Abstract. - BACKGROUND: Hemophagocytic lymphohistiocytosis (HLH) is a rare syndrome that is often fatal despite treatment. It is caused by a dysregulation in natural killer T-cell function, resulting in activation and proliferation of histiocytes with uncontrolled hemophagocytosis and cytokines overproduction. The syndrome is characterized by fever, hepatosplenomegaly, cytopenias, liver dysfunction, and hyperferritinemia. HLH can be either primary, with a genetic aetiology, or secondary, associated with malignancies, autoimmune diseases, or infections.

AIM: To focus on secondary HLH complicating zoonotic diseases.

MATERIALS AND METHODS: PubMed search of human cases of HLH occurring during zoonotic diseases was performed combining the terms (haemophagocytic OR haemophagocytosis OR hemophagocytosis OR hemophagocytic OR erythrophagocytosis OR macrophage activation syndrome) with each one of the etiological agents of zoonoses.

RESULTS: Among bacterial diseases, most papers reported cases occurring during brucellosis, rickettsial diseases and Q fever. Regarding viral diseases, most of the cases were reported in patients with avian influenza A subtype H5N1. Among the protozoan zoonoses, most of the cases were reported in patients with visceral leishmaniasis. Regarding zoonotic fungi, most of the cases were reported in AIDS patient with histoplasmosis. No cases of secondary HLH were reported in patient with zoonotic helminthes.

CONCLUSIONS: Zoonotic diseases are an important cause of HLH. Secondary HLH can delay the correct diagnosis of the zoonotic disease, and can contribute to an adverse outcome.

Key Words: Hemophagocytic lymphohistiocytosis (HLH), Zoonoses, Developing Countries, Epidemiology, Review.

Introduction

Although zoonotic infections are a major burden worldwide – both in terms of immediate and long-term morbidity and mortality1,2 and in terms of socioeconomical, ecological, and political impact3 – scientific and public health interest and funding for these diseases remain relatively minor and inadequate4. In the present review we will focus on secondary hemophagocytic lymphohistiocytosis (HLH) complicating zoonotic diseases.

HLH is a potentially fatal hyperinflammatory syndrome that is characterized by histiocyte proliferation and hemophagocytosis. HLH may be inherited (primary, familial) and occurs generally in infants or may be secondary to infection, malignancy or rheumatologic conditions, thereby, occurring at any age. The former is a syndrome associated with autosomal recessive disorders that lead to defects in apoptosis induction of virus-infected cells or tumor cells by cytolytic immune cells, including natural killer (NK) cells or cytotoxic T lymphocytes (CTL). Defects of cytotoxic activities of NK or CTL cells, X-linked lymphoproliferative syndrome type 1 (XLP1) and type 2 (XLP2) can also lead to HLH development5. Secondary HLH, called also macrophage activation syndrome (MAS), is a common finding in systemic juvenile idiopathic arthritis (sJIA) in which, an apparent
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hybrid situation is present. In fact, several mutations have been reported recently in sJIA6. Thus, as in other infection-associated hyperinflammatory syndromes7-10, activation of receptors and cells of the innate immunity system is likely to play a major role in HLH.

The most typical presenting signs and symptoms are fever, hepatosplenomegaly, and cytopenias. Less frequently observed clinical findings are neurological symptoms, lymphadenopathy, edema, skin rash, and jaundice11,12. Common laboratory findings include hypertriglyceridaemia, hyperferritinaemia, a coagulopathy with hypofibrinogenaemia, and elevated aminotransferases11,12. However, HLH is diagnosed using clinical criteria developed by the HLH Study Group of the Histiocyte Society13,14 (Table I).

Literature Review

PubMed search of human cases of HLH occurring during zoonotic diseases was performed combining the terms (haemophagocytic, or haemophagocytosis, or hemophagocytic, or erythrophagocytosis, or macrophage activation syndrome) with each one of the etiological agents of zoonoses and/or one of the diseases indicated in Tables II and III for the period January 1950 to August 2012. A study was considered eligible for inclusion in the systematic review if it reported data on patients with zoonotic diseases who had microscopic signs of hemophagocytosis and/or fulfilled the diagnostic criteria of the HLH Study Group of the Histiocyte Society.

