ORIGINAL INVESTIGATIONS

Mixed connective tissue disease with juvenile onset: results of a retrospective single-center study

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Mixed connective tissue disease (MCTD) is one of the very rare systemic autoimmune diseases; it accounts for 0.1–0.6% of cases in pediatric rheumatologists' practices. MCTD is characterized by a broad spectrum of clinical manifestations and a high frequency of extremely unspecific symptoms at the onset, with the overall picture of the disease forming slowly and gradually. The diagnosis is often delayed and confirmed only at an advanced stage of organ dysfunction with the development of irreversible changes.

Objective: *to identify a group of patients fulfilling the criteria for MCTD in an open, single-center, continuous retrospective study among anti-ribonucleoprotein (anti-RNP) antibody-positive patients and to analyze their demographic, clinical and laboratory characteristics and therapy.*

Material and methods. *All anti-RNP-positive patients admitted to the pediatric department of V.A. Nasonova Research Institute of Rheumatology from 2019 to 2023 and meeting at least one of the variants of the MCTD criteria (Kasukawa, Alarcyn-Segovia, Kahn and Sharp criteria) were included in the study.*

Results and discussion. *18 (56.25%, 17 girls and 1 boy) of 32 anti-RNP-positive patients fulfilled criteria for MCTD. Patients most frequently fulfilled a combination of criteria – Sharp and Kahn (n=8) or Alarcyn-Segovia and Kahn (n=8). The median age of onset of MCTD was 12.2 [9.7; 13.9] years. The most common clinical manifestations were arthritis (100%), various skin lesions (94.4 %), Raynaud's phenomenon (88.9%), lymphadenopathy (72.2%) and general constitutional disorders (50%). Sjögren's syndrome (SS) was diagnosed in 17 (94.4%) patients. All patients had antinuclear factor (ANF) 1/1280, and the anti-RNP level was >200 U/ml. There were also antibodies against double-stranded DNA (n=5), Ro- (n=4) and Sm- (n=5) antigens. An IgM rheumatoid factor was detected in 6 patients and hypergammaglobulinemia in 10 patients. Capillaroscopic changes in the nailfold with predominant scleroderma type were found in 77.8% of patients. The most common combination was of Raynaud's phenomenon, arthritis, SS, lymphadenopathy and hypergammaglobulinemia (50%).*

All patients received glucocorticoids, 9 – hydroxychloroquine, 8 – methotrexate, 3 – mycophenolate mofetil, 1 – cyclophosphamide, 1 – azathioprine. Biologic DMARDs (bDMARDs) were prescribed to 12 (66.7%) patients: 3 – rituximab, 8 – abatacept, 1 – belimumab, with an acceptable safety profile and initial efficacy.

Conclusion. *Most patients in the study met the Kahn criteria. Only 2 patients met all variants of the criteria, which indicates the need to use a combination of criteria when a MCTD is suspected. A combination of Raynaud's phenomenon, arthritis, SS, lymphadenopathy and hypergammaglobulinemia was observed in half of patients with MCTD. The presence of Raynaud's phenomenon and high ANF titer in children with rheumatic diseases, especially with a polymorphic clinical picture, requires the inclusion of MCTD in differential diagnosis. Preliminary results indicate the safety of the use of biologic drugs in children with MCTD.*

Keywords: *mixed connective tissue disease; antibodies against ribonucleoprotein; childhood.*
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Mixed connective tissue disease (MCTD) is a rare systemic autoimmune disease characterized by a combination of the presence of antibodies to ribonucleoprotein (anti-RNP) in high titer and manifestations of at least two of the following diseases: systemic lupus erythematosus (SLE), dermatomyositis (DM)/polymyositis (PM), systemic sclerosis (SSc) and rheumatoid arthritis [1]. Most authors describe MCTD as a distinct clinical entity [1–3], some believe that it may represent an early stage of a well-defined systemic connective tissue disease, for example, SLE or SSc. Some authors classified MCTD as one of the variants of overlap syndrome [4]. MCTD does not have unique clinical features, and there are significant individual differences in its clinical manifestations. According to R. Gunnarsson et al. [5], the incidence of MCTD in Norway was 2.1 per 1 million per year; the prevalence was 3.8 per 100 thousand for juvenile onset, and 3.4 for adult onset; 76.9% of patients were women. In this study, 10.2% of patients had onset before 18 years of old, the median age of onset was 13.0 [11.6; 14.4] years. However, the authors note that many patients had the initial manifestations of MCTD before the age of 18. (17.7%) [5]. In general, MCTD is one of rare diseases in the practice of pediatric rheumatologists, according to various data, it occurs in 0.1–0.6% of cases [4, 6]. MCTD is characterized by a wide range of various disorders and, as a rule, a high frequency of extremely non-specific symptoms at the onset with a gradual slow formation of the full picture of the disease, therefore, its diagnosis is often delayed and verified already at an advanced stage of organ disorders with the presence of persistent damage [7]. Anti-RNPs may also be present in other well-defined diseases, such as SLE, SSc, idiopathic inflammatory myopathies, Sjogren's syndrome (SS), and in some cases are associated with individual clinical manifestations, for example, with scleroderma-like changes in patients with SLE [8–10]. Currently, there are four variants of the

