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Autoimmune Response in Children with Multisystem Inflammatory Syndrome in Children (MIS-C): Clinical Significance of ANA Positivity

Multisistem Enflamatuvar Sendromlu Çocuklarda (MIS-C) Otoimmün Yanıt: ANA Pozitifliğinin Klinik Önemi

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Abstract

Objective: This study aimed to investigate autoimmune responses in children diagnosed with multisystem inflammatory syndrome (MIS-C) after severe acute respiratory syndrome coronavirus 2 infection and evaluated the clinical significance of antinuclear antibody (ANA) positivity.

Material and Methods: This retrospective study included 50 pediatric patients, aged 1 month to 18 years, who had regular follow-up visits after hospitalization for MIS-C between 2020 and 2021. Laboratory results at the sixth-month follow-up, including ANA and other autoantibody tests, as well as clinical characteristics, were evaluated.

Results: Of the 50 patients, 62% were male, and the median age was 7.9 years (interquartile range: 4.5-11.9). Eighteen percent of the patients had a history of chronic disease. ANA positivity was detected in five cases (10%), and all ANA-positive patients exhibited involvement of four or more organ systems during MIS-C (p= 0.020). Among thyroid autoantibodies, antithyroglobulin antibody (anti-Tg) positivity was identified in 3 (6.8%) patients, while anti-thyroid peroxidase antibody (anti-TPO) positivity was observed in 2 (5.6%) patients. ANA positivity was present in both patients with anti-TPO positivity, showing a statistically significant (p= 0.010) result, whereas ANA positivity was detected in one of the three patients with anti-Tg positivity (p= 0.254).

Öz

Giriş: Bu çalışmada, şiddetli akut solunum sendromu koronavirüs tip 2 enfeksiyonu sonrasında çocuklarda gelişen multisistem enflamatuvar sendrom (MIS-C) hastalarında otoimmün yanıtlar incelendi ve antinükleer antikor (ANA) pozitifliğinin klinik önemi değerlendirildi.

Gereç ve Yöntemler: Bu retrospektif çalışmaya, 2020-2021 yılları arasında MIS-C tanısı ile hastane yatışı sonrası düzenli kontrole gelen 1 ay-18 yaş aralığındaki 50 çocuk hasta dahil edildi. Olguların MIS-C sonrası altıncı ay kontrollerinde laboratuvar verileri, ANA ve diğer otoantikor testleri ile klinik özellikleri değerlendirildi.

Bulgular: Çalışmaya katılan 50 hastanın %62'si erkek olup, ortanca yaş 7.9 yıl (çeyreklikler arası aralık: 4.5-11.9 yıl) idi. Hastaların %18'inde kronik hastalık öyküsü mevcuttu. Beş olguda (%10) ANA pozitifliği saptandı; ANA pozitif tüm olgular MIS-C hastalığını dört veya daha fazla organ sistem tutulumu ile geçirdi (p= 0.020). Tiroid otoantikorlarından antitiroglobulin antikor (anti-Tg) pozitifliği üç olguda (%6.8) görülürken antitiroperoksidaz antikor (anti-TPO) pozitifliği iki olguda (%5.6) saptandı. Anti-TPO pozitifliği olan iki olgunun tamamında ANA pozitifliği saptanmış olup bu birliktelik istatistiksel olarak anlamlı bulundu (p= 0.010); anti-Tg pozitifliği olan üç olgunun yalnızca birinde ANA pozitifliği görüldü (p= 0.254).

Sonuç: Çalışmamız, MIS-C hastalarının %10'unda ANA pozitifliği olduğunu ve bunun çoklu organ tutulumu ile ilişkili olabileceğini

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Conclusion: ANA positivity was present in 10% of MIS-C patients and may be associated with multiorgan involvement. These findings suggest a possible role of an autoimmune mechanism in MIS-C. Larger prospective studies are needed to clarify the prognostic significance of ANA positivity in disease severity.