Results

The PubMed search identified 1157 papers. Duplicate publications or papers not reporting clinical cases were excluded. After a scrupulous analysis, 153 papers were further evaluated. In the Table

Table I. HLH 2004 Diagnostic criteria (modified from ref.13,14).

| The diagnosis of HLH can be established if one of either 1 or 2 below is fulfilled: |
| 1. A molecular diagnosis consistent with HLH |
| 2. Diagnostic criteria for HLH are fulfilled (five out of the eight criteria below): |

- Fever
- Splenomegaly
- Cytopenias (affecting ≥ 2 lineages in the peripheral blood):
  - Hemoglobin < 90 g/l (in infants < 4 weeks: hemoglobin < 100 g/l)
  - Platelets < 100,000/ml
  - Neutrophils < 1000/ml
- Hypertriglyceridaemia and/or hypofibrinogenaemia:
  - Fasting triglycerides ≥ 265 mg/dl
  - Fibrinogen ≤ 1.5 g/L
- Hemophagocytosis in bone marrow or spleen or lymph nodes
- Low or absent NK-cell activity
- Ferritin ≥ 500 µg/l
- Soluble CD25 ≥ 2400 U/L

Comments:

(1) If hemophagocytic activity is not proven at the time of presentation, further search for hemophagocytic activity is encouraged. If the bone marrow specimen is not conclusive, material may be obtained from other organs. Serial marrow aspirates over time may also be helpful.

(2) The following findings may provide strong supportive evidence for the diagnosis: (a) spinal fluid pleocytosis (mononuclear cells) and/or elevated spinal fluid protein, (b) histological picture in the liver resembling chronic persistent hepatitis (biopsy).