classification criteria of MCTD (Table. 1) [11]. According to R.J. Mier et al. [12], Kasukawa's criteria are more often used in pediatric practice. Problems arise not only in the diagnosis, but also in the administration of treatment for such patients, since complete clinical recommendations for the therapy of MCTD have not been developed [7]. As for the evolution of MCTD, according to S. Cappelli et al. [13], approximately 9 years after the start of follow-up, about 60% of patients still met Kasukawa's criteria, 17.3% – had progress to SSc and 9.1% – to SLE.

Over the past 20 years, only four studies of cohorts of pediatric patients with MCTD have been published in the English-language literature [4, 14–16]. Taking into account the difficulties in diagnosing this pathology in real clinical practice, the study of the features of MCTD in childhood seems to be actual.

The aim of the study: in an open single-center continuous retrospective study to identify a group of patients with anti–RNP+ who meet the criteria of MCTD and analyze their demographic, clinical and laboratory characteristics and therapy.

Material and methods: Anti-RNP+ patients were selected from the clinical database of the Pediatric Rheumatology Department of V.A. Nasonova Research Institute of Rheumatology over the period from 2019 to 2023. Patients who met at least one of the variants of MCTD criteria (the criteria suggested by Kasukawa, Alarcon-Segovia, Kahn and Sharp) were selected from this group for analysis (Table 1). Data from medical records was entered into specially designed database.

The parents of the patients signed an informed consent to the anonymous use of personal data.

All patients underwent standard clinical and laboratory-instrumental examination in accordance with the available clinical recommendations for this nosology. The examination was carried out at V.A. Nasonova Research Institute of Rheumatology: complete blood count, urine analysis, biochemical blood analysis were performed in the biochemical laboratory, immunological and genetic studies – in the laboratory of Immunology and molecular biology, X–ray examination– in the X-ray department, instrumental investigations – in the department of functional diagnostics. If there was a suspicion of SS, an in-depth dental examination was performed, including a dentist's consultation, ultrasound, sialometry, sialography, and the Schirmer test.

Statistical data processing was carried out using the Statistica v. program. 10.0 (StatSoft Inc., USA). The preliminary sample size of the study was not calculated. Quantitative variables were described using the median and the interquartile interval (Me [25th; 75th percentile]).

Results. Out of 32 anti-RNP+ patients, 18 (56.25%, 17 girls and 1 boy) met at least one of criteria of MCTD: 15 – Kahn criteria, 10 – Kasukawa criteria, 9 – Sharp criteria, 8 – Alarcon-Segovia criteria. At the same time, 4 patients fell under only one set of criteria, 6 patients – under two, and 6 more – under three sets of criteria, 2 patients – under all four sets of criteria. The most common combinations were Sharp and Kahn criteria (n=8) and Alarcon-Segovia and Kahn criteria (n=8). The median age at onset of the first symptoms of the disease was 12.2 [9.7; 13.9] years, and the median age of diagnosis verification was 14.3 [11.8; 15.7] years. In 8 (44.4%) children manifestations of SLE prevailed, in 5 (27.8%) – SSc, in 4 (22.2%) – symmetrical polyarthritis, and in 1 patient – Raynaud's syndrome and recurrent parotitis. The clinical characteristics of the patients are presented in Table 2.

