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Childhood-onset systemic lupus erythematosus in Croatia: demographic, clinical and laboratory features, and factors influencing time to diagnosis

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Abstract

Objectives

Childhood-onset systemic lupus erythematosus (cSLE) presents with diverse clinical features and often with non-classical symptoms that may delay diagnosis and increase risk of morbidity and mortality. This paper aims to analyse incidence, and clinical and laboratory features of cSLE in Croatia between 1991 and 2010, and to identify factors influencing time to diagnosis.

Results

Medical records at three university-based tertiary care centres were analysed retrospectively for 81 children with cSLE (68 girls). Mean age at onset was 13.4±2.8 yr (interquartile range 3), and annual incidence varied from 1–15 per million at risk. The most frequent clinical and laboratory features were musculoskeletal symptoms (80%) and increased erythrocyte sedimentation rate (96%). The most frequent immunological laboratory findings were the presence of antibodies against histones (86%), double-stranded DNA (73%), and Sm protein (64%), as well as low levels of C3 complement (69%). Haematuria was present in 58% of children, proteinuria in 56%, and biopsy-confirmed lupus nephritis in 43%. Median time from symptom onset to diagnosis was 2 months (range 0–96). Time to diagnosis was inversely associated with ECLAM score ($p<0.001$), but it showed no association with age, gender, clinical features or distance from the nearest paediatric centre.

Conclusion

This is the first large-scale, in-depth study of clinical and laboratory features of cSLE in Croatia. Among all demographic, laboratory and clinical features examined, ECLAM score alone was inversely associated with time to diagnosis. This highlights the need to improve detection of children with fewer symptoms early in the course of the disease, therefore serious consequences for prognosis could be avoided.

Key words

childhood-onset systemic lupus erythemathosus, Croatia, factors influencing time to diagnosis, incidence

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Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease caused by changes in the immune system that lead to inflammation of blood vessels and connective tissue in all organs and systems; therefore, SLE presents with a broad spectrum of clinical features, along with abnormal laboratory values (1).

SLE usually occurs in young women (female to male ratio 7–15:1), but in as many as 20% of cases, the first symptoms appear in children and adolescents under 18, in a form known as childhood-onset systemic lupus erythematosus (cSLE) (1-4). Most children who suffer from cSLE develop symptoms during puberty, and disease onset before the age of five years is rare (1). The prevalence of cSLE is approximately 4- to 6-fold higher among girls, and it is estimated at 6.4–50 per 100.000 (1, 5), while the annual incidence rate varies between 2.2 to 9 per million (5-7).

The first manifestations of cSLE can be quite diverse and usually include one or more of the following: fever, malar or discoid rash, polyarthralgia and arthritis (1, 3, 9). This chronic and systemic illness often leads to serious complications, including musculoskeletal, neuropsychiatric and renal problems. Cumulative organ damage occurs in up to 60% of patients, which significantly reduces quality of life (10, 11). Indeed, the initial symptoms and course of the disease are more severe than in adults, often necessitating more aggressive treatment (12, 13). Diagnosis of cSLE is made on the basis of clinical features and laboratory findings, but the diversity of cSLE symptoms and its often non-classical and misleading presentation (*e.g.* other than skin, joints, constitutional symptoms) can extend time to diagnosis and treatment considerably (8). Even though such delays are the single most important factor influencing morbidity and mortality in children with cSLE (14, 15), factors influencing time from symptom onset to diagnosis are rarely investigated.

Although the exact mechanisms and pathophysiology of cSLE remain unclear, environmental factors are known to trigger the disease in genetically and hormonally predisposed individuals. While some environmental factors such as viruses remain controversial, others are well substantiated, including several drugs and ultraviolet-B (UV-B) radiation (1). Despite of that, we are unaware of studies investigating possible differences in SLE features within a population with respect to differences in climate or solar irradiation in different parts of the same country, since the studies on (c)SLE involve the entire population of a particular country.

In the present study, we performed a 20-year retrospective analysis to establish incidence of cSLE in Croatia. We investigated clinical and laboratory features of children with cSLE in Croatia, and compared patients from parts of the country receiving different amounts of solar radiation in order to explore possible differences in cSLE features. Also, we investigated time form symptom onset and cSLE diagnosis, and examined numerous demographic, clinical and laboratory features of these cases to identify factors influencing time to diagnosis.

Patients and methods

The protocol for this retrospective study was approved by the ethics committees of all three participating hospitals (University Hospital Centre Rijeka, University Hospital Centre Split, University Hospital Centre Zagreb), as well as by the ethics committee of the University of Zagreb School of Medicine.

We reviewed medical records of all patients aged ≤18 years at disease onset who were diagnosed with cSLE during the period 1991–2010 in the paediatric departments of three university-affiliated tertiary care hospitals in the three largest cities in Croatia, namely Zagreb, Rijeka, and Split. The diagnosis of cSLE was made on clinical and immunological grounds, and in order to be included in this study, patients needed to fulfill the 1997 ACR criteria for the classification of SLE (9). The patients with overlap syndrome were not included in this study.