(1) Other abnormal clinical and laboratory findings consistent with the diagnosis are: cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash. Hepatic enzyme abnormalities, hypoproteinaemia, hyponatraemia, VLDL ↑, HDL ↓.
<table>
<thead>
<tr>
<th>Bacteria</th>
<th>References and notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaplasma phagocytophilum</td>
<td>15Review</td>
</tr>
<tr>
<td>Bartonella sp.</td>
<td>16Renal transplant recipients</td>
</tr>
<tr>
<td>Brucella sp.</td>
<td>17Lyme disease</td>
</tr>
<tr>
<td>Brucella sp.</td>
<td>18Analysis of children with brucellosis associated with pancytopenia, Turkey; 198 year-old male, Turkey; 2064 year-old female, antilymphoma chemotherapy; 21Multicenter retrospective study, Turkey; 22Retrospective study, 3 patients, Turkey; 2311 year-old boy, Turkey; 245 patients, Spain; 25,26 disseminated intravascular coagulation, Spain; 27,28 Retrospective study, Saudi Arabia; 29,30 Two and half years old female, India; 30Pulmonary involvement, Iran; 30Bone marrow biopsy findings in brucellosis patients with hematologic abnormalities, China;</td>
</tr>
<tr>
<td>Campylobacter sp.</td>
<td>31Campylobacter fetus, AIDS, USA</td>
</tr>
<tr>
<td>Capnocytophaga sp</td>
<td>32Sudden Sensorineural Hearing Loss, Japan</td>
</tr>
<tr>
<td>Clostridium sp.</td>
<td>33AIDS; 34Pancreatic carcinoma</td>
</tr>
<tr>
<td>Coxella burnetii</td>
<td>35,36,37,38,40</td>
</tr>
<tr>
<td>Ehrlichia chaffeensis and Ehrlichia ewingii</td>
<td>41Two children, USA; 42Case report, USA; 43Fatal case, USA; 4467 year-old white man, disseminated intravascular coagulopathy, USA</td>
</tr>
<tr>
<td>Leptospira sp.</td>
<td>45Ftal case, Taiwan; 46L. monocytogenes, bone marrow transplant recipient, France</td>
</tr>
<tr>
<td>Listeria sp.</td>
<td>47M. avium, AIDS; 48M. avium, Lupus erythematosus</td>
</tr>
<tr>
<td>Mycobacterium avium</td>
<td>50-53</td>
</tr>
<tr>
<td>Orientia tsutsugamushi</td>
<td>54Murine typhus in returned travelers; 55MSF, Italy; 56,57Rickettsia conorii; 58MSF; 59MSF, Israel; 60Fulminant Rocky Mountain spotted fever</td>
</tr>
<tr>
<td>Rickettsia spp</td>
<td>60Child suffering from chronic granulomatous disease, associated with septicemia due to Salmonella typhi murium; 61</td>
</tr>
<tr>
<td>Salmonella sp. (excluding S. typhi)</td>
<td>62-64Turkey</td>
</tr>
<tr>
<td>Hantaviruses</td>
<td>66Hemorrhagic fever with renal syndrome, South Korea. 67Hemorrhagic fever with renal syndrome Japan. 68Influenza A virus H5N1 subtype; 69Fatal case of swine influenza virus in an immunocompetent host, USA</td>
</tr>
<tr>
<td>Influenza viruses</td>
<td>70-71China, Taiwan</td>
</tr>
<tr>
<td>SARS coronavirus</td>
<td>72</td>
</tr>
<tr>
<td>Protozoa Babesia</td>
<td>81Splenectomized renal allograft recipient, USA</td>
</tr>
<tr>
<td>Leishmania spp.</td>
<td>82Children with HLH treated at the University Children’s Hospital in Belgrade; 83Chronic granulomatous disease; 84Four childhood cases; China; 85Immunocompetent adult-case report and review; 86A review of situation in Thailand; 87Retrospective study Clinical analysis on 28 patients with hemophagocytic lymphohistiocytosis syndrome, China; 88Two cases, India; 89AIDS, India; 9028 years man, India; 91Cases, India; 92Illustrative case and review, India; 93Fatal case, India; 94Retrospective study, India; 95Cerebrospinal fluid involvement, Oman; 96Nine cases, Saudi Arabia. 974.5 month-old infant associated with H1N1 virus infection, Turkey; 98Adolescent, Turkey; 99Child, Turkey; 100Child; Turkey; 1014 year-old boy travel history, Turkey; 1024 year-old boy, Turkey; 10318 Turkish children (2 weeks-72 months); 104Child, pseudomonas septicemia, myelodysplasia, Turkey 105Greece; 106Epstein Barr, Cyprus; 1072 year-old child, Israel; 10846 year-old woman, Israel;</td>
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II are reported all the agents of zoonoses associated with secondary HLH, while in the second column of Table III are listed zoonotic agents not associated with secondary HLH.

Among bacterial diseases, 15 papers reported cases occurring during brucellosis, 16 during rickettsial diseases (Rickettsia spp, Orientia sp, Erlichia spp and Anaplasma spp), 6 during Q