Keywords: ANA, anti-Tg, anti-TPO, child, MIS-C

düşündürdüğünü göstermektedir. Bu bulgular, MIS-C'de otoimmün yanıtın rolüne işaret etmekte olup ANA pozitifliğinin hastalık şiddeti üzerindeki prognostik değerinin anlaşılabilmesi için daha geniş prospektif çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: ANA, anti-Tg, anti-TPO, çocuk, MIS-C

Introduction

Multisystem inflammatory syndrome in children (MIS-C) is a recently defined, severe inflammatory response observed in children following severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection (1). MIS-C is characterized by fever, multiple organ involvement, and elevated inflammatory markers, and cardiac, hematological, gastrointestinal, dermatological, and neurological involvement are frequently observed during its course (2).

The pathophysiology of multisystem inflammatory syndrome is not fully understood. Some findings suggest that the adaptive immune response may be dysregulated following SARS-CoV-2 infection and that this may be associated with an increased cytokine response, inflammation, activation and migration of lymphocytes and myeloid cells, and mucosal immune dysfunction (3). These mechanisms suggest that an excessive immune response and adaptive immune activation may play an important role in the pathogenesis of the disease (1,4,5).

Various autoantibodies are important in the differential diagnosis of inflammatory processes and autoimmune diseases. Antinuclear antibodies (ANA) are autoantibodies formed against antigens found in cell nuclear structures, such as deoxyribonucleic acid, histones, and centromeres, and are one of the serological markers commonly used in the diagnosis of connective tissue diseases. However, low levels of ANA positivity can be detected in 10-30% of healthy individuals (6). Although ANA positivity is rarely seen in healthy children, it can be detected at higher rates in children with rheumatological diseases (7). The ANA test can be performed using the enzyme-linked immunosorbent assay (ELISA) method, as well as indirect immunofluorescence microscopy (IIF) using human epithelioma Type 2 (HEp-2) cells as a substrate. The IIF-HEp-2 technique is considered the gold standard for ANA detection (8).

Various studies have reported the presence of autoantibodies in patients who have had SARS-CoV-2 infection (9,10). It has been reported that patients with COVID-19 who test positive for ANA tend to have a more severe clinical condition and a worse prognosis (9). Furthermore, it has been found that those with ANA positivity at 12 months after COVID-19 tend to continue symptoms such as fatigue and dyspnea, and that laboratory markers such as tumor necrosis factor and C-reactive protein (CRP) predict high ANA titers at 12 months (10).

MIS-C shows significant similarities to Kawasaki disease (KD) in terms of clinical findings (11,12). Fever, mucocutaneous findings, cardiac involvement, and systemic inflammation can be seen in both conditions, which can complicate differential diagnosis, especially in atypical cases (12). Due to this clinical overlap, immunological markers such as autoantibodies are thought to be areas that could potentially contribute to understanding the immunological differences between MIS-C and KD; however, MIS-C-specific distinguishing autoantibody profiles have not yet been clearly defined. There is no specific study in the literature that directly examines the relationship between MIS-C history and ANA positivity. However, there are case reports showing that MIS-C after COVID-19 can clinically overlap with rheumatological diseases (13,14). This suggests that the pathogenesis of MIS-C is not limited to healthy children and that careful clinical follow-up is necessary in children with a history of rheumatological or inflammatory diseases.

This study aims to evaluate the frequency of autoantibodies, particularly ANA positivity, in MIS-C patients and the relationship between this positivity and the clinical features of MIS-C. Furthermore, by examining the possible links between ANA positivity and the degree of multisystem involvement in MIS-C, the study aims to provide baseline data for future research. This study does not include a healthy control group, and the baseline ANA levels of the patients prior to MIS-C are unknown. Therefore, our findings do not establish causality but only provide preliminary observations regarding possible clinical associations.

Materials and Methods

This retrospective study was conducted at Health Sciences University İzmir Tepecik Training and Research Hospital. Our hospital served as a pandemic center for COVID-19, with an average of 77,000 admissions and a capacity of 910 beds.