Skin involvement included sclerodactyly (n=7), telangiectasias (n=2), malar rash (n=5), heliotropic rash and Gottron papules (n=4), erythema nodosum (n=1), livedo reticularis (n=2). SS was diagnosed in 17 (94.4%) patients, of whom 15 had isolated involvement of the salivary glands and 2 had combined involvements of salivary and lacrimal glands.

According to laboratory examination, the level of anti-RNP in all patients exceeded 200 U/ml (the norm is 0–25 U/ml), ANA was detected on a culture of Hep-2 cells in a titer ≥1/1280. Speckled type of ANA was detected in 77.8% of patients, mixed type (homogeneous + speckled + cytoplasmic) – in 22.2%. Antibodies to double-stranded DNA (anti–dsDNA) were found in 5 (27.8%) patients, antibodies to Ro (anti-Ro) in 4 (22.2%), and anti–Sm in 5 (27.8%) patients. IgM rheumatoid factor (RF) was detected in 6 (33.3%) patients, the median level was 47.25 [27.75; 54.90] IU/ml. A decrease in C3 component of the complement was found in 11.1% of children, hypergammaglobulinemia in 55.6% (n=10). Capillaroscopic changes in the nail folds were present in 77.8% of patients: in 5 – nonspecific abnormalities, in 5 – an early scleroderma pattern, in 3 – late scleroderma pattern with a myopathic component and in 1 – changes occurring in juvenile DM (see figure). Scleroderma pattern was detected in 71.4% of patients with lung involvement. The most common combination of symptoms of MCTD was Raynaud's syndrome, arthritis, SS, lymphadenopathy and hypergammaglobulinemia (n=9, 50%).

All patients received glucocorticoids (GC) per os, 9 patients – hydroxychloroquine, 8 – methotrexate (MT), 3 – mycophenolate mofetil (MMF), 1 – cyclophosphamid, 1 – azathioprine. Twelve patients (66.7%) received biologics. The reason for their use in 9 patients was insufficient effectiveness of the previous therapy, which included GCs and synthetic disease-modifying antirheumatic drugs (sDMARDs), in 3 children the presence of initially high disease activity required simultaneous administration of sDMARD and biologics. Three patients received rituximab (RTM), 8 – abatacept (ABA), 1 – belimumab with good efficacy and safety.

Discussion: In a single-center open retrospective study, we analyzed the clinical features and data of laboratory and instrumental examination of patients with high titer of anti-RNP (>200 U/ml) who met at least one set of MCTD diagnostic criteria. It is important to note, that MCTD criteria were developed for adult patients, so they may have certain limitations when used in pediatric practice [17]. Based on clinical manifestations and data of laboratory examination, the conformance of each patient to MCTD classification criteria was assessed. At the same time, we took into account that the presence of high titer anti-RNP is not a pathognomonic sign of MCTD. In the study P. Ungprasert et al. [18], which included of 264 anti-RNP+ patients, only 18.9% met at least one set of MCTD criteria, but the majority (58%) were patients with SLE. Interestingly, the proportion of patients with SSc was only 2% and with SS – 7%. I. Elhani et al. [19] analyzed the data of 36 anti-RNP+ patients over the age of 14, 50% of whom met at least one set of MCTD criteria, but 61% of them met the criteria of ACR/EULAR (American College of Rheumatology / European Alliance of Associations for Rheumatology)-2019 for SLE. In the work of D. Isenberg [20], anti-RNP+ was detected in 35% of patients with SLE. T. Miyamae et al. [9] based on a study of 80 children with a confirmed diagnosis of SLE, 27.5% of whom were positive for both anti-RNP and anti-dsDNA, and 12.5% – only for anti-RNP in the absence of anti-dsDNA, concluded that anti-RNP+ and anti-dsDNA+ children should be observed as having two competing diagnoses.

Table 1. Classification criteria of the MCTD [11]

