TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2015) 45: 1192-1197

doi:10.3906/sag-1404-150

The prevalence of infectious agents in patients with systemic sclerosis

Hüseyin BİLGİN[1,*], Hilal KOCABAŞ[2], Recep KEŞLİ[1]

[1]Department of Pediatrics, Faculty of Medicine, Turgut Özal University, Ankara, Turkey
[2]Division of Rheumatology, Department of Physical Medicine and Rehabilitation, Meram Faculty of Medicine, Necmettin Erbakan University, Konya, Turkey
[3]Department of Medical Microbiology, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

Received: 25.04.2014 • **Accepted/Published Online:** 30.11.2014 • **Printed:** 31.12.2015

Background/aim: Systemic sclerosis (SSc) is an autoimmune disease characterized by microvascular injury, excessive extracellular matrix deposition, and fibrosis in the skin and internal organs. Bacterial and viral infectious agents have been suspected to be contributing factors in the development and progression of the pathologic features of SSc.

Materials and methods: In this study, 30 SSc patients who were admitted to the rheumatology unit of the Konya Training and Research Hospital and 30 healthy controls were included. The presence of 9 different antibodies (IgM and IgG) against *Helicobacter pylori*, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and parvovirus B19 were investigated in sera samples obtained from the 60 participants using an enzyme-linked immunosorbent assay method. The characteristics of current and past infections with *H. pylori*, CMV, EBV, and parvovirus B19 were evaluated by determining the seropositivity of the tested bacterial and viral agents.

Results: The prevalences of *H. pylori*, CMV, EBV, and parvovirus B19 were determined to be higher in patients with SSc than in the control group.

Conclusion: SSc is associated with a higher rate of certain infections, which deserves further investigation in order to assess the role of infections in disease etiology/pathogenesis.

Key words: Systemic sclerosis, infection, etiology

1. Introduction

The relationship between infection and autoimmunity has been increasingly defined in recent years. Bacterial, viral, and parasitic infections are known to induce and exacerbate autoimmune diseases (1,2). Systemic sclerosis (SSc) is an autoimmune disease characterized by microvascular injury, excessive extracellular matrix deposition, and fibrosis of the connective tissues of the skin, lungs, kidneys, heart, and gastrointestinal tract. The etiology of SSc remains uncertain. Increasing evidence suggests that there are many potential environmental triggers for SSc and that host factors determine the susceptibility to disease in response to these triggers. Bacterial and viral infectious agents have been suspected to be contributing factors in the development and progression of the pathologic features of SSc (3).

The potential role of infectious agents in SSc has been poorly investigated. The aim of the present study was to evaluate the possible association between infection and the etiology of the SSc.

2. Materials and methods

2.1. Groups of patients

Thirty SSc patients who were admitted to the rheumatology unit of the Konya Training and Research Hospital between April 2009 and March 2012 and 30 sex- and age-matched healthy controls were included in the study. All patients with SSc met the American Rheumatism Association criteria for diagnosis (4). The study group was classified according to the extent of skin involvement (diffuse vs. limited). The participants in this study had not received a transfusion of blood or blood products.

2.2. ELISA assays

The presence of 9 different antibodies was examined in sera samples from the 60 participants using an enzyme-linked immunosorbent assay (ELISA) method. The sera were tested against antibodies that reflect the existence of current and past infections of *Helicobacter pylori*, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and parvovirus B19.

* Correspondence: hubilgin@hotmail.com

The following agents were used for the detection of antibodies: anti-CMV (IgM and IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Lübeck, Germany), EBV EBNA-1 (IgM) (IBL International GmbH, Hamburg Germany), EBV EBNA-1 (IgG) (EUROIMMUN Medizinische Labordiagnostika AG), EBV-CA (IgM) (EUROIMMUN Medizinische Labordiagnostika AG), anti-*H. pylori* (IgM and IgG) (EUROIMMUN Medizinische Labordiagnostika AG), and antiparvovirus B19 (IgM and IgG) (EUROIMMUN Medizinische Labordiagnostika AG). All tests were evaluated by using a microplate reader (Biotek ELx 800, BioTek Instruments, Inc., Winooski, VT, USA). Interference from rheumatoid factor was avoided by the addition of sheep IgG to the sample dilution buffer. The participants underwent comprehensive examinations and laboratory evaluations, including full blood count, erythrocyte sedimentation rate, and renal and liver functions.