The study included children aged 1 month to 18 years who were diagnosed with MIS-C and examined and treated at Health Sciences University İzmir Tepecik Training and Research Hospital between 2020 and 2021 and who regularly attended the Pediatric Infectious Diseases and Pediatric Rheumatology outpatient clinics after discharge. Laboratory data from the sixth-month outpatient follow-up visits after discharge due to MIS-C were considered. The cases were evaluated based on physical examination findings, height, weight, and laboratory data, including hemogram values, kidney function tests (urea, creatinine), blood ions such

as sodium, potassium, and calcium, liver function tests [aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase, total bilirubin, direct bilirubin]; albumin, CRP, erythrocyte sedimentation rate (ESR), ferritin, fibrinogen, D-dimer, prothrombin time, activated partial thromboplastin time, direct Coombs test, rheumatoid factor (RF), ANA, anti-double-stranded DNA antibody (anti-ds-DNA), anti -cardiolipin immunoglobulin M (IgM) and immunoglobulin G (IgG), anti-beta-2-glycoprotein-1 antibodies IgM, IgG, and immunoglobulin A (IgA), anti-thyroid peroxidase (anti-TPO), and anti-thyroglobulin (anti-Tg) antibodies.

The sixth-month follow-up for MIS-C evaluation was preferred because it represents a period when the acute inflammatory phase has largely subsided and late-phase immune responses can be assessed more stably. The literature reports that the 3-6-month interval is appropriate for evaluating immune responses after MIS-C and COVID-19 in terms of subacute and early late-stage immune changes. It has been suggested that autoantibody positivity detected in the early period is more likely to reflect a transient inflammatory response, while autoantibodies detected at six months may reflect a more stable immune activation (9,10,15).

The exclusion criteria for the study were defined as failure to attend regular outpatient clinic visits for Pediatric Infectious Diseases and Pediatric Rheumatology, and incomplete laboratory data. Thirteen cases who did not attend regular follow-up visits after discharge, five cases who moved to different provinces, and two cases who died from various complications after MIS-C were excluded from the study.

This study was conducted with the permission of the Non-Interventional Research Ethics Committee of İzmir Tepecik Training and Research Hospital (Ethics decision number: 2021/04-22).

Statistical Analysis

Statistical analyses were performed using SPSS 24.0 (IBM Corp, Armonk, NY). Mean ± standard deviation was used for continuous data following a normal distribution, while median and interquartile range (IQR, Q1-Q3) were used for data not following a normal distribution. Categorical data were expressed as number (n) and percentage (%). Categorical comparisons were performed using the chi-square test or Fisher's exact test. A significance level of $p< 0.05$ was selected.

ANA evaluation

ANA screening was performed using IIF-HEp-2 cell substrates (Euroimmun, Lübeck, Germany). The test procedure and evaluation were performed according to the manufacturer's instructions. A 1:100 dilution was used for screening, and titers below this threshold were considered negative. Positive samples were evaluated according to the international consensus on the characterization of ANA patterns (see: www.ANApatterns.org for the classification algorithm and representative images) (16).

Results

Of the 50 patients included in the study, 31 were male (62%), and the median age of the cases was 7.9 years (IQR: 4.5-11.9 years). The median age of female cases was significantly higher than that of male cases (female: 9 years, male: 7.5 years, $p< 0.001$). Nine (18%) cases had a history of chronic disease. Seven patients were overweight (≥85th percentile) or obese (≥95th percentile). In addition, one case had a history of acute lymphoblastic leukemia and one case had juvenile idiopathic arthritis (JIA). Eleven (22%) cases required treatment in the intensive care unit due to MIS-C (Table 1).

ANA positivity was detected in five cases, with 1/100 nucleolar, 1/100 homogeneous, 1/320 homogeneous, 1/320 nuclear dots, and 1/1000 diffuse granular dots observed in one case each. Demographic data of the cases according to their ANA results are summarized in Table 1.

When the number of organ systems involved in hospital admissions for MIS-C was evaluated, it was seen that all ANA-positive cases had involvement in four or more organs, and this result was found to be statistically significant ($p= 0.020$). The ANA positivity in the JIA-diagnosed case was homogeneous speckled at a titer of 1/320. Since thyroid autoantibodies were not tested in all patients, anti-Tg was found positive in three cases, and this rate was calculated as 6.8% out of 44 patients who underwent anti-Tg testing. Similarly, anti-TPO was positive in two cases, representing 5.6% of the 36 patients tested for anti-TPO. Both anti-Tg and anti-TPO positivity were observed together in one of these cases; additionally, this patient's mother had a history of hypothyroidism. ANA positivity was determined in both of the two anti-TPO positive cases, while ANA positivity was observed in only one of the three anti-Tg positive cases ($p= 0.010$ and $p= 0.254$, respectively) (Table 2). There was no statistically significant difference between sex and anti-TPO or anti-Tg positivity ($p= 0.674$ and $p= 0.882$, respectively). ds-DNA and RF were negative in all cases. Direct Coombs positivity was detected in only one case, and the ANA value of this case was negative. Anti-beta-2-glycoprotein-1 IgM, IgG, and IgA values were negative in all cases. The laboratory characteristics of the cases are presented in Table 3. Laboratory results (leukocyte count, absolute neutrophil count, hemoglobin value, platelet count, CRP value, ESR, AST, ALT, ferritin, fibrinogen, D-dimer, APTT, PT, urea, creatinine, direct bilirubin) were compared, no statistically significant differences were found between the two groups ($p> 0.050$ for all).

