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High prevalence of streptococcal or Epstein-Barr virus infections in children with acute non-septic monoarthritis

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SUMMARY

To investigate associations between infections and acute monoarthritis, we performed a prospective study on 32 children consecutively hospitalized and 32 age-matched controls. Among 26 (81%) children having infections, the most frequent agents were Group A β-hemolytic Streptococcus (GAS: 53%) and Epstein-Barr virus (EBV: 37.5%). Among controls, only 5 (16%) were infected with GAS and 2 (6%) with EBV (P<0.005). The most frequently involved joints were hip in 15 children and ankle in 10 children. Our study showed that acute monoarthritis in children may be frequently associated with streptococcal or EBV infections.

KEY WORDS: Acute monoarthritis, Coxarthritis, Group A β-*hemolytic Streptococcus*, Epstein-Barr virus, Cytomegalovirus.

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In childhood, acute arthritis occurs at a rate of 71 per 100,000 subjects, is monoarticular in more than three quarters of the cases and is generally not associated with a specific etiology (Riise *et al.*, 2008). In addition to bacterial infections, mostly due to group A β-*hemolytic Streptococcus* (GAS), viral infections are rarely associated with acute arthritic syndromes that recall early-onset of rheumatoid arthritis or other chronically-evolving diseases (Thompson *et al.,* 2009; Kim *et al.,* 2009; Pezzone *et al.,* 2009; Riise, Lee *et al.,* 2008; Franssila *et al.,* 2006; Vassilopoulos *et al.,* 2008). Since infections are common in childhood and have a wide spectrum of diseases, we performed a prospective study to assess their possible association with the development of acute monoarthritis in childhood.

The study included patients, who were admitted and followed up in the University Pediatric Unit of the San Salvatore Hospital in L'Aquila, Italy, from January 1, 2007 to December 31, 2012. We recruited 32 children (19 boys: 59.4%) aged 1 to 9 years (mean: 4.3 years) with acute non-septic monoarthritis, as shown by the following features lasting less than 6 weeks and not associated with a traumatic event:

1) joint swelling;
2) pain and limited range of motion in one joint or walking with a limp or other functional limitations affecting a higher or lower limb;
3) joint involvement demonstrated by ultrasound or magnetic resonance imaging.

Septic arthritis was excluded by negative blood culture, Gram stains and joint aspirate culture. Two groups of patients were distinguished: preschool (≤5 years old) and school children (>5 years old). As controls 32 age and sex-matched children without articular manifestations underwent the same investigations of the patients. These children were evaluated concurrent with each case of arthritis. The study was approved by the University Ethical Committee.

GAS infection and post-streptococcal reactive arthritis (PSRA) were diagnosed on the basis of the presence of ≥2 of the following findings:

1) positive GAS in throat culture;

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2) increasing antistreptolysin-O (ASO) titer of ≥2 dilution steps between the acute and convalescent phases; or (3) ASO titer ≥200 IU/mL (Special Writihg Group 1992; Ayoub *et al.,* 1996).

Juvenile Idiopathic Arthritis (JIA) was classified according to the International League of Associations for Rheumatology criteria, that is arthritis of unknown etiology persisting for ≥6 weeks with onset before the age of 16 years (Mackie *et al.,* 2004). Investigations for *Borrelia Burgdorferi, Mycoplasma pneumoniae,* and other possible bacterial agents included antibody detection, throat and blood culture. The association between viral infections and arthritis was based on the detection of virus-specific DNA and/or IgM antibodies, followed by the development of or an increase in the titer of specific IgG antibodies. IgG and IgM antibodies against Epstein-Barr virus (EBV) viral capsid antigens (VCA) and EB nuclear antigens (EBNA), cytomegalovirus (CMV) and parvovirus B19 were examined in serum samples, using commercial enzyme immuno-assays from Radim (Pomezia, Italy) and Sorin (Saluggia, Italy). EBV-DNA, CMV-DNA and parvovirus B19-DNA were detected in blood, urine and/or saliva with the use of a Real-Time (RT) polymerase-chain-reaction (PCR) from BioSorin. Other studies included detection of rotavirus in stool samples, IgG and IgM antibodies to influenza, adenovirus and herpes simplex virus (HSV) types 1/2.