A power analysis was conducted after recruitment. Using an α level of 0.05, β level of 0.20, and sample size of 30, power was found as 70.7% for the study. Therefore, the sample size of n = 30 may be sufficient. SSc is a rare disease with an incidence rate of 2.3–10 per million (5). Hence, the maximum amount of patients had been detected to be included in the study.

2.2.1. ELISA measurements

Patient samples were diluted 1:101 with a sample buffer (10 µL of serum in 1.0 mL of sample buffer), and photometric measurements were made at wavelengths of 450 nm and 630 nm for all the tests that were included in the study.

2.2.2. Anti-CMV IgM assay

The measurement unit was a ratio (extinction of the control or patient samples/extinction of calibrator 2). Results were evaluated as follows: <0.80 ratio was negative, ≥0.80 to <1.1 ratio was borderline, and ≥1.1 ratio was positive. The detection limit of the test was a 0.05 ratio, intraassay coefficients of variation (CVs) were 6.1%–11.3%, and interassay CVs were 6.2%–9.3%. Specificity and sensitivity rates of the test were 100% and 98.4%, respectively.

2.2.3. Anti-CMV IgG assay

The measurement unit was relative unit per milliliter (RU/mL). Results were evaluated as follows: <16 RU/mL was negative, ≥16 to <22 RU/mL was borderline, and ≥22 RU/mL was positive. Linearity of the test was 2–200 RU/mL, the detection limit of the test was 0.04 RU/mL, intraassay CVs were 4.2%–5.4%, and interassay CVs were 5.7%–11.2%. Specificity and sensitivity rates of the test were 100% and 98.4%, respectively.

2.2.4. EBV EBNA-1 IgM assay

The measurement unit was the cut-off index (COI). Results were evaluated as follows: <0.8 COI was negative, 0.8–1.2 COI was equivocal, and >1.2 COI was positive. Intraassay CV was 7.8%, and interassay CV was 12.1%. Specificity and sensitivity rates of the test were >95% for each.

2.2.5. EBV EBNA-1 IgG assay

The measurement unit was RU/mL. Results were evaluated as follows: <16 RU/mL was negative, ≥16 to <22 RU/mL was borderline, and ≥22 RU/mL was positive. The linearity of the test was 12–126 RU/mL, the detection limit of the test was 0.9 RU/mL, intraassay CVs were 4.8%–8.9%, and interassay CVs were 8.4%–10.9%. Specificity and sensitivity rates of the test were 100% for each.

2.2.6. EBV CA IgM assay

The measurement unit was a ratio (extinction of the control or patient samples/extinction of calibrator 2). Results were evaluated as follows: <0.80 ratio was negative, ≥0.80 to <1.1 ratio was borderline, and ≥1.1 ratio was positive. The detection limit of the test was a 0.01 ratio, intraassay CVs were 3.3%–5.8%, and interassay CVs were 5.5%–6.9%. Specificity and sensitivity rates of the test were 100% for each.

2.2.7. EBV CA IgG assay

The measurement unit was RU/mL. Results were evaluated as follows: <16 RU/mL was negative, ≥16 to <22 RU/mL was borderline, and ≥22 RU/mL was positive. The linearity of the test was 2–200 RU/mL, the detection limit of the test was 1 RU/mL, intraassay CVs were 4.2%–7.4%, and interassay CVs were 3.2%–8.2%. Specificity and sensitivity rates of the test were 100% for each.

2.2.8. Anti-*H. pylori* IgM assay

The measurement unit was the COI. Results were evaluated as follows: <0.8 COI was negative, 0.8–1.2 COI was equivocal, and >1.2 COI was positive. Intraassay CV was 8.1%, and interassay CV was 11.3%. Specificity and sensitivity rates of the test were >95% for each.

2.2.9. Anti-*H. pylori* IgG assay

The measurement unit was RU/mL. Results were evaluated as follows: <16 RU/mL was negative, ≥16 to <22 RU/mL was borderline, and ≥22 RU/mL was positive. Detection limit of the test was 0.7 RU/mL, intraassay CVs were 3.1%–3.2%, and interassay CVs were 3.4%–4.6%. Specificity and sensitivity rates of the test were 100% for each.

2.2.10. Antiparvovirus B19 IgM assay

The measurement unit was a ratio (extinction of the control or patient samples/extinction of calibrator 2). Results were evaluated as follows: <0.80 ratio was negative, ≥0.80 to <1.1 ratio was borderline, and ≥1.1 ratio was positive. The detection limit of the test was a 0.1 ratio, intraassay CVs were 5.2%–6.0%, and interassay CVs were 5.6%–7.7%. Specificity and sensitivity rates of the test were 97.9% and 100%, respectively.