Table 1. Characteristic features of cases diagnosed with multisystem inflammatory syndrome based on antinuclear antibody (ANA) results

	Total n= 50	ANA Positive** n= 5	ANA Negative n= 45	p
Age, year, median (IQR)*	7.9 (4.5-11.9)	9 (5.1-13.9)	7.5 (4.5-11.7)	0.900
Sex, n (%)				
Female	19 (38)	3 (60)	29 (64.4)	0.355
Male	31 (62)	2 (40)	16 (35.6)	
Chronic diseases n (%)				
Overweight/obese	7 (14)	1 (20)	6 (13.3)	0.216
Juvenile idiopathic arthritis	1 (2)	1 (20)	-	
Acute lymphoblastic leukemia	1 (2)	-	1 (2.2)	
Length of hospital stay, median (IQR)	9 (6.7-11.5)	8 (5.5-16)	9 (6.5-11)	0.638
Intensive care unit requirement, n (%)	11 (22)	2 (40)	9 (20)	0.301
Number of systems involved				
2-3	26 (52)	-	26 (57.8)	0.020
≥4	24 (48)	5 (100)	19 (42.2)	
Treatment				
Intravenous immunoglobulin n (%)	44 (88)	4 (80)	40 (88.9)	0.487
Corticosteroids n (%)	39 (78)	4 (80)	35 (77.8)	1.000
Immunomodulatory therapy n (%)	3 (6)	1 (20)	2 (4.4)	0.276

*Interquartile range, **Calculations were performed using Fisher's exact test.
ANA: Antinuclear antibody, IQR: Interquartile range.

Table 2. Clinical and autoantibody characteristics of anti-nuclear antibody positive cases

No. of Patients	Age (year)	Sex	Underlying Disease	ANA Titer and Pattern	Anti-Tg	Anti-TPO	Other Autoantibodies	Organ System Involved (n)	ICU Admission
1	9	F	None	1/1000 granular	Positive	Positive	All negative	5	No
2	7.3	M	None	1/100 homogenous	Positive	Negative	All negative	5	Yes
3	2.9	F	JIA	1/320 homogenous	Negative	Negative	All negative	4	No
4	15	M	Obesity	1/100 nucleolar	Negative	Negative	All negative	5	Yes
5	12.8	F	None	1/320 nuclear dots	Negative	Negative	All negative	5	No

The clinical and autoantibody characteristics of these five patients are summarized in Table 2. All ANA-positive cases had involvement of four or more organs. Only one patient (patient no. 3) had a previously known JIA diagnosis. Other autoantibodies (anti-dsDNA, aCL IgM/IgG, β2GP1 IgM/IgG/IgA, RF, Coombs).
ANA: Antinuclear antibody, Anti-Tg: Anti-thyroglobulin antibody, Anti-TPO: Anti-thyroid peroxidase antibody, JIA: Juvenile idiopathic arthritis, ICU: Intensive care unit, F: Female, M: Male.