G.C. Sharp et al., 1972	D. Alarcyn-Segovia and M. Villareal, 1987	R. Kasukawa et al., 1987	M.F. Kahn et al., 1989
Major criteria: 1) severe myositis; 2) lung involvement; 3) Raynaud's syndrome or esophageal dysmotility; 4) swelling of fingers or sclerodactyly; 5) anti-U1-RNP positive, anti-Sm negative	*Serologic criteria:* anti-U1-RNP at a titer of ≥1:1,600	1. *General symptoms:* a) Raynaud's syndrome; b) swelling of fingers 2. Anti-U1-RNP positive	*1. Serologic criteria:* anti-U1-RNP at a titer of ≥1:2000, corresponding to the speckled type of ANA
Minor criteria: 1) alopecia; 2) leukopenia; 3) anemia; 4) pleurisy: 5) pericarditis; 6) arthritis; 7) trigeminal neuritis; 8) malar rash; 9) thrombocytopenia; 10) mild myositis; 11) the history of the swollen hands	*Clinical criteria:* a) swelling of hands; b) synovitis; c) myositis; d) Raynaud's syndrome; e) acrosclerosis with/without proximal sclerodactyly	*3. Symptoms:* **SLE**: a) polyarthritis; b) adenopathy; c) malar rash; d) serositis; e) leukopenia/ thrombocytopenia **SSc:** a) sclerodactyly; b) pulmonary fibrosis or restrictive lung changes; c) esophageal dysmotility or dilation **PM:** a) muscle weakness; b) an increase in the level of enzymes; c) myopathic changes on EMG	2. *Clinical criteria:* a) Raynaud's syndrome; b) synovitis; c) myositis; d) swelling of fingers
Reliable diagnosis: 4 major criteria, anti-U1-RNP>1:4000, absent anti-Sm *Possible diagnosis:* 3 major criteria and absence of anti-Sm or 2 major criteria + 1 minor criterion, anti- U1-RNP >1:1000	*Reliable diagnosis:* serological criteria + 3 clinical criteria. If a, d, e criteria are present, criteria b, c are mandatory	*Reliable diagnosis:* 1 out of 2 general symptoms + anti-U1-RNP + 1 of symptoms of 2 connective tissue diseases: SLE, SSc, PM	*Reliable diagnosis:* anti-U1-RNP positive+ Raynaud's syndrome + 2 of the other 3 criteria

Annotation: Anti-Sm – antibodies to Smith antigen; anti-RNP – antibodies to ribonucleoprotein; EMG – electromyography; ANA – antinuclear factor.

In our study, the vast majority of patients met Kahn's criteria, Kasukawa's criteria took the second place. There are very few studies in the literature that would use all four sets of criteria. A comparison of four sets of classification criteria demonstrated that Alarcon-Segovia's and Kahn's criteria are the most acceptable for diagnosis in terms of sensitivity and specificity [21]. Most other authors compared three earlier sets of criteria. Thus, S. Cappelli et al. [[13] It is reported that Kasukawa's criteria were more sensitive (75%) than Alarcon-Segovia's criteria (73%) and Sharp's criteria (42%). But P. Ungprasert et al. [18] decided that Alarcon-Segovia's and Kasukawa's criteria were equally sensitive (72%), and Sharp's criteria were the least sensitive (28%). The criteria of Kasukawa [4, 14–16] and Alarcon-Segovia [4] were used in pediatric cohort studies.

Girls predominated among our patients, and in studies of other authors girls also constituted about 85-100% of patients [4, 14–16]. In our study group, the duration of the disease at the time of diagnosis was about 2 years, which is also comparable with literature data: the period from the onset of the first symptoms of the disease to diagnosis ranged from 2 to 3 years [4, 15].

At the time of diagnosis, the majority of our patients had symptoms classified as SLE, similar results were received by E.D. Batu et al. [4] and S.O. Hetlevik et al. [15]. The most common manifestations were arthritis, Raynaud's syndrome, and various skin lesions, which were described in previously published studies. The frequency of detection of Raynaud's syndrome ranged from 75% to 98%, arthritis – from 75% to 87% [4, 15, 16]. The incidence of skin lesions was 96.7% [4]. Only in the study by Y.Y. Tsai et al. [14] these manifestations were less common: Raynaud's syndrome and arthritis – in 58% of cases, skin lesions – in 50%. The most common skin lesions in our study, as in the data of other authors, were malar rash (25-53,3% [4, 16]), sclerodactyly (26,6–53,3% [4, 16]), heliotropic rash (55% [16]). Lymphadenopathy turned out to be a very common clinical manifestation in our patients (72%), but according to the literature, its frequency in children was 15-23% [4, 15, 16]. We found a high incidence of SS, while in other studies it was detected in 16.7–20% of cases [4, 16]. Such differences may be due to the fact that sialometry and sialography were performed in all our patients with suspected MCTD, which was probably not one of the routine methods in the studies of other cohorts. Interestingly, E.D. Batu et al. [4] discovered hypolacrimia more often (13.3%) than impaired salivary gland function (10%). More than a third of the patients in our cohort had lung involvement, which corresponds to the literature data (15–41,7%) [4, 14, 16], at the same time, kidney involvement was detected only in a small part of patients, which is consistent with the results of most studies (less than 10%) [7, 15], and only the study by

E.D. Batu et al. [4] demonstrated a greater incidence – 30%.

