CASE REPORT

Kawasaki disease and Anaplasma sp. infection of an infant in Cyprus

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On admission, liver enzymes, direct and total bilirubin, and the inflammatory markers erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were raised. Tests (by PCR and immunofluorescence assay [IFA]) for cytomegalovirus (CMV), Epstein–Barr virus (EBV), enterovirus, adenovirus, parvovirus and mycoplasma were all negative, as was blood culture. Antibodies against Coxiella burnetii phase II, Rickettsia conorii and Rickettsia typhi were negative, as were the corresponding PCR amplifications. Antibodies against Anaplasma phagocytophilum were revealed (IgG negative, IgM 1/20), together with a positive PCR.

The child was initially started on cefuroxime IV. However, the persistence of fever for longer than five days, and the development of red cracked lips and desquamation of the perianal area added Kawasaki disease (KD) and Stevens Johnson’s syndrome to the differential diagnosis. Therefore, intravenous immune globulin (IVIG) at a dose of 2 g/kg was given on day 3 of hospitalization. Despite the fact that the liver enzymes and bilirubin gradually returned to normal levels, and the CRP started to decrease after an initial
increase, the clinical condition of the child did not appear to improve; the fever and rash persisted and there were still findings suggestive of liver dysfunction, such as moderately raised prothrombin time and low albumin. She also started to have abdominal distention and diarrhea.

As a result, the child was transferred to the Intensive Care Unit, where the antibiotic treatment was switched to piperacillin/tazobactam and vancomycin; later, metronidazole was also added. 

Clostridium difficile toxin in stools was negative.

As a result of the persistence of fever and the strong suspicion of KD, a second course of IVIG was given to the child on day 8 of hospitalization. ESR and CRP gradually fell, whereas the platelets increased on day 10 after admission. WBC count remained high, but the differential changed to 75% instead of 90% (the initial finding). ASTO titer was low. Chest X-ray and ultrasound of abdomen were normal.

A new echo performed on day 14 of the disease revealed aneurysms of the coronary vessels. Therefore, the diagnosis of refractory KD was confirmed and the child was started on steroids, after which she became afebrile, the rash and other symptoms resolved, and the laboratory findings returned to normal.

In a short epidemiological survey, conducted in areas adjacent to the residence of the patient, 51 samples (41 bovine and 10 sheep) from four flocks were collected and tested for A. phagocytophilum by PCR and IFA. None of the samples was positive by PCR. The overall seroprevalence against the pathogen was 56.8% (29/51), 68% (28/41) bovine and 10% (1/10) sheep.

All tests were performed at the Archbishop Makarios Hospital, apart from those concerning C. burnetii, R. conorii, R. typhi and A. phagocytophilum, which were conducted at the laboratory of Clinical Bacteriology, Parasitology, Zoonoses and Geographical Medicine (Crete, Greece).

Regarding the A. phagocytophilum tests, the presence of IgG and IgM antibodies against the agent in patient serum samples was detected using indirect IFA with a commercial kit (Focus Diagnostics, USA), according to the manufacturer’s recommendations. IgG titers ≥1/64 and IgM titers ≥1/20 were considered positive. DNA extraction from blood samples was carried out using the QIAamp DNA blood mini-kit (Qiagen, Germany). PCR amplification was carried out using the primer set EHR16SD (GGT-ACC-YAC-AGA-AGA-AGT-CC) and EHR16SR (TAG-CAC-TCA-TCG-TTT-ACA-GC), targeting a 345 bp fragment of the Anaplasmatic 16s rRNA gene, using the conditions previously described.1 Distilled water was used as a negative control.

The animal sera were tested for the presence of IgG antibodies against A. phagocytophilum antigen by IFA using a commercial kit (VMRD Inc., Veterinary Medical Research & Development, USA). IgG titers ≥1/100 were considered positive. Regarding animal blood samples, four pools were formed depending on animal species. DNA extraction and PCR amplification were carried out as described above. The positive PCR product was purified using the QIAquick PCR purification spin kit (Qiagen, Germany) and directly sequenced using the CEQ 8000 Beckman Coulter sequencer (BioAnalytika—GenoType, Athens). The sequence revealed (GenBank accession no. EU448141) was 100% identical to Anaplasma sp. when compared using nucleotide BLAST (National Center for Biotechnology Information; http://www.ncbi.nlm.nih.gov/BLAST).

