisms might be operative in different organ systems. As our cohort did not show high disease activity in all 8 organ systems according to BILAG, we performed statistical analysis for renal, mucocutaneous, and musculoskeletal disease. We found kidney disease was associated with systemic levels of sTNFR-55 and sTNFR-75, and musculoskeletal involvement was correlated with IL-1ra levels. As expected, ds-DNA correlated with sTNFR but not with IL-1ra. These findings support the hypothesis of different pathological mechanisms in the different organ systems. In contrast to renal and musculoskeletal disease, there was no significant association between mucocutaneous disease activity and any of the measured laboratory variables. We have obtained similar results in a cohort of patients with Behçet's disease (unpublished data). It appears likely that in mucocutaneous diseases locally produced cytokines and adhesion molecules do not reach concentrations that are detectable systemically.

Our results indicate that several immunoinflammatory markers may help to detect subclinical disease activity in SLE. Thus these markers may add information to well established disease activity assessments such as the BILAG score. The difference in correlations between immunoinflammatory markers and organ involvement supports the concept of different pathological mechanisms in SLE according to the organ system involved. Further, the more significant correlations between immunoinflammatory markers and BILAG organ/system scores emphasizes that using only total BILAG and neglecting organ/system BILAG scores leads to a loss of important information. Finally, the findings suggest a biological basis for the epidemiological fact that SLE is an independent risk factor for coronary artery disease and that this risk increases with active disease.

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