All children were admitted to one of these three hospitals after being re-

ferred by a primary care practitioner or a physician at a local secondary care hospital for suspected cSLE, or simply for "fever of unknown cause", "arthralgia/arthritis" or "rash". In the Croatian health care system, referring physicians usually suspect cSLE, but do not make the final paediatric rheumatology diagnosis or initiate treatment. Instead, patients presenting with uncertain or serious diagnoses are referred to a hospital, which may be one of the three tertiary care centres in this study in the case of patients living in or near Split, Zagreb, or Rijeka, or it may be a secondary care centre in the case of patients living outside the three cities examined here – these patients were subsequently referred to one of the three tertiary centres involved in this study. Thus, all patients suspected for cSLE in Croatia were eventually referred, admitted to and evaluated in one of the tertiary care centres involved in this study.

Although several co-authors retrieved and prepared medical documentation of patients with cSLE from the archives of three tertiary care hospitals involved in this study, and different paediatric departments, only one author reviewed the medical documentation of each patient and did the actual data collection, while the decision about the recruitment of the patients in the study was made by the paediatrician with 10-year experience in paediatric rheumatology.

Clinical presentation was defined as all symptoms and signs were (a) reported by the child or the parents at disease onset; (b) observed by physicians during examinations in the primary, secondary or tertiary care centres; and (c) present at the time of diagnosis in the tertiary care referral centre. Time to diagnosis was defined as the interval between the time of onset of the first cSLE symptoms and the time when cSLE diagnosis was established in one of the three referral hospitals.

cSLE activity at the first in-hospital evaluation in the tertiary referral centre was manually calculated according to the European Consensus Lupus Activity Measurement (ECLAM) index (16), which has been validated for retrospective analysis of disease activity (17). The medical records of all children in the study were complete enough to provide all laboratory data for calculating the ECLAM score. These included data on proteinuria, haematuria and urinary casts, erythrocyte sedimentation rate (ESR), serum creatinine levels, numbers of erythrocytes, leukocytes and thrombocytes, concentration of C3 complement, and total complement activity (CH50).

Annual incidence of cSLE was calculated over the entire study period based on population data for the population at risk (5–18 years of age) provided by the Croatian Bureau of Statistics (personal communication).

Laboratory findings

Relevant laboratory findings were defined to be those present ***a***) at the first in-hospital evaluation of the child in one of the tertiary care referral centres and ***b***) at the time of diagnosis in the referral centre. These findings included: erythrocyte sedimentation rate, numbers of erythrocytes, leukocytes, and thrombocytes, proteinuria (>0.5 g/24 h), haematuria (>5 red blood cells (RBC) seen per high power field, HPF), increased serum creatitine (>80 mmol/L), low concentrations of complement C3 (<0.9 g/L), complement C4 (<0.1 g/L), and rheumatoid factor (RF >14 IU/mL).

ANA were determined by indirect immunofluorescence (IIF) using commercially available slides of HEp-2 cells (Euroimmun, Lubeck, Germany). Patients sera were diluted 1:100 for screening. All positive samples were titrated in doubling dilutions until end point. Results were reported as negative (<1:100) or positive (expressed as a antibody titer). Starting in 2004, all positive ANA sera by IIF were further tested by antigen-specific ELISA (Euroimmun, Lubeck, Germany) or by AtheNA Multi-Lyte ANA-test system, a multiplexed, microparticle Luminex-based immunoassay (Zeus Scientific Inc, New Jersey, USA) that allows for the simultaneous detection of 9 antibodies of established clinical significance (antibodies to dsDNA, histone, SS-A, SS-B, Sm, RNP, Scl-70, Jo-1 and centromere B). The results were expressed in international units. Samples were considered positive when values were greater than 20 IU/ml (for ELISA) and greater than 120 IU/ml (for Luminex). ELISA tests for anti-aCL IgG, and anti-aCL IgM were purchased from Hycor Biomedical Inc (Garden Grove, USA), and specific enzyme labelled anti-IgG and anti-IgM conjugate were used. Sera were considered to be positive for anti-aCl IgG when values were greater than 40 U/ml, and for anti-aCl IgM when values were greater than 30 U/ml.

Lupus anticoagulant (LAC) was detected by coagulation assays including prothrombin time, an activated partial tromboplastin time and dilute Russell s viper venom time.

Presence of lupus nephritis was evaluated by pathohistological examination of biopsied renal tissue by light and electron microscopy, as well as by immunofluorescence to label IgA, IgG, IgM, C3 and C1q. Renal biopsy was performed on the bases of clinical and laboratory indication, as well as in all children with proteinuria >0.8 g/day. The slides were examined by experienced pathologists, and lupus nephritis was classified according to pre-2004 standards of the World Health Organisation (WHO) (18) and post-2004 standards of the International Society of Nephrology (ISN) (19), using the following typology (WHO/ISN): type 2, pure mesangial alterations/mesangial proliferative lupus nephritis; type 3, focal segmental glomerulonephritis/focal lupus nephritis; type 4, diffuse glomerulonephritis/diffuse lupus nephritis; type 5, diffuse membranous glomerulonephritis/membranous lupus nephritis; and type 6, advanced sclerosing glomerulonephritis/advanced sclerosing lupus nephritis.