2.2.11. Antiparvovirus B19 IgG assay

The measurement unit was international units (IU/mL). Results were evaluated as follows: <4 IU/mL was negative,

≥4 to <5.5 IU/mL was borderline, and ≥5.5 IU/mL was positive. The linearity of the test was 1–100 IU/mL. The detection limit of the test was 0.1 IU/mL, intraassay CVs were 2.5%–7.7%, and interassay CVs were 1.6%–6.0%. Specificity and sensitivity rates of the test were 100% for each.

2.3. Ethical approval

Ethical approval was provided by the Ethics Committee of Meram Medical School, Selçuk University (Konya, Turkey) (ref: 2011/259). The patients provided both verbal and written consent prior to their participation.

2.4. Statistical analysis

The results of tests were expressed as the number of observations (n), mean ± standard deviation, median, and min–max values. The results of the homogeneity (Levene test) and normality tests (Shapiro–Wilk) were used to decide which statistical methods to apply in the comparison of the study groups. Normally distributed groups and groups with homogeneous variances were compared using the Student t-test. According to those tests results, parametric test assumptions were not available for some variables, so the comparisons of 2 independent groups were performed by Mann–Whitney U test. Categorical data was analyzed with the Fischer exact test and chi-square test. $P < 0.05$ was considered statistically significant. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

3. Results

The mean age of the subjects was 35 ± 15.25 years (19 females and 11 males, age range 7–60) for the SSc patients and 37 ± 16.35 years (18 females and 12 males, age range 8–59) for the control group. Table 1 summarizes the demographic characteristics of the study population.

In our population, 73.3% of SSc patients had *H. pylori* antibodies (IgM and/or IgG), compared with 46.6% of the control group ($P < 0.05$). Anti-CMV antibodies (IgM and/or IgG) were detected in 93.3% of SSc patients, compared with 73.3% of the control group ($P < 0.05$). EBV antibodies (IgM and/or IgG) were detected in 93.3% of SSc patients, compared with 66.6% of the control group ($P < 0.05$). Parvovirus B19 antibodies (IgM and/or IgG) were detected in 86.6% of SSc patients, compared with 63.3% of the control group ($P < 0.05$). Table 2 shows the prevalence of antibodies against these infections in patients with SSc and patients in the control group.

There was no statistically significant difference between the limited SSc and diffuse SSc patients for *H. pylori*, CMV, EBV and parvovirus B19 infections. The prevalence of recent infections (IgM-positive but IgG-negative) was similar between the SSc patients and controls.

4. Discussion

Infections can trigger autoimmune diseases. This fact is well established in the literature. SSc is among the less investigated autoimmune diseases, and not much information exists on the disease pathogenesis and triggers (6–8). In the current study, we investigated the relationship between past and recent infections and the etiology of SSc.

Many agents have been postulated as being involved in the cause of the disease. Bacterial and viral infectious agents have been suspected to be contributing factors in the development and progression of SSc. The rationale for this infection hypothesis is that many SSc-like symptoms are transiently elicited by infectious agents in otherwise healthy individuals. Some researchers have suggested that the production of specific autoantibodies in SSc patients is the result of an antigen-driven response caused by molecular mimicry. Molecular mimicry is a mechanism of autoimmune disease that may explain the pathogenicity of antibodies against viral proteins in SSc patients (9). Self-reactive antibodies against a virus may induce endothelial cell apoptosis through the interaction with the integrin α3β1 and α6β1 NAG-proteins complex (9). Endothelial injury represents one of the first steps in the pathogenesis of SSc. Endothelial cells may be infected by bacteria or viruses that may be instrumental in inducing vasculitis (10).

Attempts to establish a link between the *H. pylori* infection status and SSc have yielded conflicting results. One study identified higher incidence rates of *H. pylori*

Table 1. Demographic characteristics of the study population.

Characteristics	SSc			Controls	P-value Limited+diffuse SSc vs. controls
	Limited	Diffuse	Limited+diffuse		
No. of patients	18	12	30	30	
Female/male	1.57	2	1.72	1.5	P = 0.792
Mean age, years	34 ± 16.25	36 ± 13.45	35 ± 15.25	37 ± 16.35	P = 0.529
Disease duration, years	2.7	3.1	2.9	-	

Table 2. Prevalence of antibodies against infections in patients with systemic sclerosis and control groups.