Discussion

In our study, ANA positivity was detected in 5 (10%) of 50 children diagnosed with MIS-C, and involvement of four or more organ systems was observed in all cases with ANA positivity. Although this finding cannot establish a definitive relationship due to the limited sample size and the absence of a control group, it suggests a possible link between ANA positivity and disease severity in MIS-C. Original studies examining the relationship between MIS-C and ANA in the literature are quite limited (17,18). However, ANA positivity in these case reports may reflect pre-existing autoantibody positivity due to underlying rheumatological conditions such as systemic lupus erythematosus or Crohn's disease, rather than a newly developed finding associated with MIS-C. Therefore, these cases do not provide evidence for a direct relationship between MIS-C and ANA positivity, but rather reflect the difficulty of differential diagnosis. However, there are publications reporting increased autoantibody responses in children following SARS-CoV-2 infection, particularly antibodies against nuclear antigens, including ANA (9). This suggests that SARS-CoV-2 may play a role in the pathogenesis of MIS-C and possible autoimmune processes; however, due to the lack of a control group, larger studies are needed to confirm this relationship. Our study serves as a preliminary

Table 3. Laboratory characteristics of cases diagnosed with multisystem inflammatory syndrome

	No. of Patients (%) n= 50
ANA positive	5 (10)
Rheumatoid factor positive	1 (2)
Anti-dsDNA positive	0
Direct Coombs positive	1 (2)
Cardiolipin IgM positive	0
Cardiolipin IgG positive	0
Anti-beta-2 glycoprotein 1 IgM positive	0
Anti-beta-2 glycoprotein 1 IgA positive	0
Anti-beta-2 glycoprotein 1 IgG positive	0
C3 low	0
C4 low	0
Anti-TPO positive*	2 (5.6)
Anti-Tg positive*	3 (6.8)
Leukocyte count, median (IQR), mm^3/uL	7200 (5575-8775)
Absolute neutrophil count, median (IQR) mm^3/uL	3600 (2600-4650)
Hemoglobin value, median (IQR), g/dL	13.1 (11.5-13.6)
Platelet value, median (IQR), mm^3/uL	328 000 (283 000-388 750)
C-reactive protein, median (IQR), mg/L	1.3 (0.4-3.1)
ESR, median (IQR), mm/hour	15 (10-20)

*Anti-T: Anti-thyroglobulin antibody; Anti-M: Antimicrosomal antibody. Anti-Tg was tested in 44 patients, Anti-TPO in 36 patients; statistical analysis was performed based on these numbers.
ANA: Antinuclear antibody, Anti-Tg: Anti-thyroglobulin antibody, Anti-TPO: Anti-thyroid peroxidase antibody, IQR: Interquartile range, ESR: Erythrocyte sedimentation rate.

observation pointing to a possible association between ANA positivity and more severe multisystem involvement. It is not yet clear whether ANA positivity detected in MIS-C patients reflects a transient or persistent immune response. Studies in adult and pediatric cohorts after COVID-19 have reported that autoantibody positivity detected early on is often transient; however, ANA positivity persisting months after infection, particularly at 6-12-month follow-ups, may be associated with more persistent immune activation (9,10). However, data including long-term and serial ANA measurements specific to MIS-C are still limited. Therefore, interpretations regarding the temporal course of ANA positivity detected in our study can only be made in light of data obtained from the post-COVID-19 literature, and this should be considered an important limitation of our study.

In the literature, the vast majority of MIS-C cases have been described in children who were previously healthy and had no known comorbidities (2). However, there are reports of MIS-C developing after COVID-19 in children who have been diagnosed with inflammatory or rheumatological diseases. For example, a case report from New York City, USA, described a pediatric patient with Crohn's disease who was diagnosed with MIS-C and had a positive ANA test (17). In a case report from South Africa, MIS-C developed in a patient being monitored for systemic lupus erythematosus who tested positive for ANA, and the patient was successfully treated (18). In our series, a patient who tested positive for ANA had a previous diagnosis of JIA. This situation shows that MIS-C can also develop in children with a history of rheumatological or inflammatory diseases and that careful clinical follow-up is necessary in this group.