Table II. (Continued). Clinical significant agents of zoonoses found associated with secondary HLH.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>References and notes</th>
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<tbody>
<tr>
<td><strong>Protozoa</strong></td>
<td></td>
</tr>
<tr>
<td><em>Leishmania</em> spp.</td>
<td>112 20 month-old boy, Tunisia; 119 2 year-old boy, Tunisia; 117 Tunisia; 118 2 severe cases, Tunisia; 126 15 month-old girl, Travel Spain, Norway; 120 7 year-old previously healthy Czech boy, travel in Italy; 121 16 month-old girl, Spain; 123 Spain; 124 Spain; 125 Pericardial effusion, Spain; 126 Rheumatoid arthritis, adalimumab, Spain; 127 Steroid, bronchial asthma, Spain; 128 France; 129 France; 130 12 month-old girl, France; 131 12 cases, France</td>
</tr>
<tr>
<td><strong>Toxoplasma gondii</strong></td>
<td>132,133 Primary disseminated toxoplasmosis; 134,16,135 Renal transplantation; 136 Bone marrow transplantation; 137,138,139 AIDS</td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>140 A child case, cryptococcal meningocencephalitis, Japan</td>
</tr>
<tr>
<td><em>Histoplasma capsulatum</em></td>
<td>139, 141-149 AIDS; 150 AIDS Reconstitution inflammatory syndrome; 151 Pediatric AIDS</td>
</tr>
<tr>
<td><strong>Penicillium marneffei</strong></td>
<td>152,153 Leukemia; 154 21-year-old man with Still’s disease; 155,156 Adult-onset Still’s disease, adalimumab, 157 Kidney transplant recipients, USA; 158 Heart transplant recipient, USA; 159 7 year-old boy with chronic mucocutaneous candidiasis, USA; 160 Sarcoïdosis on chronic steroid treatment, USA; 161 France; 162 Immunocompetent, India; 163 2 cases, India; 164 Fungal endocarditis, chronic hepatitis C, cryoglobulinemia, renal failure and <em>Staphylococcus aureus</em> perinephric abscess and bacteremia</td>
</tr>
<tr>
<td><strong>Bacteria</strong></td>
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<tr>
<td><em>Bacillus antraci</em>, <em>Chlamydophila psittaci</em>, <em>Corynebacterium ulcerans</em>, <em>Escherichia coli O157H7</em>, <em>Francisella tularensis</em>, <em>Helicobacter sp</em>, <em>Mycobacterium bovis</em>, <em>Mycobacterium caprae</em>, <em>Mycobacterium marinum</em>, <em>Mycobacterium microti</em>, <em>Mycobacterium ulcerans</em>, <em>Mycobacterium genavense</em>, <em>Pasteurella sp</em>, <em>Shigella sp</em>, <em>Staphylococcus aureus</em> (clearly associated to animal reservoir), <em>Streptococcus suis</em>, <em>Streptococcus canis</em>, <em>Streptococcus acidominimus</em>, <em>Streptococcus bovis</em>, <em>Vibrio</em> spp. (excluding <em>Vibrio cholera</em>), <em>Xersinia sp</em></td>
<td></td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td>Borna disease virus, California serogroup viruses, Chikungunya virus, Cowpox virus, Ebola virus, Hendra virus, Japanese encephalitis virus, Kyasanur forest disease virus, Lassa virus, Lymphocytic choriomeningitis virus, Marburg virus, Monkeypox virus, Nipah virus, Omek haemorrhagic fever virus, Oropouche virus, Rabies and lyssaviruses, Rift Valley fever virus, Ross River virus, Sindbis virus, Tick-borne encephalitis, Venezuelan equine encephalitis virus, West Nile virus, Yellow fever virus, Zika virus</td>
</tr>
<tr>
<td><strong>Protozoa</strong></td>
<td><em>Balantidium coli</em>, <em>Blastocystis hominis</em>, <em>Cryptosporidium parvum</em>, <em>Giardia lamblia</em>, <em>Plasmodium knowlesi</em>, <em>Trypanosoma brucei</em>, <em>Trypanosoma cruzi</em></td>
</tr>
<tr>
<td><strong>Fungi</strong></td>
<td><em>Basidiobolus ranarum</em>, <em>Malassezia</em> spp., <em>Microsporum</em> spp., <em>Paracoccidioides brasiliensis</em>, <em>Trichophyton</em> spp</td>
</tr>
</tbody>
</table>
fever, 2 during leptospirosis. One paper each reported papers during Lyme disease or \textit{Capnocytophaga} sp. infection. Most of the above papers described cases of secondary HLH occurring in immunocompetent patients without important comorbidities. Cases of \textit{Bartonella}, \textit{Clostridium}, \textit{Listeria}, \textit{Mycobacterium}, \textit{Salmonella} spp and \textit{Campylobacter fetus} infections were less reported and most of them occurred in patients with major comorbidities. Among the zoonotic mycobacterial diseases, HLH was reported only in patients with \textit{Mycobacterium avium} infection affected by HIV infection or by systemic lupus erythematosus.

Among viral diseases, cases were reported in patients with avian influenza A subtype H5N1, swine influenza, SARS coronavirus, Crimean-Congo haemorrhagic fever, hepatitis E virus. Most of cases occurred in immunocompetent patients without important comorbidities.