Subgroup analysis by sunlight exposure

We divided our study population into subgroups by sunlight exposure in order to determine whether such exposure was associated with particular clinical or laboratory features of cSLE. Using a solar irradiation map from the Croatian Meteorological and Hydrological Service (20), children were divided into two groups depending on whether they lived in a continental climate,

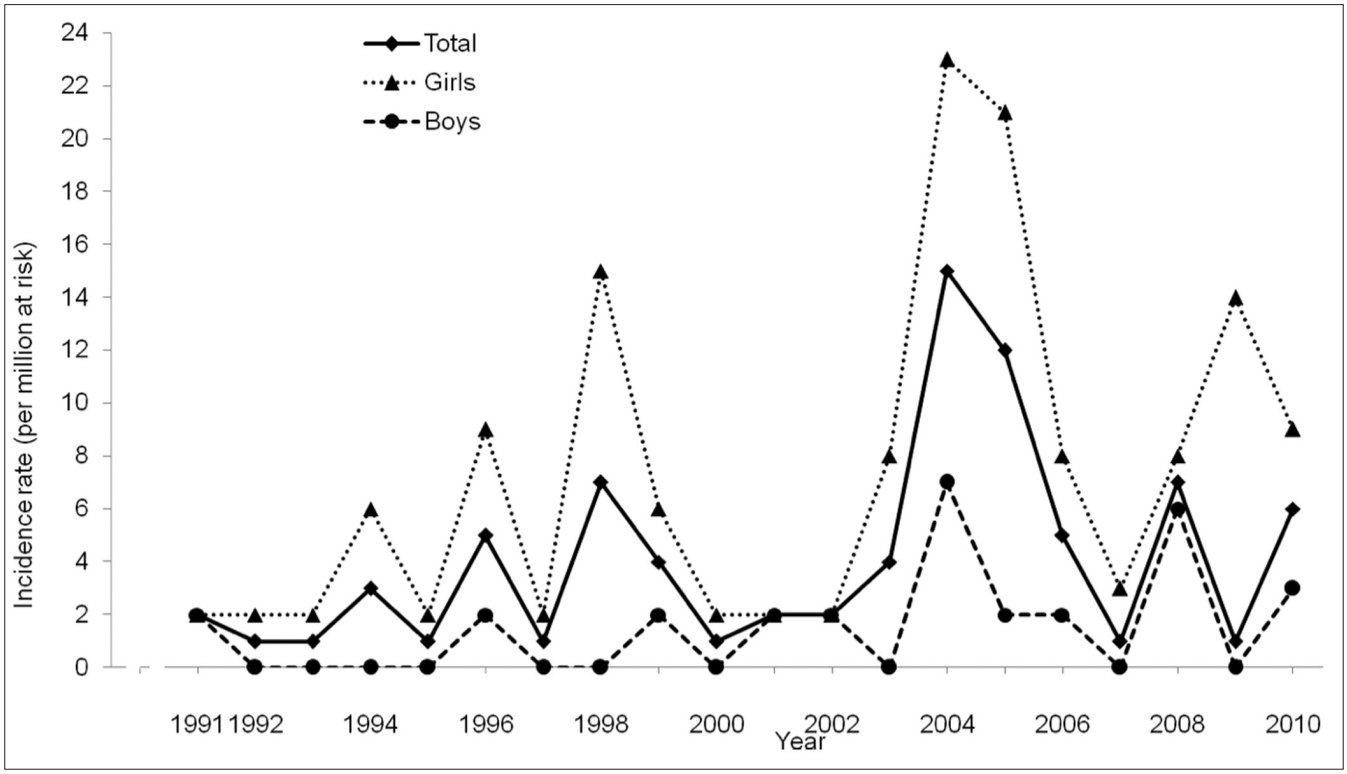

Fig. 1. Annual incidence rates of juvenile systemic lupus erythematosus among Croatian children, 1991–2010.

which received annual solar energy of 3161–4680 MJ/m^2, or a Mediterranean climate, which received annual solar energy of 4681–5760 MJ/m^2.

Statistical analysis

Demographic, clinical and laboratory features were summarised using descriptive statistics. Differences in clinical and laboratory features were analysed by Fisher's exact test and the χ^2-test. Categorical variables were compared using Fisher's exact test, while continuous variables were compared using the *t*-test for independent samples. Associations between continuous variables were assessed using the rank correlation test (Spearman's correlation test). All statistical analyses were performed using MedCalc 9.5.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

Patients

During the 20-year study period, 81 children (68 girls, 84%) met our inclusion criteria. Mean age (±SD) at the disease onset was 13.4±2.8 yr for both genders, 13.6±2.7 for girls (range 6–17), and 12.3±3.1 for boys (range 7–18). There were 15 children aged between 6–11 yrs, 64 between 12–17 yrs, and 2 aged 18 yrs (interequartile range 3 yrs).

The median time from symptom onset to diagnosis was 2 months (range 0–96 months). In 9 children (11%), time to diagnosis was longer than 1yr, in 1 child (1.2%) it was 2 yr, and in another child it was 8 yr.

One girl aged 10 yr at symptom onset was diagnosed with cSLE and was positive for antinuclear antibodies. At the time of diagnosis, the girl was being treated with carbamazepine for epilepsy, and cSLE symptoms and laboratory findings resolved after she was taken off carbamazepine; therefore she was excluded from the study.