	SSc (30)			Controls (30)	P-value Limited+diffuse SSc vs. controls
	Limited (18)	Diffuse (12)	Limited+diffuse (30)		
Prevalence of *H. pylori* antibodies, % of patients					
IgM+, IgG-	5.5	8.3	6.6	3.3	P = 1.0
IgM+, IgG+	16.6	16.6	16.6	10	P = 0.706
IgM-, IgG+	50	50	50	33.3	P = 0.190
IgM+ and/or IgG+	72.2	75	73.3	46.6	P = 0.035
Prevalence of CMV antibodies, % of patients					
IgM+, IgG-	0	0	0	3.3	P = 1.0
IgM+, IgG+	22.2	16.6	20	13.3	P = 0.731
IgM-, IgG+	72.2	83.3	76.6	56.6	P = 0.100
IgM+ and/or IgG+	94.4	91.6	93.3	73.3	P = 0.038
Prevalence of EBV antibodies, % of patients					
IgM+, IgG-	5.5	8.3	6.6	6.6	P = 1.0
IgM+, IgG+	11.1	16.6	13.3	6.6	P = 0.671
IgM-, IgG+	72.2	83.3	76.6	56.6	P = 0.100
IgM+ and/or IgG+	94.4	91.6	93.3	66.6	P = 0.010
Prevalence of parvovirus B19 antibodies, % of patients					
IgM+, IgG-	5.5	0	3.3	0	P = 1.0
IgM+, IgG+	5.5	8.3	6.6	3.3	P = 1.0
IgM-, IgG+	72.2	75	73.3	60	P = 0.273
IgM+ and/or IgG+	88.8	83.3	86.6	63.3	P = 0.037

infection in patients with rheumatic diseases, including SSc, as detected by serologic analysis (11). In contrast, 2 larger studies discovered there was no difference in the *H. pylori* infection rates between patients with SSc with Raynaud phenomenon and healthy controls (12,13). A recent study (14) indicated that, although there is no difference in the *H. pylori* infection rate between control subjects and patients with SSc, 90% of patients with SSc were infected with the virulent CagA strain compared with 37% of infected control subjects. In a study of patients with primary Raynaud phenomenon, the eradication of *H. pylori* infections was associated with the complete disappearance of the episodes of Raynaud phenomenon in 17% of treated patients and a reduction in symptoms in an additional 72% (15). In the present study the prevalence of *H. pylori* antibodies (both IgM and IgG) was significantly higher among SSc patients than in controls.

CMV may play a role in SSc pathogenesis due to its ability to infect both endothelial and monocyte/macrophage cells, and through the upregulation of fibrogenic cytokines and the induction of immune dysregulation (16,17). CMV, as a possible trigger for SSc, has been suggested by the reported appearance of SSc shortly after acute episodes of viral infections (18). The most direct evidence of a link between CMV and SSc is the presence of high-titer IgG antibodies against the polyglycine motifs of CMV (19). In addition, antitopoisomerase I (Scl-70) autoantibodies, which are characteristic of diffuse SSc, can cross-react with a peptide sequence of the UL70 protein of CMV (20). One difficulty in establishing a clear association between CMV and SSc is the fact that 60% to 90% of adults display serologic evidence of past CMV infection, yet SSc affects at most 3 in 10,000 people. We discovered that the prevalence of anti-CMV antibodies (both IgM and IgG) is higher among SSc patients than in controls.

EBV DNA has been detected in the lungs of SSc patients (21). It has been observed that an antibody against hnRNP autoantigen called p542 may develop in mononucleosis, and IgG anti-p542 has been discovered in SSc (22). In these patients, autoantibodies against a 60–62-kDa cellular protein, which cross-reacts with the glycine/alanine repeat in EBNA-1, may have been detected (19). Farina et al. (23) showed that SSc fibroblasts have greatly diminished IFN-inducible gene response upon TLR7/9 agonist stimulation. This is possibly one of the reasons why EBV is able to infect and persist in SSc fibroblasts. They suggested that EBV infection could contribute to fibrosis in SSc skin. In the current study, the prevalence of EBV antibodies (both IgM and IgG) was significantly higher among SSc patients than in controls.