**Discussion**

The first description of anaplasmosis in humans was communicated in the USA in 1994; the first confirmed case in Europe was described in Slovenia in 1997.2 Since then, a number of

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**Table 1** Biochemical and hematological tests performed during hospitalization (Hb: hemoglobin; INR: international normalized ratio; ALP: alkaline phosphatase; γGT: γ-glutamyl transferase; AST: aspartate aminotransferase; ALT: alanine aminotransferase; LDH: lactate dehydrogenase)

<table>
<thead>
<tr>
<th>Test [normal values]</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 8</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl) 11</td>
<td>8.5</td>
<td>8.9</td>
<td>10.9</td>
<td></td>
</tr>
<tr>
<td>WBC × 10⁹/l 17</td>
<td>23</td>
<td>28.5</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Neutrophils (%) 71</td>
<td>90</td>
<td>85</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Platelets × 10⁹/ul 267</td>
<td>261</td>
<td>280</td>
<td>426</td>
<td></td>
</tr>
<tr>
<td>ESR (mm) 61</td>
<td>59</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dl) ≤0.5 12</td>
<td>38</td>
<td>11</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Prothrombin time (s) 17</td>
<td>15</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR 1.4</td>
<td>1.29</td>
<td>1.2</td>
<td></td>
<td></td>
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<tr>
<td>Fibrinogen 543</td>
<td>434</td>
<td>347</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl) [11–25] 20</td>
<td>19</td>
<td>10</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl) [0.2–0.9] 0.5</td>
<td>0.4</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/dl) [5.8–7.2] 7.2</td>
<td>6.3</td>
<td>5.9</td>
<td>7.5</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl) [4–5] 4.1</td>
<td>2.7</td>
<td>2.4</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Bilirubin total (mg/dl) [0.3–1.2] 6.2</td>
<td>5.4</td>
<td>2.9</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Bilirubin direct (mg/dl) [0.5–1.5] 3.8</td>
<td>2.8</td>
<td>1.4</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>ALP (U/L) [74–390] 278</td>
<td>172</td>
<td>147</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>γGT (U/L) [9–35] 153</td>
<td>56</td>
<td>125</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>AST (U/L) [15–40] 255</td>
<td>36</td>
<td>50</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L) [3–37] 434</td>
<td>99</td>
<td>40</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>LDH (U/L) [260–600] 642</td>
<td>401</td>
<td>485</td>
<td>427</td>
<td></td>
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</table>
Anaplasmosis is a clinical syndrome most commonly manifested by non-specific fever, chills, headache and myalgias. Leukopenia, a left shift, thrombocytopenia and hepatic transaminase elevations are present in the majority of patients. Infection by the pathogen can be severe, with nearly half of patients requiring hospitalization. The diagnosis can be confirmed by PCR, especially during early infection when antibodies are not yet detectable. The recommended therapy for children with acute KD is 100 mg doxycycline given orally at 12-hour intervals.

KD was first described and reported by Tomisaku Kawasaki in 1967. It is an acute febrile multisystem vasculitis that almost exclusively affects infants and young children. The diagnosis of KD is considered to be confirmed by the presence of fever for five days and four of the remaining five criteria: 1) bilateral conjunctival injection; 2) changes of the mucous membranes of the upper respiratory tract: injected pharynx, injected fissured lips, strawberry tongue; 3) changes of the peripheral extremities: peripheral edema, peripheral erythema, periungual desquamation; 4) polymorphous rash; and 5) cervical adenopathy. Furthermore, there must be no alternative explanation of the findings. As regards therapy for the disease, in 1988 the Committee on Infectious Diseases of the American Academy of Pediatrics endorsed IVIG treatment as the recommended therapy for children with acute KD.

The cause of KD remains unknown, although recent studies suggest that exposure to an infectious agent in a genetically vulnerable child is required for the pathogenesis of KD. It is hypothesized that a non-specific immune activation triggered by various agents might constitute the common pathway for the manifestation of the disease. The assumption of the association of an infectious agent is based on several observations. These include a seasonal peak in the winter to spring months in most geographical areas; epidemics with a clear epicenter, the peak incidence in certain age groups, with only rare cases in infants, three-month-olds and adults, suggesting a role for transplacental antibodies in conferring protection; and the similarity of many of the clinical features of KD to those of other infectious diseases.

The idea postulated is that bacterial toxins act as superantigens that can trigger the cascade of events that eventually leads to KD. Despite several disputes, attempts have been made to implicate bacterial agents, such as *Bartonella* sp. and *Ehrlichia*, C. burnetii, *R. conorii* and *R. typhi*. Nevertheless, no specific microorganism has been consistently detected in children with KD, making the above hypothesis a controversial one.

To our knowledge, this is the first report describing a case of KD with a concomitant infection by *Anaplasma* sp. confirmed by PCR. Although no association between bacterial infection and KD can be proven by this case, this report reveals once more the need for the investigation of the underlying cause of KD.

**Conflict of interest:** No conflict of interest to declare.

**References**