Epidemiology

In this study of a predominantly Caucasian population, the ratio of females to males was 5.2:1. The overall annual incidence rate varied from 1 to 15 per million at risk (Fig. 1). Annual incidence rates among boys ranged from 0 to 7 per million at risk, while annual incidence rates in girls ranged from 2 to 23 per million at risk, with several peaks (Fig. 1). Starting from year 2004, annual incidence rates were significantly higher than before 2004 (p=0.019, *t*-test for independent samples) (Fig. 1). Among our 81 patients, 3 (3.7%) had a first-degree relative with SLE. One boy had both his mother and sister who developed SLE as adults, and two girls were twins from the same family. Clinical and laboratory manifestations of children with positive family history did not differ from those in children with negative family history.

Clinical features prior to referral

Most of the children complained of arthropathia/arthritis (54%), rash (35%), and general symptoms including fever, malaise and weight loss (36%). One-fifth was referred due to abnormal laboratory findings, including increased ESR, cytopenia, haematuria, and proteinuria. Small numbers of children were referred because of headache (n=3), abdominal pain (4), lymphadenopathy (11), Raynaud's phenomenon (9), and oedema (3).

Prior to referral, children had a median of 1 symptom (range 1–4): 53% had one symptom, 31% two symptoms; 11% 3

Table I. Clinical and laboratory signs during the first in-hospital evaluation in the tertiary referral centre of 81 Croatian children with cSLE.

	Signs (n)*				
Clinical symptoms	% of tested	Laboratory features	% of tested	Antibodies	% of tested
Musculoskeletal (81)	80	Accelerated ESR (77)	96	Anti-nuclear (79)	95
Skin and mucose (81)	65†	Low C3 complement (70)	69	Anti-histones (21)	86
Constitutional (81)	62	Low C4 complement (70)	44	Anti-dsDNA (37)	73
Fever (81)	31	Hematuria (81)	58	Anti-Sm (33)	64
Fatigue (81)	26	Proteinuria (81)	56‡	Anti-RNP (27)	48
Malar rash (81)	52	Anaemia (78)	43§	Anti-SS-A (Ro) (29)	35
Renal (81)	58	Thrompocytopenia (76)	31	Anti-Scl-70 (19)	32
Immunological** (81)	26	Leukopenia (78)	22††	Anti-SS-B (La) (29)	23
Abdominal (81)	22	Pancytopenia (76)	15	Anti-Jo-1 (21)	19
Cardiovascular (81)	20	Elevated serum creatinine (81)	20	Anticardiolipin	
Vascular (81)	15			IgG (41)	32
Raynaud's phenomenon (81)	14			IgM (41)	29
Neuropsychiatric (81)	7			LAC (41)	20
Respiratory (81)	4				

ESR: erythrocyte sedimentation rate, LAC: lupus anticoagulant, RF: rheumatoid factor.
*Number of tested children; †Girls (62%) *vs*. boys (90%), p=0.028, Fisher's exact test; ‡Girls (32 %) *vs*. boys (69 %), p=0.025, Fisher's exact test; §Girls (40%) *vs*. boys (69%), p=0.029, χ^2 test; **Splenomegaly, lymphadenopathy, antiphospholipid syndrome and hypercoagulability characterised with deep vein thrombosis, microvascular thrombosis in renal, skin and pulmonary vessels, and positive LAC and anti-CL IgG antibodies; ††Girls (20%) *vs*. boys (46%), p=0.017, χ^2 test.

symptoms; and 5% 4 symptoms. Most of the children with one complaint had musculoskeletal (n=18), skin (n=11), or constitutional (n=6) symptom; children with 2 complaints had musculoskeletal symptom combined with constitutional (n=9), or skin (n=5) symptom; while children with 3 symptoms mostly had musculoskeletal and constitutional symptoms combined with skin (n=3) or laboratory (n=2) symptom. Of the children with 4 symptoms, one had a combination of musculoskeletal, constitutional, renal and laboratory symptoms, another a combination of musculoskeletal, skin, renal and immunological symptoms, while two had musculoskeletal, constitutional and skin symptoms combined with neurological or renal symptom (1 of each).

Clinical features at diagnosis

The most frequent initial symptoms at the time of diagnosis were musculoskeletal, occurring in 80% of children, followed by skin and mucosa involvement (65%) and constitutional symptoms (62%) (Table I). The least frequent initial symptoms were respiratory (4%). Symptoms and signs did not differ significantly between girls and boys, except that skin and mucosa involvement was more frequent among boys (90% *vs*. 62%, p=0.028).

Of the 65 children with musculoskeletal symptoms, 55 (85%) showed joint involvement, while 42 (79%) of 53 children with skin and mucosa involvement presented with malar rash. Additionaly, less frequent symptoms included abdominal, cardiovascular, and neuropsychiatric involvement, as well as immunological abnormalities (splenomegaly, lymphadenopathy, and antiphospholipid syndrome and hypercoagulability characterised with deep vein thrombosis, microvascular thrombosis in renal, skin and pulmonary vessels, and positive LAC and anti-CL IgG antibodies).