Routine evaluations were leukocyte and platelet counts, erythrocyte sedimentation rate (ESR) and C-reative protein (CRP). Leukocytosis was diagnosed if leukocyte values were higher than the upper normal limits for patients' age. Thrombocytosis was categorized as mild (400-600 $\times 10^3/mm^3$), moderate (600-900 $\times 10^3/mm^3$) and severe (>900 $\times 10^3/mm^3$). Antinuclear antibody (ANA) titer of ≥1/40 and IgM rheumatoid factor (RF) of ≥14 IU/mL were considered positive.

Statistical tests were performed using Fisher's exact test.

Among 32 children with monoarthritis, 26 (81%) had infections, all caused by GAS or EBV, apart from one who had a coinfection by CMV in addition to GAS infection (Table 1). The most frequent infection was by GAS, which was detected in 17 children (53%): of these, 5 (16%) had a coinfection by GAS and EBV, and one further child had both GAS and CMV (3%). Among 17 children infected by GAS, 8 (47%) had a positive throat swab and 15 (88%) had a high ASO on admission. GAS infections were treated with a single intramuscular injection of benzathine penicillin G, which was repeated in two children with persistently elevated ASO titer and arthralgias. An EBV infection was diagnosed in 12 children (37.5%), 7 of whom had EBV alone (22%) and 5 (16%) were coinfected by GAS.

The diagnosis of EBV infection was based on the detection of increased VCA-IgM in 9 children and viral DNA detection in 5 children. Among 5 patients with coinfection by GAS and EBV, VCA-IgM antibodies were elevated in 4 children, and EBV-DNA was positive in three. Two single CMV infections (6%) also occurred (Table 1), as inferred by IgM antibodies in one child and CMV-DNA in saliva from the other patient. Overall, a viral involvement was found in 15 children (47%). No child had other viral or bacterial infections.

Five (16%) of the healthy control children were infected with GAS. This rate was significantly lower than the 53% rate for the cases (P=0.0037) Only 2 (6%) of the healthy control children were infected with EBV. This rate was significantly lower than the 38% rate for the cases (P=0.005).

The most frequently involved joints were hip (15 children: 47%), and ankle (10: 31%). Table 2 shows the association between infections and joint involvement. Among the 32 patients acute monoarthritis occurred significantly ($p<0.0001$) more frequently in pre-school (21 patients) than in school children (11 patients). EBV infection was predominant in children less than 5 years of age (75%), and all single EBV infections occurred in this age group. GAS infections, however, were equally distributed between preschool and older children. All children recovered from monoarthritis within 16 to 34 days (mean 21).

Respiratory manifestations occurred in 27 patients (84%), who showed pharyngotonsillitis concurrent or prior to the onset of joint involvement. Lymphadenitis was noted in 15 children (46.9%). Fever was present in 11 children (34%). Gastrointestinal manifestations, such as

TABLE 1 - *Clinical manifestations and current infections associated with acute monoarthritis in 32 children on admission.*