Regarding the protozoan zoonoses, most of the cases were reported in patients with visceral leishmaniasis. Only in few cases of the 46 articles reporting such cases, comorbidities were present. Nine papers reported cases occurring in patients with toxoplasmosis and most of them were immunocompromised. Two cases were reported in patient with babesiosis.

Regarding zoonotic fungi, 23, four and one paper reported cases occurring in patients with \textit{Histoplasma capsulatum}, \textit{Penicillium marneffei} and \textit{Cryptococcus neoformans} infections, respectively. Most of them occurred in immunocompromised patients. No cases of secondary HLH were reported in patient with zoonotic helminths.

In one case each, a double infection with Epstein Barr virus\textsuperscript{112}, H1N1 virus\textsuperscript{103}, \textit{Pseudomonas} septicemia,\textsuperscript{130} and \textit{Staphylococcus aureus} perinephric abscess\textsuperscript{164}, in addition to the zoonotic agent, were reported.

Regarding comorbidities, five papers reported cases occurring in kidney transplant recipients\textsuperscript{16,134,135,157}, one in a heart transplant recipient\textsuperscript{158} and two in bone marrow transplant recipients\textsuperscript{47,136}. Twenty papers reported cases occurring in HIV-infected patients\textsuperscript{33,48,95,137-151,165,166}, of them, one occurred in a pediatric patient\textsuperscript{165}, and one in the course of AIDS Reconstitution Inflammatory Syndrome\textsuperscript{150}. In two and four papers leukaemia\textsuperscript{152,153} and chronic granulomatous disease (CGD)\textsuperscript{60,87-89}, respectively, were present. Other major comorbidities reported were rheumatologic diseases\textsuperscript{39,126,154,156}, which in three cases were under treatment with adalimumab\textsuperscript{26,155,156}. Other major comorbidities/immunosuppressive conditions were chronic steroid treatment\textsuperscript{125,160}, chronic mucocutaneous candidiasis\textsuperscript{159}, antilymphoma chemotherapy\textsuperscript{20}, and pancreatic carcinoma\textsuperscript{36}. Five papers reported cases occurring after travel to endemic zones\textsuperscript{54,80,107,119,169}.

### Discussion

Zoonotic infections are defined, in general, as infections transmitted from animal to man (and less frequently vice versa), either directly (through direct contact or contact with animal products) or indirectly (through an intermediate vector as an arthropod or an insect)\textsuperscript{170}. The main zoonotic features of influenza are represented by the role of animal hosts as reservoirs and substrates for the development of novel strains, and their role in the introduction of these strains into human pathology. Avian H5N1 influenza is a typical zoonotic infection, requiring close contact with infected animal hosts\textsuperscript{1}. The current H1N1 pandemic strain stopped being zoonotic after human-to-human transmission emerged as the cause of the pandemic. The single non-human hosts for each of influenza B and influenza C viruses play a minimal role in human disease\textsuperscript{1}. Avian influenza A subtype H5N1 infection and severe acute respiratory syndrome (SARS) due to coronavirus (SARS-CoV) share similar pathologic features. Pneumocytes are the primary target of infection, resulting in diffuse alveolar damage. Systemic cytokine activation results in hemophagocytic syndrome, lymphoid depletion, and skeletal muscle fiber necrosis\textsuperscript{171}. However, HLH has also been found in fatal cases of H1N1 infection during the pandemic which emerged in April 2009\textsuperscript{172}.

HIV infection alone or in the presence of other opportunistic and non-opportunistic infections or malignancies has been associated with HLH, and HLH has also been described in the setting of immune reconstitution inflammatory syndrome\textsuperscript{150,173,174}.

Also rotavirus infection can cause secondary HLH\textsuperscript{175}, but this is not a frequent finding\textsuperscript{176-180}. Of note, animal rotaviruses might be able to cross species barriers, and lack of systematic surveillance of rotavirus infection in small animals hinders the ability to establish firm epidemiological connections\textsuperscript{178,180-182}.