During the first in-hospital evaluation in the tertiary referral centre, the mean number of affected organ systems was 4 (range 1–7). The two genders were similar in the number of organs (p=0.061) and in which organs were affected (p>0.175), except that boys had more heart symptoms (p=0.003). The mean ECLAM score was 5.1 (SD 2.4, median 5, range 1–10). ECLAM score did not vary significantly with gender (p=0.962) or age (p=0.850 using a cut-off of 14 yr). Of the 81 children, 10 (12%) presented with non-classical symptoms, including torticolis, oedemas in various locations, chorea with ataxia, dysarthria and dysphonia, icterus, and sepsis. Small numbers of children had serious conditions as part of the onset of cSLE symptoms: lupus encephalitis (n=2), tetraparesis (1), seizures (2), antiphospholipid syndrome and hypercoagulability (3), hypertension (2), cardiomyopathy (1), growth retardation (1), and osteoporosis (2).

More than one-third of the children (28 girls, and 3 boys) had other co-morbidity (*e.g*. organ involvement/conditions usually not associated to cSLE), such as IgA hypoglobulinemia (n=6), autoimmune thyroiditis (n=9), autoimmune hepatitis (n=5), juvenile idiopathic arthritis (enthesitis-related arthritis, HLA-B27+ [2]), hyperinsulinism (n=1), Sjögren disease (n=1), celiac disease (n=1), thoracic scoliosis (n=1), pollen allergy (n=1), seborrhea (n=1), gigantismus (n=1), obesity (n=1), and knee tumour (n=1). There was no difference in the occurrence of other conditions between genders (p=0.206).

Laboratory features

The most frequent abnormal finding was increased ESR, present in 74 of 77 tested children (96%) (Table I). Nearly half (43%) of 78 tested children had anaemia, which was more frequent in boys than in girls (69% *vs*. 40%, p=0.029, χ^2 test). Similarly, leukopenia was significantly more common in boys (46% *vs*. 20%, p=0.017,

χ^2-test). Of children with anaemia, 6 had antibodies against erythrocytes, of children with leukopenia or thrombocytopenia only 2 had antibodies against leukocytes or thrombocytes, while of children with pancytopenia only 1 had antibodies against all three blood cells. Different types of cytopenias did not show any association with the presence of antibodies against erythrocytes, leukocytes, or thrombocyte, or with hepato- or splenomegaly.

Hypocomplementemia was frequent: out of 70 tested children, 69% had low concentrations of complement C3, while 44% had low concentrations of complement C4 (Table I). Lupus anticoagulant antibody was present in 20% of 41 tested children, and 31% of 64 tested children had increased RF levels (Table I). The concentration of anti-CL IgG antibody was very high (>40 U/mL) in 13 of 41 (32%) tested children, while concentration of anti-CL IgM antibody was very high (>30 U/mL) in 12 of 41 (29%) patients (Table I).

Of 79 children, 95% were positive by the ANA screen, with a median titer of 1:1 280 (range1:20 to 1:20480). The anti-nuclear antibodies most frequently present were against histones (18 of 21 children, 86%) and dsDNA (27 of 37, 73%); the anti-nuclear antibody least frequently found was against Jo-1 (4 of 21 children, 19%) (Table I).

Renal involvement

Kidneys were affected in 47 of 81 children (58%), which usually manifested as haematuria (47 of 81, 58%) and proteinuria (>0.5 g/24 h, 45 of 81, 56%) (Table II). Renal symptoms occurred equally frequently in girls and boys, except for proteinuria, which was twice as frequent in boys (9 of 13, 69%) than in girls (22 of 68, 32%, p=0.025).

On the basis of clinical and laboratory indications renal biopsy was performed in 35 of 81 patients (43%): pathohistological examination revealed type 2–6 glomerulonephritis in all cases. The most frequent types of glomerulonephritis were types 3 and 4 in girls (9 and 7 of 29, respectively) and types 4 and 5 in boys (2 and 2 of 6, respectively, Table II).

Two children had suffered renal failure by the time of the first in-hospital evaluation in the tertiary referral centre, and one had to be dialysed.

Table II. Renal signs during the first in-hospital evaluation in the tertiary referral centre of 81 Croatian children with cSLE.

Signs (n)*			
Clinical symptoms	% of tested	Pathohistological finding	% of tested
Renal (81)	58	Glomerulonephritis (81)†	43
Haematuria (81)	58	Type 2 (35)	9
Proteinuria (81)	56‡	Type 3 (35)	28
Elevated serum creatinine (81)	20	Type 4 (35)	26
Oedema (81)	11	Type 5 (35)	20
Hypertension (81)	4	Type 6 (35)	17
Nephritic syndrome (81)	2		
Nephrotic syndrome (81)	1		
Renal failure (81)	2		

*Number of tested children; †Renal biopsy was performed on the basis of clinical and laboratory indications; ‡‡Girls (32 %) *vs*. boys (69 %), p=0.025, Fisher's exact test.

Treatment and outcome

The first in-hospital evaluation in the tertiary referral centre lasted a median of 66 days (range 11–146). Before the year 2004, none of 29 children stayed in hospital for less than a month, 4 (14%) stayed between 30–60 days, 9 (31%) between 60–90 days, while 16 (55%) children stayed in hospital more than 3 months (median 99, range 37–132 days). In 2004 and after, the admission time was significantly shorter compared to admission time before 2004 (median 54, range 11–146 days, p<0.001, t-test for independent samples): 17 (33%) children stayed in the hospital for less than a month, 21 (40%) between 30–60 days, 10 (19%) between 60–90 days, and 4 (8%) more than 90 days.