Patient (years) Sex	*Joint*	*Concomitant clinical manifestations*	*Current infections*	*Positive examinations*	*Inpatient (days)*
1. (8.10) M	Hip	Pharyngotonsillitis	None		no
2. (3.7) F	Hip	Absent	EBV	IgM	3
3. (5.1) M	Hip	Pharyngotonsillitis - lymphadenitis	GAS	ASO 696*	4
4. (1) F	Hip	Pharyngotonsillitis	EBV	IgM	7
5. (3.5) F	Hip	Pharyngotonsillitis - fever	EBV	IgM	5
6. (4.2) M	Hip	Absent	GAS	ASO 520*	no
7. (7.1) M	Hip	Pharyngotonsillitis	GAS	ASO 514*	no
8. (6.5) M	Hip	Pharyngotonsillitis - fever - lymphadenitis - diarrhea	EBV	IgM	3
9. (7.8) M	Hip	Pharyngotonsillitis - lymphadenitis	EBV	IgM	no
			GAS	ASO 548*	
10. (3.3) M	Hip	Pharyngotonsillitis - lymphadenitis - diarrhea	GAS	ASO 2922*	5
11. (3.3) F	Hip	Pharyngotonsillitis - lymphadenitis	EBV	IgM	10
			GAS	Throat swab, ASO 554*	
12. (4.4) M	Ankle	Pharyngotonsillitis - lymphadenitis - vomiting	GAS	Throat swab, ASO 479*	5
13. (6.9) F	Ankle	Pharyngotonsillitis - fever Henoch-Schonlein purpura - diarrhea - vomiting	GAS	ASO 409*	4
14. (2.3) F	Knee	Absent	None		6
15. (3) M	Knee	Pharyngotonsillitis - lymphadenitis	EBV	DNA saliva (18480)**, IgM	no
16. (4.4) F	Ankle	Pharyngotonsillitis - lymphadenitis	EBV	DNA saliva (51330)**, IgM	no
17. (3.6) M	Ankle	Pharyngotonsillitis - Henoch-Schonlein purpura	GAS	Throat swab, ASO 753*	no
18. (6.4) F	Ankle	Pharyngotonsillitis - Henoch-Schonlein purpura - vomiting	EBV	DNA saliva (214357)**, IgM	7
			GAS	Throat swab, ASO 422*	
19. (4.1) M	Wrist	Pharyngotonsillitis - Henoch-Schonlein purpura - fever	CMV	IgM	5
20. (5.9) F	Ankle	Pharyngotonsillitis - Henoch-Schonlein purpura - fever	GAS	Throat swab, ASO 598*	6
21. (1) M	Ankle	Pharyngotonsillitis - lymphadenitis - fever	None		5
22. (1.6) F	Ankle	Pharyngotonsillitis	GAS	Throat swab	4
23. (6.5) M	Hip	Pharyngotonsillitis - lymphadenitis - fever	EBV	DNA saliva (256800)**	4
			GAS	ASO 575*	
24. (2.6) M	Hip	Pharyngotonsillitis	CMV	DNA saliva (158930)**	no
			GAS	Throat swab	
25. (4.8) F	Ankle	Pharyngotonsillitis - lymphadenitis - Henoch-Schonlein purpura	None		no
26.(1.10) M	Hip	Pharyngotonsillitis -lymphadenitis	EBV	DNA saliva (122300)**, IgM	5
27. (4.4) M	Knee	Fever	None		5
28. (2.5) M	Hip	Pharyngotonsillitis - lymphadenitis	EBV	IgM	no
			GAS	ASO 756*	
29. (2) F	Knee	Fever	CMV	DNA saliva (1112000)**	6
30. (2) M	Elbow	Pharyngotonsillitis - lymphadenitis - fever	EBV	IgM	8
31. (6) M	Ankle	Pharyngotonsillitis - Henoch-Schonlein purpura	GAS	Throat swab, ASO 499*	5
32. (9,7) F	Knee	Pharyngotonsillitis - lymphadenitis - fever	GAS	Throat swab, ASO 1511*	3

*IU/mL. **Genome copy number/ml.

TABLE 2 - *Current infections in 32 control children without arthropathy.*

Patient	*Age (years)*	*Sex*	*Current infections*	*Positive examinations*	*Concomitant clinical manifestations*
1.	8.1	M			
2.	4.3	F	CMV	IgM	Pharyngotonsillitis
3.	5.1	M			
4.	1.1	F			
5.	2.3	F			
6.	4.3	M			
7.	7.1	M	GAS	ASO 502*	Pharyngotonsillitis
8.	6.3	M			
9.	7.4	M	GAS	ASO 893*	Pharyngotonsillitis
10.	3.4	M			
11.	2.5	F	GAS	ASO 440*	Lymphadenitis
12.	4.4	M	CMV	DNA saliva (52030)**	Pharyngotonsillitis
13.	7.8	F			
14.	2.1	F			
15.	3.2	M	EBV	DNA saliva (132000)**	Pharyngotonsillitis
16.	5.3	F			
17 .	3.5	M			
18.	5.7	F			
19.	4.6	M			
20.	5	F			
21.	1.1	M			
22.	1.2	F			
23.	6	M	GAS	ASO 630*	Pharyngotonsillitis
24.	2.7	M			
25.	3.8	F			
26.	1.7	M			
27.	3.7	M	EBV	DNA saliva (206320)**	Pharyngotonsillitis - lymphadenitis
28.	2.3	M			
29.	2.1	F			
30.	1.9	M			
31.	5.10	M			
32.	10.1	F	GAS	ASO 1272*	Pharyngotonsillitis

*IU/mL. **Genome copy number/ml.

diarrhea, vomiting and abdominal pain, were reported in 10 children (31%). Seven patients (21%) had Henoch-Schonlein purpura (Table 1). Cardiac or other complications were excluded by ultrasound, radiologic and electrocardiographic examinations. Among controls, 8 (25%) had pharyngotonsillitis, and 2 (6%) had lymphadenitis.