Almost all the cases associated with bacterial infections were due intracellular organisms frequently causing epatosplenomegaly and leuko-
nia such as Brucella and Rickettsia spp. Early treatment of brucellosis with appropriate antibiotics will be life-saving. We believe that HLH should always be considered in the severe cases of rickettsial diseases, especially if associated with pancytopenia. More studies are needed to understand whether immunosuppressive treatments (e.g., with steroids) could be beneficial (as we suspect), especially in those cases not responding promptly to antibiotic therapy.

No cases were reported in the course of Escherichia coli O157:H7 infection, even though in a cirrhosis patient a case caused by E. coli infection secondary to bacterial translocation has been described.

Leishmania donovani and Leishmania infantum can cause HLH. Moreover Leishmania infection by itself can mimic the syndrome, especially in the presence of organomegaly and cytopenia. A bone marrow aspirate determines the correct diagnosis. Treatment of leishmaniasis-associated haemophagocytic syndrome with amphotericin B results in cure.

Pulmonary involvement in HLH has been reported in some severe cases. Occurrence of pulmonary involvement is quite frequent especially in HLH triggered by viral infections. Being rare itself, HLH is diagnosed almost exclusively in seriously ill, hospitalized patients. Diagnosis of secondary HLH may be laborious, but the primary source must be continually sought, particularly in the case of rare pathogens. Furthermore, it should be stressed that the identification of hemophagocytosis in bone marrow aspirate represents only one of 5-8 criteria needed for the diagnosis of HLH. Treatment of secondary HLH is dependent on its cause. Infectious agents should be eradicated promptly, along with administering supportive care. There are no randomized trials for primary HLH, due to the rarity of this disease. Treatment is based on the combination of immune suppression (such as cyclosporin A) and chemotherapy (such as etoposide). Intravenous immunoglobulins may also be beneficial. Treatment should be started without delay, yet it should be kept in mind that the use of immunosuppression may further delay diagnosis and definitive treatment.

Conclusions

Zoonotic diseases are an important cause of HLH. Secondary HLH can delay the correct diagnosis of the zoonotic disease, and can contribute to an adverse outcome. Further studies are needed to understand whether an immunosuppressive treatment could be beneficial in those cases that do not promptly respond to anti-infective therapy.

References


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75) HENTER JI, CHOW CB, LEUNG CW, LAU YL. Cytotoxic therapy for severe avian influenza A (H5N1) infection. Lancet 2006; 367: 870-873.


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100) BHUTANI V, HALDAR D, VARMA N, MARWANA R, VARMA S. A case series highlighting the relative frequencies of the common, uncommon and atypical/unusual hematological findings on bone marrow examination in cases of visceral leishmaniasis. Medit J Hematol Infect Dis 2011; 3: e2011035.


125) **Cerdan Vera MT, Bernal Ferrer AM, Segui Canet JM, Serre Aranda M.** Pericardial effusion in a case of hemophagocytic lymphohistiocytosis secondary to leishmaniasis. *An Pediatr (Barc)* 2012. doi.org/10.1016/j.anpedi.2012.05.011


149) **Khandekar MM, Deshmukh SD, Holla VV, Rane SR, Kakrani AL, Sangale SA, Habibu AA, Pandit DP, Bhore AV, Sastry J, Phadekar MA, Boillinger RC.** Profile of bone marrow examination in HIV/AIDS patients to detect opportunistic infections, especial-
Secondary hemophagocytic lymphohistiocytosis in zoonoses: A systematic review


171) Ng W, To KF, Lam WY, Ng TK, Lee KC. The comparative pathology of severe acute respiratory syndrome and avian influenza A subtype H5N1--a review. Hum Pathol 2006; 37: 381-390.


176) Arista S, Vizzini E, Ferraro D, Caccio A, Di Stefano R. Distribution of VP7 serotypes and VP4 genotypes among rotavirus strains recovered from


189) CASCIO A, IARIA C. Epidemiology and clinical features of Mediterranean spotted fever in Italy. Parassitologia 2006; 48: 131-133.