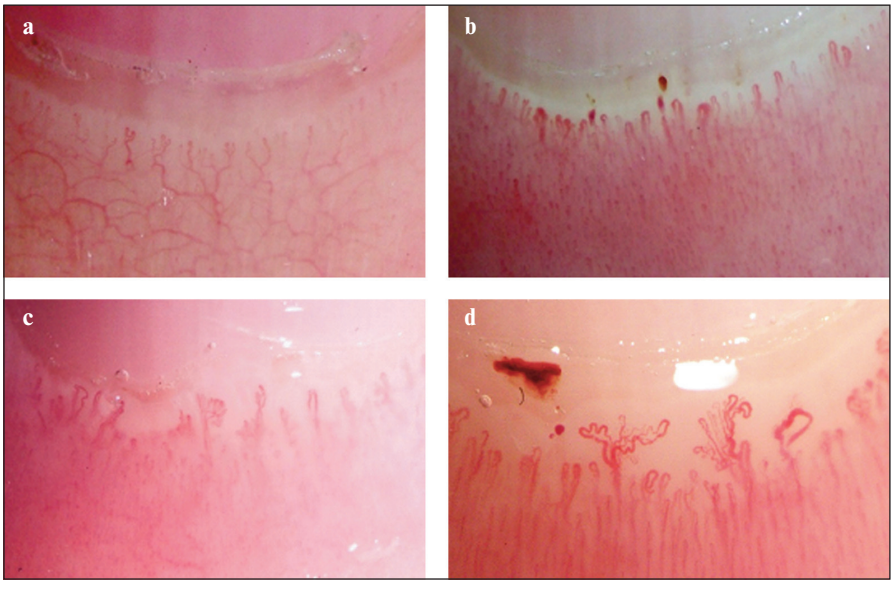
More than a third of our patients had leukopenia, which coincides with the data of similar studies (25-43,3%) [4, 14–16], however, S.O. Hetlevik et al. [15] detected leukopenia significantly less frequently – in 17% of cases. IgM RF was present in a third of our patients, which is comparable with the results of E.D. Batu et al. [4], but differs from the data obtained in other studies – from 50% to 78.3% [14–16]. According to S.O. Hetlevik et al. [15], IgM RF may be a prognostic marker of ongoing active disease. According to the literature, anti-Sm (10%), anti-dsDNA (20%) and anti-Ro (13%) may also be detected in children with MCTD [12]. In the study of P. Ungprasert et al. [18], which included patients over the age of 18, IgM RF was detected in 24% of patients who met the criteria of MCTD, anti-Ro in 14%, anti–Sm in 18%, hypocomplementemia in 3%. In our study these immunological parameters were somewhat more common. E.D. Batu et al. [4] demonstrated similar frequency of anti-Ro (27.6%) and a significantly higher frequency of anti-Sm (51.7%).

Raynaud's syndrome, as mentioned above, is the main clinical feature of MCTD, regardless of the age of onset [17]. The nail fold capillaroscopy is an inexpensive non–invasive and reproducible imaging method that allows assessing structural changes in peripheral microcirculation that may be associated with Raynaud's syndrome [22]. Raynaud's syndrome in patients with MCTD can be observed earlier than other symptoms, so capillaroscopy provides an ideal opportunity to diagnose the earliest stages of microcirculation changes. In our study, the majority of patients had capillaroscopic changes in the nail folds either of early or late type of sclerodermic patterns, which can serve as an additional argument supporting further possible evolution of the disease towards the predominance of SSc. In a study by A. Felis-Giemza et al. [23], an early scleroderma pattern in capillaroscopy was detected in 44% of patients with MCTD. According to I.M. Markusse et al. [24], the "early" pattern is associated with the presence of anti-RNP positivity. In a large prospective study involving 3,029 patients with primary Raynaud's syndrome (mean follow–up period was 4.8 years), a scleroderma pattern was also statistically significantly associated with the development of MCTD, and the number of patients who met the criteria of MCTD and had scleroderma pattern on capillaroscopy increased over time [25]. According to M Celinska-Löwenhoff et al. [26], an active scleroderma pattern with the presence of giant capillaries, detected by capillaroscopy, may be a promising marker of interstitial lung disease in patients with MCTD, especially with a short duration of the disease. In this regard, it should be noted that a significant part of our patients with lung involvement had a scleroderma pattern on capillaroscopy. A significant proportion of children with a scleroderma pattern and Raynaud's syndrome in this study suggests a high probability of disease evolution towards the predominance of the sclerodermic component, as shown in a prospective study by S.O. Hetlevik et al. [15].