Of 81 children in this study, 79 were discharged as being in "improved condition/remission". Five girls were transferred to other paediatric wards, namely cardiology (n=2), dialysis (n=2), and nephrology (n=1). After the first in-hospital evaluation, 6 children were re-admitted because of relapses: 1 boy and 5 girls had relapse after a median of 12 months (range 4–48), while another girl relapsed more than 5 times in the next two years.

The children received treatment after the clinical assessment and after the blood and urine samples were taken for the laboratory evaluation in the tertiary referral centre.

Most children were treated with glucocorticoids (methylprednisolone or prednisone, 73%), azathioprine (27%), cyclophosphamide (24%), hydroxychloroquine (21%), non-steroidal anti-inflammatory drugs (NSAIDs, 19%), or combinations of these. In some cases, one or more of these drugs was used in combination with other immunomodulatory agents: methotrexate (n=4), human immunoglobulins (4), mycophenolate mofetil (2), or chimeric monoclonal antibody against CD20 protein (2).

Of the total group of 81 children, 13 (16%) needed additional therapy, such as transfusion (erythrocytes [n=5], platelets [n=1], albumins [n=1]), plasmapheresis (n=1), pericardiocentesis (n=2), or dialysis (n=3). Less than 4 children required physical therapy to correct already impaired limb function caused by arthralgia/arthritis or contractures.

During the first in-hospital evaluation in the tertiary referral centre, 2 of 13 boys died, corresponding to 15% of the boys in the study and 3% of the entire study population. One boy died 28 days after the admission because of catastrophic antiphospholipid syndrome. His first symptoms included arthralgia/arthritis and recurrent fever, but during the next 2 months and prior the admission in tertiary referral centre, and developed CNS vasculitis with chorea, ataxia, disarthria, and dysphonia, along with aggresive microvascular occlusive disease, characterised with microvascular thrombosis in renal, skin and pulmonary vessels; ANA titre was 1:8192, concentration of anti-dsDNA 268 IU/mL, RF 48 IU/mL,

Table III. Association of specific symptoms with auto-antibodies or patient location in 81 Croatian children with juvenile systemic lupus erythematosus*.

Symptoms / Signs	Anti-SS-A	Anti-Sm	Anti-dsDNA	Anti-Scl70	Anti-Jo	Anti-histones	Low C3	Low C4	Patient location*
Skin and mucose	•	•	•	<0.001	•	•	•	•	•
Raynaud's phenomenon	•	•	•	•	0.009	•	0.051	•	•
Neuropsychiatric	<0.001	0.003	0.011	•	•	•	•	•	•
Lupus nephritis	•	0.002	•	•	•	•	•	•	•
Abdominal	•	•	•	•	•	•	0.013	•	•
Antiphospholipid syndrome	•	0.003	•	0.001	•	•	•	•	•
Thrombocytopenia	•	•	•	•	•	•	0.039	0.002	•
Pancytopenia	•	•	•	•	•	•	0.037	0.002	•
Patient location	•	<0.001	0.050	•	•	0.048	•	•	-----

*Children (n=81) were divided into two groups based on the presence/absence of symptoms, and the levels of each protein were compared between the groups. Significant differences between the two groups are indicated with the corresponding *p*-value, whereas non-significant differences are represented with a dot (*t*-test for independent samples). A similar approach was used to compare boys with girls and to compare children in continental areas with children in Mediterranean areas.

complement C3 0.35 IU/mL, and anti-CL IgG 35 U/mL.

The other boy died of renal failure. He presented with a 2-week history of arthralgia/arthritis and fatigue prior the first in-hospital evaluation in tertiary referral centre, but during his stay in hospital he developed acute renal failure, with the concentration of antibodies against SS-A 724I U/mL, SS-B 780 U/mL, Sm 412U/mL, RNP 282 U/mL, dsDNA 95, both CL IgM and IgG 11 U/mL, and complement C3 0.5g/L, and C4 0.05 g/L. Because there was no transplant option, he was treated with dialysis, but he died 52 days after the admission.

Relationship of clinical and laboratory features with the presence of auto-antibodies and C3 and C4 complement levels

Elevated concentrations of at least one autoantibody and/or low complement were associated with symptoms affecting skin and mucosa, the nervous or abdominal systems, and kidneys (Table III). Anti-SS-B, anti-RNP, anti-CL IgM, and anti-CL IgG were not associated with any of clinical symptoms, patients' gender or their location.

Girls and boys showed similar distributions of autoantibodies.

Influence of annual solar irradiation on the clinical and laboratory presentation of cSLE

In order to explore whether sunlight exposure affects clinical and laboratory features of cSLE, we divided patients into two subgroups based on their residence: the continental subgroup (n=46) received lower annual solar irradiation, while the Mediterranean subgroup (n=35) received higher annual solar irradiation. The two subgroups were comparable in size (p=0.267), age (p=0.103), and gender (p=0.135).

The two subgroups did not differ in ECLAM score (p=0.955) or in clinical or laboratory presentation of cSLE (Table III). However, the Mediterrean subgroup showed significantly higher frequencies of several anti-nuclear antibodies: anti-Sm (p<0.001), anti-dsDNA (p=0.050), and anti-histone (p=0.048) (Table III).