Parvovirus B19 has been proposed as a causative agent in rheumatoid disease and other vascular injury syndromes, including Wegener granulomatosis (24), microscopic polyarteritis nodosa (25), Henoch–Schönlein purpura (26), and dermatomyositis (27) in SSc. A direct correlation between the extent of degenerative endothelial cell alterations and the degree of B19RNA expression suggested a causal role of B19 in the propagation of endothelial cell dysfunction (28). A possible role for parvovirus B19 infection in the evolution of SSc is suggested by the presence of B19 at a high percentage (57%) in bone marrow biopsies from unselected SSc patients in the absence of B19 viremia (29). It has been suggested that bone marrow may represent a reservoir from which the B19 virus could spread to SSc target tissues (30). We detected a significantly higher prevalence of parvovirus B19 antibodies (both IgM and IgG) in SSc patients than in controls.

We discovered a higher prevalence of infectious agents in SSc patients than in healthy controls. SSc likely occurs as a result of interactions between the infectious agent and a cascade of host-specific factors and events. This is not surprising, because the immune response to infection is highly individual. It is controlled by multiple genes, age, and the route of infection. In a disease as varied, complex, and rare as SSc, infection prevalence alone should not be expected to provide sufficient evidence for or against a pathological role in the disease. Therefore, additional factors such as coinfections, differences in the strains of infectious agents, and immunological and genetic host factors will have to be further identified and controlled to understand the role of infectious agents in SSc.

Our study had several limitations. First, the relationship between exposure time to the viral infections and clinical onset of the disease was not determined. Second, the study group was small owing to the rarity of the disorder. This condition is estimated to occur in 2.3–10 people per 1 million (5). Third, the impact of environmental factors in the onset of SSc was not analyzed.

References

1. Hannu T, Hedman K, Hedman L, Leirisalo-Repo M. Frequency of recent parvovirus infection in patients examined for acute reactive arthritis. A study with combinatorial parvovirus serodiagnostics. Clin Exp Rheumatol 2007; 25: 297–300.
2. Namboodiri AM, Rocca KM, Kuwana M, Pandey JP. Antibodies to human cytomegalovirus protein UL83 in systemic sclerosis. Clin Exp Rheumatol 2006; 24: 176–178.
3. Moroncini G, Mori S, Tonnini C, Gabrielli A. Role of viral infections in the etiopathogenesis of systemic sclerosis. Clin Exp Rheumatol 2013; 31 (Suppl. 76): 3–7.
4. Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). Arthritis Rheum 1980; 23: 581–590.
5. Black CM. Systemic sclerosis and pregnancy. Clin Rheumatol 1990; 4: 105–124.
6. Amital H, Gershwin EM, Shoenfeld Y. Reshaping the mosaic of autoimmunity. Semin Arthritis Rheum 2006; 35: 341–343.
7. Shoenfeld Y, Blank M, Abu-Shakra M, Amital H, Barzilai O, Berkun Y, Bizzaro N, Gilburd B, Zandman-Goddard G, Katz U et al. The mosaic of autoimmunity: prediction, autoantibodies, and therapy in autoimmune diseases–2008. Isr Med Assoc J 2008; 10: 13–19.
8. Randone SB, Guiducci S, Cerinic MM. Systemic sclerosis and infections. Autoimmun Rev 2008; 8: 36–40.
9. Tachibana I, Bodorova J, Berditchevski F, Zutter MM, Helmer ME. NAG-2, a novel transmembrane-4 superfamily (TM4SF) protein that complexes with integrins and other TM4SF proteins. J Biol Chem 1997; 272: 181–189.
10. Zhou YF, Show M, Harrell RF, Yu ZX, Unger EF, Epstein SE. Chronic non-vascular cytomegalovirus infection: effects on the neointimal response to experimental vascular injury. Cardiovasc Res 2000; 45: 1019–1025.
11. Aragona P, Magazzu G, Macchia G, Bartolone S, Di Pasquale G, Vitali C, Ferreri G. Presence of antibodies against *Helicobacter pylori* and its heat-shock protein 60 in the serum of patients with Sjogren's syndrome. J Rheumatol 1999; 26: 1306–1311.

12. Savarino V, Sulli A, Zentilin P, Raffaella Mele M, Cutolo M. No evidence of an association between *Helicobacter pylori* infection and Raynaud phenomenon. Scand J Gastroenterol 2000; 35: 1251–1254.

13. Sulli A, Seriolo B, Savarino V, Cutolo M. Lack of correlation between gastric *Helicobacter pylori* infection and primary or secondary Raynaud's phenomenon in patients with systemic sclerosis. J Rheumatol 2000; 27: 1820–1821.