ANA can be detected in healthy individuals. In a study conducted in China, among 25.110 healthy individuals undergoing routine screening, the positivity rate for ANA titer >1:100 was 14%, while the positivity rate for ANA titer >1:320 was 5.93%, and these rates were found to be significantly higher in women than in men (19.05% vs. 9.04%, respectively; p< 0.01) (19). In another study, ANA positivity was found to be approximately 7.09% in the healthy population, with a rate of 10.2% in women and 4.6% in men (20). In this study, the authors also found that inflammatory and immunological markers were more pronounced in the ANA-positive population and concluded that high ANA levels may be associated with inflammatory and immunological dysfunction (20). A review of peer-reviewed literature published between 1961 and 2025 concluded that although a positive ANA test result may suggest autoimmune disorders, its presence and titer should be interpreted in conjunction with clinical findings, and that low titers in particular are generally not diagnostically significant (21). The authors concluded that ANA titers higher than 1:160 may provide greater specificity in distinguishing true positives from false positives in healthy individuals (21). In a pediatric center study conducted in Türkiye, positivity was detected in 113 of 409 patients who underwent the desired ANA test; however, it was reported that the positive predictive value of this positivity for connective tissue diseases was quite low (16%). Specifically, the predictive value for systemic lupus erythematosus was only 13%, which suggests that ANA positivity alone has limited diagnostic value and should be interpreted with caution in the clinical setting. However, the same study emphasized that high-titer ANA positivity increases the likelihood of autoimmune disease (7).

KD is a rare disease in children with many clinical features overlapping with MIS-C. Although its etiology is not fully understood, it has been reported to be associated with viral infections (22,23). The role of autoantibodies in KD has been studied for a long time; however, ANA positivity has generally been reported as a rare finding (24). In this cohort study reported from Italy, various immunological markers were evaluated in children diagnosed with KD during the acute and convalescent phases. The study showed that ANA was completely negative in both the KD and febrile control groups.

In contrast, other immune abnormalities, such as circulating immune complexes, anticardiolipin antibodies, anti-endothelial cell antibodies, and T-cell subset abnormalities, were frequently detected, particularly in the acute phase, while a significant decrease in the frequency of these findings was observed in the convalescent phase. However, ANA positivity was not detected, and none of the evaluated immunological parameters showed a significant association with coronary artery involvement or disease prognosis. These findings suggest that ANA has limited diagnostic or prognostic value in KD (23). In a prospective follow-up study from North India, ANA positivity was observed in only 6% of 50 KD patients approximately six years after diagnosis (25). Similarly, ANA positivity was found at a low rate of 4% in children followed up long-term at a center in South India (24). Although different autoantibody profiles have been reported in KD, the diagnostic value of the ANA test is limited. In our study, ANA positivity was detected in 10% of MIS-C patients, and multiple organ involvement was observed in all of these cases. This finding suggests that the autoimmune response in MIS-C may be related to disease severity. Therefore, the rarity of ANA positivity in KD and its more pronounced occurrence in MIS-C supports immunopathological differences between the two diseases and suggests that MIS-C may be more closely related to autoantibody-mediated mechanisms.

There is no specific study in the current literature that directly examines the relationship between MIS-C and ANA positivity. However, there are publications on the presence of various autoantibodies, such as anti-Ro/SSA, rheumatoid factor, lupus anticoagulant, and anti-interferon antibodies, in addition to ANA positivity with SARS-CoV-2 infection (9,26-29). The inflammatory response, which plays an important role in the pathogenesis and mortality of SARS-CoV-2 infection, is generated by the innate and adaptive immune systems. Autoantibody activation triggered by a cytokine storm due to adaptive immune system activation may be related to mechanisms such as molecular similarity between viral proteins and human proteins and continuous exposure to viral antigens (26,30,31). In a study by Duran et al., the ANA-IFA positivity rate among 105 COVID-19 patients was found to be 19%, and although the ANA-IFA positivity rate was higher in patients with pulmonary symptoms, this difference was not statistically significant (26). In a study by Stefano Netti et al. involving 638 COVID-19 patients, it was found that the 30-day survival rate was significantly lower in COVID-19 patients who were ANA-positive (20%) and did not have an autoimmune disease (64.4% vs. 83.0%), and the likelihood of experiencing serious respiratory complications during hospitalization was even higher in ANA-positive patients (35.4% vs. 17.0%) ($p< 0.001$) (9). The fact that the relationship between MIS-C and ANA positivity has not been sufficiently investigated in the literature suggests that addressing this issue in detail in the future could be valuable in terms of both pathogenesis and clinical approach.