Leukocytosis occurred in 11 children (34%): neutrophilia in 12 (37%) and lymphocytosis in 4 children (13%), respectively. Thrombocytosis was mild in 10 children (31%), moderate in 2 (6.2%), and high in one child. ESR and CRP were both elevated in 21 patients (66%) on admission, and in 14 (44%) and 4 of them (13.5%), respectively, at follow-up. Among 11 children with only GAS infection, ESR was elevated in 8 (73%), while CRP was increased in 7 children (64%). Of the 7 children with single EBV infection, 3 showed high ESR and CRP. The evaluation of the immunoglobulin levels did not show significant alterations. ANA and RF were negative, except for one child who developed mono-articular JIA.

Rheumatic diseases are frequent in children, and may be associated with significant morbidity, decreased quality of life, and monetary costs. Acute monoarticular arthritis is a relatively common clinical condition, which may be associated with infections. Although hampered by the small number of patients, our prospective study showed an infectious association in 81% of them. GAS infection predominated occurring in 53% of the patients. A reactive arthritis after a streptococcal infection and not fulfilling the Jones criteria for diagnosis of acute rheumatic fever, was identified and termed "post-streptococcal reactive arthritis" (PSReA) (Ayoub *et al.,* 1997; Prakken *et al.,* 2004; Mackie *et al.,* 2004). PSReA may occasionally be migrant and associated with carditis, but our patients were all free of complications. Inflammatory processes, including immune-mediated and bacterial-induced increase in vascular permeability and tissue damage, are the most likely pathogenic mechanisms (Rasmussen *et al.,* 2002). In addition to anti-pathogenic, immunomodulatory and chemotactic activities of many antimicrobial peptides, streptococcal virulence factors, including soluble M1-protein, cysteine protease, superantigens, and DNAses can play an important pathogenic role (Beisswenger *et al.,* 2005; Johanson *et al.,* 2010).

Comprehensive serologic and virologic tests unique to our study showed that EBV infection was the second possible cause of monoarthritis, occurring in 37.5% of the children. Although EBV infection has been occasionally reported in children with acute or chronic arthritis, previous studies have shown parvovirus B19 and rubella as the most involved viruses, based only on serologic investigations in adult patients (Franssila *et al.,* 2006; Philips, 1997; Lehmann *et al.,* 2003; Smith *et al.,* 2006; Stahl *et al.,* 2000). None of our patients had B19 or rubella infections. The possible pathogenic mechanisms for EBV-associated monoarthritis, as single or concomitant factors, include a direct infection of synovial cells or immune-mediated damage of the synovial tissue following the antiviral immune response. EBV-infected monocytes and macrophages may secrete proinflammatory cytokines such as interleukin 6 and tumor necrosis factor α, which can induce arthritis, even rheumatoid arthritis (Franssila *et al.,* 2006). The clinical course of arthritis associated with viral infections is typically self-limited, with arthropathy usually lasting only a few weeks. Coinfection by GAS and EBV occurred in 16% of our patients, probably as a result of synergistic effects on inflamed pharyngeal and tonsillar tissue (Rush *et al.,* 2003).

Like previous studies, we observed a peak incidence of monoarthritis in patients younger than 5 years of age, particularly in EBV-infected children, presumably because of the great exposure of pre-school-children to this infection (Riise *et al.,* 2008). Like others, we observed that 45% of our patients had monoarthritis in autumn (data not shown), and the concomitant or recent occurrence in 84.4% of them of pharyngotonsillitis (Riise *et al.,* 2008; Riise, Lee *et al.,* 2008). Involvement of the knee was commonly reported previously but the joints most affected in our children were hip (46.9%) and ankle (31.2%) (Riise *et al.,* 2008; Thompson *et al.,* 2009; Sharma *et al.,* 1999).

In conclusion, our study showed that acute non-septic monoarthritis in children is frequently associated with streptococcal or EBV infections. Testing for EBV-specific antibodies and DNA, in addition to ASO and throat cul-

ture, should enhance diagnosis, prognosis, and management.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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