Specific protocols of treatment for MCTD have not been developed, and drugs aimed at eliminating the leading manifestations of the disease are used. According to the literature, the vast majority of patients receive low and medium doses of GC and hydroxychloroquine [4, 12, 14, 27], which corresponds to our results. As S.O. Hetlevik et al. [15] point out, the frequency of GC use increased significantly with long-term follow-up. All sDMARDs are used in patients with MCTD, but predominantly MT, which is also confirmed by our results [12, 15, 27]. Mostly, it is due to a high incidence of arthritis in MCTD. Recently, there has been an increase in the administration of MMF [4]. Two thirds of our patients received biologics. There are reports of suc-

Table 2. Clinical characteristics of the patients (n=18)

Characteristics	The number of patients, %
Arthritis	100
Sjogren's syndrome	94.4
Raynaud's syndrome	88.9
Lymphadenopathy	72.2
Skin and mucous involvement	94.4
General constitutional disorders, *including:* Febrile fever	50 22.2
Lung involvement	38.8
Myositis	27.8
Nephritis	11.1
Myocarditis	11.1
Pericarditis	5.6
Leukopenia	38.9
Thrombocytopenia	11.1



Capillaroscopic changes in patients with MCTD: a – non-specific changes; b – early scleroderma type; c – late scleroderma type with myopathic component; d – changes occurring in juvenile DM

cessful use of TNF-α inhibitors [15] and RTM [4, 28] in MCTD. An analysis of the literature data allows us to conclude that there is a distinct increase in the number of RTM prescriptions for the treatment of MCTD, including the juvenile onset, despite the status of "of label". If in the study by S.O. Hetlevik et al. [15], published in 2017, RTM was used in only 2% of children with MCTD, in the work of E.D. Batu et al. [4], which appeared in 2023, it was used in 27.6%. In 2023 the results of the use of RTM in combination with MMF in adults having MCTD with lung involvement were published, confirming the high efficacy with an acceptable safety profile [29]. There is evidence of high efficacy of RTM in severe Raynaud's syndrome and thrombocytopenia in patients with MCTD [28, 30, 31]. We have not found information in the literature about the use of ABA in MCTD, however, there is a successful experience of its use in juvenile idiopathic arthritis [32], juvenile DM [33], SSc [34], overlap syndromes [35], interstitial lung disease in children with immunodeficiency [36]. Previously, we reported good efficacy and safety of ABA in SS and idiopathic inflammatory myopathies in children [37, 38]. Considering that ABA, unlike RTM, is allowed for use in pediatric practice, as well as the high incidence of arthritis in the study group, the administration of this drug to a large number of patients seems justified.

The small number of our patients certainly limits the interpretation of our data, so further accumulation of material will increase the reliability of the results. The main objective of the study was to describe the real picture of clinical and laboratory changes in patients with such a rare pathology as MCTD. In addition, we tried to show the complexity of classifying such patients using various available sets of criteria.

Conclusion. The majority of patients with MCTD in our study met Kahn's criteria. The results obtained demonstrate the possibility of patients with MCTD to match various combinations of criteria. Only 2 children fit all sets of criteria, which indicates the need to use several sets of criteria for diagnosis of MCTD. A combination of Raynaud's syndrome, arthritis, SS, lymphadenopathy and hypergammaglobulinemia was observed in half of the patients with MCTD. The presence of Raynaud's syndrome and a high ANA titer in children with rheumatic diseases, especially with a polymorphic clinical picture, requires inclusion of MCTD in the range of differentiable conditions. Preliminary results demonstrated the safety of the use of biologics in children with MCTD.

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