In our study, anti-TPO positivity among thyroid autoantibodies was found to be statistically significant with ANA positivity, whereas anti-Tg positivity was not found to be statistically significant. Data on the relationship between MIS-C and thyroid autoantibodies are limited in the literature (32). In a study by Elvan-Tüz et al. evaluating thyroid function in MIS-C cases, anti-TPO and anti-Tg levels were reported, but no significant relationship was found between autoantibody positivity and clinical severity or autoimmune response (32). Similarly, a review examining thyroid function in children who had COVID-19 reported that anti-thyroid autoantibody positivity could be observed (33). Furthermore, it has been reported that non-thyroidal illness syndrome may occur in MIS-C patients, and it has been suggested that this condition is related to systemic inflammatory response and critical illness stress (15). In this context, the fact that anti-TPO was found to be more significant than anti-Tg in our study suggests that anti-TPO in particular should be considered in thyroid autoimmunity after MIS-C. However, due to the limited number of cases, this relationship needs to be confirmed in larger series.

The co-detection of anti-TPO positivity and ANA positivity in our study may reflect a broader immune activation rather than an organ-specific autoimmune disease. It is known that acute and post-infectious inflammatory conditions such as MIS-C can trigger transient autoantibody production and simultaneously activate different immune pathways (30). However, due to the limited number of patients and the absence of serial measurements showing the course of autoantibodies over time, it is not possible to say whether this association represents a persistent autoimmune tendency or a transient immune response. Prospective studies involving larger patient groups and long-term follow-up are needed to establish the clinical significance of this relationship.

Our study has certain limitations. First, autoantibody levels were measured in only a small number of MIS-C patients. However, MIS-C is a rare and newly defined disease. Furthermore, ANA levels were not measured at regular intervals in MIS-C cases. Therefore, no direct conclusions can be drawn about the presence or absence of a possible autoantibody response. Furthermore, the timing of sampling is a critical factor; autoantibodies may not yet be detectable in the early phase of the immune response, and autoantibody levels may have decreased in tests performed in the late phase. Differences in the test methods used (e.g., IIF-HEp-2 substrate, ELISA, or immunoblot techniques) and variability in cut-off values between laboratories may also affect positivity rates. Furthermore, the clinical interpretation of low-titer positivity is uncertain. All of these factors may have limited our ability

to establish a possible relationship between MIS-C and ANA positivity. The absence of a healthy control group in this study and the lack of knowledge about the patients' baseline ANA levels prior to MIS-C limit our ability to comment on whether ANA positivity is a new finding associated with MIS-C. Since ANA positivity can also be seen in healthy children, our findings do not show a definitive relationship but only provide preliminary data for generating hypotheses. Since the patients' baseline ANA levels prior to MIS-C are not available, it cannot be definitively shown whether ANA positivity reflects a new finding that emerged after MIS-C or a pre-existing condition. Therefore, our results should be interpreted within the context of differential diagnosis and clinical context.

Conclusion

In our study, ANA positivity was detected in 10% of children diagnosed with MIS-C, and involvement of four or more organ systems was observed in all cases with ANA positivity. This finding suggests that ANA positivity may be associated with MIS-C severity; however, larger and prospective studies are needed to determine whether this association is causal. Furthermore, the presence of a prior JIA diagnosis in one of the ANA-positive cases indicates that MIS-C can also develop in children with a history of rheumatological disease.

It is known that ANA testing can be low-level positive even in healthy individuals. However, the high-titer ANA positivity observed in our study, associated with the clinical severity of MIS-C, suggests a possible role for this autoantibody in the pathogenesis of MIS-C. In addition, the relationship found between anti-thyroid autoantibodies and ANA positivity is noteworthy and suggests that post-COVID-19 autoimmune mechanisms are not limited to connective tissue diseases but may also have implications in the endocrine system. However, the interpretation of this possible relationship should be approached with caution, as it is based on the evaluation of ANA titers in a limited number of positive cases; our results do not show a statistical correlation but only provide a descriptive observation.

These data suggest that, while not diagnostic on its own, the ANA test may contribute to predicting disease severity and the degree of systemic involvement in the clinical follow-up of MIS-C patients. However, the relatively limited number of patients and the lack of serial monitoring of autoantibody levels limit the generalizability of the findings.

In conclusion, these findings, which may indicate a possible relationship between ANA positivity and clinical severity in MIS-C, should be confirmed in larger patient groups through prospective and long-term follow-up studies. This would shed light on the pathophysiology of MIS-C and clarify the potential role of autoantibodies in determining prognosis.

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