Factors influencing time to cSLE diagnosis

The ECLAM score was inversely associated with time to diagnosis (p<0.001, Spearman's rho -0.397). None of the other tested factors showed an association with time to diagnosis, including age, gender, distance from the nearest tertiary referral centre, or clinical symptoms (Table IV).

Discussion

This is the first in-depth analysis of clinical and laboratory manifestations of cSLE in Croatian children, a predominantly Caucasian population. The results indicate that cSLE features are similar to those reported in other countries, and by the time of diagnosis cSLE involved multiple organ systems. Of all clinical, laboratory, and demographic characteristics we examined, only the ECLAM score was (inversely) associated with this interval. Our findings highlight the need for early diagnosis not only in children with very active disease, but also in children presenting with just few symptoms.

The annual incidence rate of cSLE fluctuated between 1 and 15 per million children at risk during the 20-year study period, which is comparable to 2.2 and 2.8 per million reported for Canada (5, 7, 21), and 9 per million for Finland (6). As it was shown in Figure 1, there was a significant increase of the annual incidence rate starting with the high peak in year 2004. This increasing trend could be explained by the introduction of routine antibody screening in children suspected of cSLE: starting with the year 2004, all sera positive by IIF were further tested for auto-antibodies specific for SLE. In addition, primary care physicians probably became more aware of paediatric rheumatology diseases and more sensitive to cSLE symptoms.

The median interval between symptom onset and cSLE diagnosis was 2 months in our study, which is shorter than the 3.5–60 months reported in studies in Iran, South Africa, and even developed countries like France, Spain and the UK (3, 7, 8, 22-24). In fact, in our study, time to diagnosis was relatively short even when initial symptoms were non-classical, whereas non-classical symptoms in South Africa extended time to diagnosis to an average of 8 months (8). This may be because only 12% of our children presented with non-classical symptoms, which is much lower than the 30% reported in a French study (3) or 46% in a South African study (8).

In an effort to identify factors influencing time to diagnosis, we tested numerous clinical, laboratory and demo-

graphic factors. Of all factors tested, only one was associated with time to diagnosis: the ECLAM score was inversely associated with this interval, with weak power of the association (Table IV). A study of 257 children of Caribbean/African and Asian ethnicity in the UK found both lupus nephritis at presentation and the type of referring physician to be strong independent predictors of time to diagnosis (24). However, our data did not show an association between the presence of lupus nephritis and time to diagnosis. This difference may be due to the fact that in the UK study, shorter time to diagnosis was confounded with African ethnicity, which was also a predictor of time to diagnosis, since lupus nephritis occurs more often, at earlier ages and with greater severity in African adults and children with cSLE (25, 26). Conversely, people of European ancestry are "protected" from renal disease in SLE (27). We could not assess whether an association existed between the type of referring physician and time to diagnosis in our study population because these data were not recorded for most of our patients. Nevertheless, we do know that some children were referred by a general practitioner and the others by a paediatrician, so it would be interesting to look for differences in time to diagnosis depending on the speciality of referring physician in future work.

Several additional factors may have contributed to extended time to diagnosis in our study. One possibility is multiple referrals. Approximately 80% of children in our study were from outside Zagreb and surrounding areas, meaning that they probably went through a "multi-referral pathway" in which they were referred first to a local secondary care centre, and then to one of the tertiary referral centres involved in our study, where they were ultimately diagnosed with cSLE. Usually, each of these steps requires making an appointment up to several weeks ahead. The remaining children were from Zagreb and surrounding areas, so they were probably referred in a single step to the tertiary centre there. Other factors that may have postponed diagnosis could be socioeconomic: less-educated, younger parents living in rural areas, with more than one child may not be aware of cSLE signs in their child, or may not take them seriously enough for a longer period of time. These aspects should be included in future work to gain a more complete picture of factors influencing time to diagnosis.

The median time of first in-hospital evaluation in tertiary care centre was 66 days. This appears very long, but it was mostly influenced by long admission times before 2004: namely, with the development of paediatric rheumatology in Croatia, in recent years hospital admission time of children with cSLE is has shortened.

Two children in our study died during initial assessment in the tertiary referral centre, one because of catastrophic antiphospholipid syndrome and the other because of terminal renal failure. Even though the boys who died were aged 7 and 8 yr, we found no difference in major organ involvement between children younger and older than 14, which was the median age of cSLE onset in our study. These findings contrast with those of a US study that reported that younger children required more aggressive treatment and more admissions to the paediatric intensive care unit (28). Although the two deaths in our study were boys, we found no significant difference between girls and boys in the number of major organs involved or in the clinical and laboratory presentation of cSLE. This is in contrast with a Brazilian study that reported male gender to be an independent factor associated with death (29).

After the first in-hospital evaluation in the tertiary care centre, all children were subsequently seen by the paediatric rheumatologist in regular check-ups, but only six children were re-admitted because of one or more relapses. Unfortunately, these are the data for paediatric tertiary care centres only, but we do not have the data about the admission because of cSLE relapses in secondary care hospital or adult rheumatology wards (after the patients turned 18), because follow-up of cSLE patients was not within the scope of this study.