14. Danese S, Zoli A, Cremonini F, Gasbarrini A. High prevalence of *Helicobacter pylori* type I virulent strains in patients with systemic sclerosis. J Rheumatol 2000; 27: 1568–1569.

15. Gasbarrini A, Massari I, Serricchio M, Tondi P, De Luca A, Franceschi F, Ojetti V, Dal Lago A, Flore R, Santoliquido A et al. *Helicobacter pylori* eradication ameliorates primary Raynaud's phenomenon. Dig Dis Sci 1998; 43: 1641–1645.

16. Vaughan JH, Shaw PX, Nguyen MD, Medsger TJ, Wright TA, Metcalf JS, Leroy EC. Evidence of activation of 2 herpes virus, Epstein–Barr virus and cytomegalovirus, in systemic sclerosis and normal skins. J Rheumatol 2000; 27: 821–823.

17. Pandey P, LeRoy EC. Human cytomegalovirus and the vasculopathies of autoimmune diseases (especially scleroderma), allograft rejection, and coronary restenosis. Arthritis Rheum 1998; 41: 10–15.

18. Ferri C, Cazzato M, Giuggioli D, Sebastiani M, Magro C. Systemic sclerosis following human cytomegalovirus infection. Ann Rheum Dis 2002; 61: 937–938.

19. Vaughan JH, Smith EA, LeRoy EC, Arnett FC, Wright TA, Medsger TA. An autoantibody in systemic scleroderma that reacts with glycine rich sequences. Arthritis Rheum 1995; 38 (Suppl. 9): S255.

20. Muryoi T, Kasturi KN, Kafina MJ, Cram DS, Harrisson LS, Sasaki T, Bona CA. Antitopoisomerase I monoclonal autoantibodies from scleroderma patients and tight skin mouse interact with similar epitopes. J Exp Med 1992; 175: 1103–1109.

21. Tsukamoto K, Hayakawa H, Sato A, Chida K, Nakamura H, Miura K. Involvement of Epstein–Barr virus latent membrane protein 1 in disease progression in patients with idiopathic pulmonary fibrosis. Thorax 2000; 55: 958–961.

22. Rhodes GH, Valbrach JR, Nguyen MD, Vaughan JH. The p452 gene encodes an autoantigen that cross reacts with EBNA-1 of the Epstein–Barr virus and which may be a heterogeneous nuclear ribonucleoprotein. J Autoimmun 1997; 10: 447–454.

23. Farina A, Cirone M, York M, Lenna S, Padilla C, McLaughlin S, Faggioni A, Lafyatis R, Trojanowska M, Farina GA. Epstein-Barr virus infection induces aberrant TLR activation pathway and fibroblast-myofibroblast conversion in scleroderma. J Invest Dermatol 2014; 134: 954–964.

24. Nikkari S, Mertsola J, Korvenranta H. Wegener's granulomatosis and parvovirus B19 infection. Arthritis Rheum 1994; 37: 1707–1708.

25. Finkel TH, Torok TJ, Ferguson PJ, Durigon EL, Zaki SR, Leung DY, Harbeck RJ, Gelfand EW, Saulsbury FT, Hollister JR et al. Chronic parvovirus B19 infection and systemic necrotising vasculitis: opportunistic infection or aetiological agent. Lancet 1994; 343: 1255–1258.

26. Veraldi S, Mancuso R, Rizzitelli G, Gianotti R, Ferrante P. Henoch-Schönlein syndrome associated with human parvovirus B19 infection. Eur J Dermatol 1999; 9: 232–233.

27. Crowson AN, Magro CM, Dawood MR. A casual role for parvovirus B19 infection in adult dermatomyositis and other autoimmune syndromes. J Cutan Pathol 2000; 27: 505–515.

28. Magro CM, Nuovo GJ, Ferri C, Crowson AN, Giuggioli D, Sebastiani M. Parvoviral infection of endothelial cells and stromal fibroblasts: a possible pathogenetic role in scleroderma. J Cutan Pathol 2004; 31: 43–50.

29. Ferri C, Zakrzewaka K, Longombardo G, Giuggioli D, Storino FA, Pasero G, Azzi A. Parvovirus B19 infection of bone narrow in systemic sclerosis patients. Clin Exp Rheumat 1999; 17: 718–720.

30. Cassinotti P, Burtonoboy G, Fopp M, Siegl G. Evidence for persistence of human parvovirus B19 DNA in bone narrow. J Med Virol 1997; 53: 229–232.