Although the children in our study presented clinical and laboratory features of cSLE similar to those of children in other countries (3, 7, 21-23, 30-34), they showed some differences. Croatian children presented most frequently with musculoskeletal symptoms, while children in numerous other studies mostly had skin-related symptoms (3, 7, 21, 30, 31) or constitutional symptoms (23, 32, 33). In addition, Croatian children experienced lung, neuropsychiatric, and non-classical symptoms less frequently than the chil-

Table IV. Analysis of factors influencing time to cSLE diagnosis in 81 Croatian children.

Variable	*p*-value* (Spearman's rho)
Distance from the nearest tertiary paediatric centre	0.611 (-0.063)
Patient's age	0.467 (-0.090)
European Consensus Lupus Activity Measurement (ECLAM) score	<0.001 (-0.397)
	p-value†
Gender	0.289
Clinical symptoms and signs	
Constitutional	0.327
Musculoskeletal	0.392
Skin and mucose	0.205
Heart	0.668
Lung	0.539
Vascular	0.749
Raynaud's phenomenon	0.973
Immunological	0.601
Neuropsychiatric	0.693
Renal	0.501
Lupus nephritis	0.433
Abdominal	0.569

*Rank correlation test; †*t*-test for independent samples.

dren in other studies (3, 8, 21-24, 30-32, 24). While we observed a diversity of non-classical symptoms in 12% of children, such as torticolis, oedemas in various locations, sepsis, icterus, and chorea with ataxia, dysarthria and dysphonia, in the French study one third of children presented with no-classical symptoms, predominantly abdominal (3). This difference may indicate that Croatian physicians are less sensitive to abdominal symptoms when certain other symptoms are present.

Prevalence of renal involvement and renal failure at cSLE presentation in Croatian children were comparable to prevalence found in numerous other studies (3, 8, 15¸ 21, 30-34).

The fact that more than a third children in our study had other co-morbidites usually not found in cSLE patients (*e.g.* autoimmune thyroiditis, autoimmune hepatitis, seronegative JIA, Sjögren disease coeliac disease) may be in concordance with ongoing debate "whether SLE may be considered not as a single disease, but rather as a single syndrome, which defines by a set of signs, symptoms, or phenomena that occur together and suggest a particular abnormality" (35). Also, it would be interesting to explore presence of organ-specific auto-antibodies in children with other autoimmune diseases, such as autoimmune thyroiditis or hepatitis, coeliac disease, in Croatian children with cSLE, as it has been recently done by Aikawa *et al*. (36), but given the retrospective nature of this study, this was not possible.

Since many children with cSLE have multiple organ systems involved and subsequently lowered quality of life, an effort should be made to develop and examine the usefulness of tracking the quality of life of cSLE patients with multidimensional instruments like the Juvenile Arthritis Multidimensional Assessment Report does (37): a similar multidimensional tool for cSLE would take into account the perspectives of the patient and parents, unlike the British Isles Lupus Assessment Group (BILAG) index (38) or the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (39), which both reflect only physician's assessment.

The most frequent autoantibodies in Croatian children were against histones, SS-B Smith and RNP antigen, while the predominant auto-antibodies in other studies were against dsDNA (3, 7, 21-24, 30-32, 34). Almost all autoantibodies were associated with the presence of at least one clinical or laboratory manifestation of cSLE or patient location. Similarly, almost all clinical and laboratory manifestations were associated with the presence of at least one autoantibody, and some of them with more than one. Unfortunately, only few studies have investigated the association of clinical and laboratory features of cSLE with the presence of specific auto-antibodies. In our study, lupus nephritis was associated with high levels of only anti-Sm antibody, while in a Canadian study (21) it was associated with high levels of anti-Sm, anti-RNP, and anti-CL IgM, and in a Belgian study (32) it was associated with high levels of anti-dsDNA antibodies.

Frequencies of clinical and laboratory features (including skin symptoms) did not vary significantly between Croatian children living in continental and Mediterranean climates.

This result was unexpected, given the strong evidence implicating UV-B radiation as a trigger in cSLE onset (1). This discrepancy may reflect the fact that Croatia is a small country spreading over only 2.5 degrees of latitude.

Except of this geographical limitation and its retrospective nature, this study has several other limitations: first: it involved only 81 patients; second: just a proportion of children were tested for auto-antibodies – some of the associations might have been different in larger cohorts; and third: we compared our results with the result of other studies that might have detected antibodies using methods other that ELISA (*e.g.* immunoblotting, RIA, and multiplex and array technologies). In general, results obtained with the different methods correlate well, but discrepancies may arise due to different source of antigen, presentation of the antigen to the antibody, reaction conditions, or the avidity of the antibodies detected (40-42). Nevertheless, since the results in this study are similar to the results in other studies, we believe that the aforementioned limitations do not preclude us from drawing valid conclusions.

Even though diagnosis occurred within 2 months of symptom onset in our study, more than a half of Croatian children had involvement of more than 3 organ systems by the time of diagnosis. During the months before correct diagnosis, most children with arthralgia/arthritis adapted their behaviour to avoid using affected joints, which led to restricted movement in those joints, and in some cases to shortening of tendons with contractures. Moreover, more than half of the children in our study developed lupus nephritis by the time of diagnosis.

Conclusion

This work highlights the need for further research to identify other factors, in addition to ECLAM score, that may be related to extended time to diagnosis. Such studies may help clinicians to detect children with cSLE early in the course of the disease, when the disease is still mild or fewer organ systems are involved.

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