Human Granulocytic Anaplasmosis in Europe

ECCMID 2013
Berlin

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Anaplasma phagocytophilum

- **Order**: Rickettsiales

- **Outer cell wall structure**:
  - typical of Gram-negative bacteria
  - no peptidoglycan

- **Size**: 0.3 - 1 µm

- **Obligate intracellular pathogen (granulocytes)**

- **Morulae**: 2 - 7 µm
Anaplasmosis / Ehrlichiosis...

Human Granulocytic Ehrlichiosis

- US, 1990-94
- US, 1970

E. phagocytophila

- Europe, 1932
- 16S rDNA
gro ESL

Reorganization of genera in the families Ricketsiaceae and Anaplasmataceae in the order Rickettsiales [...] 

JS Dumler, AF Barbet et al. (IJSEM, 2001)

Human Granulocytic Ehrlichiosis agent

Tick-borne fever

E. equi

US, 1970

Equine ehrlichiosis

Anaplasma phagocytophilum

E. phagocytophila
Transmission

- **Vectors:**
  - Europe: *Ixodes ricinus*
  - United States: *Ixodes scapularis*  
    *Ixodes pacificus*

- **Reservoirs:**
  - small mammals: rodents
  - others: sheep, lamb, goat, cattle, roe deer

→ persistently infected carriers?
→ source of continuous transmission
Anaplasmosis in the USA

Geographic distribution of anaplasmosis incidence in 2010 (CDC)

Anaplasmosis cases, 1994-2010 (CDC)

1st characterization of HA, 1990-94

<table>
<thead>
<tr>
<th>Year of report</th>
<th>Nb of cases</th>
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<tbody>
<tr>
<td>1994</td>
<td>1</td>
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<td>2008</td>
<td>15</td>
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<td>2009</td>
<td>16</td>
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<tr>
<td>2010</td>
<td>17</td>
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</tbody>
</table>
Anaplasmosis in Europe

- 1st European case: Slovenia, 1997
- Since 1997, ~100 confirmed or probable cases
  - Central Europe (Slovenia) and Scandinavia ++
  - Sporadic cases all over Europe
HA in Europe: geographic distribution

- Number of cases

- **Norway**: ≈ 25 cases
- **Slovenia**: 25 cases
- **Italy**: 7 cases
- **France**: 6 cases
- **Spain**: 2 cases
- **UK**: 13 cases
- **Germany**: 10 cases
- **Belgium**: 1 case
- **Croatia**: 6 cases
- **Serbia**: 8 cases
- **Greece**: 2 cases
- **Alb.**: 2 cases
- **Russia**: 1 case
- **Ukraine**: 1 case
- **Czech R.**: 10 cases
- **Hungary**: 6 cases
- **Slovakia**: 1 case
- **Bulgaria**: 1 case
- **Belarus**: 1 case
- **Latvia**: 1 case
- **Lithuania**: 1 case
- **Poland**: 1 case
- **Estonia**: 1 case
- **Finland**: 1 case
- **Sweden**: 1 case
- **Denmark**: 1 case
- **Austria**: 6 cases
- **Switzerland**: 1 case
- **Netherlands**: 1 case
- **N. Ireland**: 1 case
- **W. Ireland**: 1 case
# Case definition (ESCMID guidelines, 2004)

## Proposed case definition for Human Anaplasmosis

| Confirmed HA | Febrile illness with a history of tick bite or tick exposure  
|             | and: 
|             | ✓ Seroconversion or ≥ 4-fold change in Ab titre  
|             | or: 
|             | ✓ Positive PCR assay demonstrating *A. phagocytophilum* specific DNA in blood  
|             | or:  
|             | ✓ Isolation of *A. phagocytophilum* in blood culture |

| Probable HA | Febrile illness with a history of tick bite or tick exposure  
|            | and: 
|            | ✓ Presence of significant *A. phagocytophilum* Ab titre (>4-fold above cut-off value)  
|            | or:  
|            | ✓ Presence of intracytoplasmic morulae in a blood smear |
Pubmed literature query for HGA, HGE, E. phagocytophila, A. phagocytophilum in Europe
Period : 1997 - 2013

≈ 30 reports of series or individual cases
58 patients with data fulfilling the ESCMID guidelines

- Confirmed or probable cases of HA
- Epidemiological data
- Clinical manifestations
- Laboratory features
- Microbiological diagnosis
Epidemiology
(58 patients)

- **Seasonality:**
  - Between April and October
  - Peak between June and August

- **Most patients recall:**
  - History of tick bite 5 - 30 days before onset of illness

- **Exposure to ticks:**
  - Living in rural areas
  - Work: hunter, farmer, forestry worker
  - Leisure outdoor activities (country, wooden areas): forest walk, athletics, camping

- **Median time from bite to onset of symptoms:** 14 days
Clinical Manifestations
(58 patients)

- Clinical presentation: acute, nonspecific febrile illness

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>% of patients</th>
</tr>
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<tbody>
<tr>
<td>High-grade fever (&gt; 38.5°C)</td>
<td>98 %</td>
</tr>
<tr>
<td>Headache</td>
<td>85 %</td>
</tr>
<tr>
<td>Malaise / asthenia</td>
<td>72 %</td>
</tr>
<tr>
<td>Arthralgia / myalgia</td>
<td>64 %</td>
</tr>
<tr>
<td>Nausea / vomiting / abdominal pain / diarrhea</td>
<td>50 %</td>
</tr>
<tr>
<td>Cough</td>
<td>22 %</td>
</tr>
<tr>
<td>Atypical pneumonitis (X-Ray established lung infiltrate)</td>
<td>9 %</td>
</tr>
<tr>
<td>Enlarged lymph nodes or enlarged spleen or liver</td>
<td>29 %</td>
</tr>
<tr>
<td>Cutaneous rash</td>
<td>9 %</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>9 %</td>
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</tbody>
</table>
## Laboratory features
(58 patients)

<table>
<thead>
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<th>Laboratory findings</th>
<th>% of patients (n = 58 patients)</th>
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</thead>
<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>69 %</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>59 %</td>
</tr>
<tr>
<td>Increased values of ASAT / ALAT</td>
<td>74 %</td>
</tr>
<tr>
<td>Increased LDH activity</td>
<td>26 %</td>
</tr>
<tr>
<td>Increased CRP values</td>
<td>67 %</td>
</tr>
</tbody>
</table>

CSF analysis = normal
Bone marrow analysis = normal
Outcome (58 patients)

- Mild disease, 100% complete recovery
- Resolve quickly even in the absence of adapted antibiotics
- Treatment: doxycycline 100 mg twice daily for at least 7 days
  - 2/3 (37/58) patients only received specific ATB treatment
- Quick clinical improvement shortly after initiation of doxycycline
  - Symptoms usually resolve within 24-48h of ATB (100% cases)
- Prolonged course with relapsing fever in 12/58 patients
- Long-term outcome: favorable, regardless of antibiotic therapy
- No death and no long-term consequences
- ≠ USA: 5% patients require intensive care (case fatality rate < 1%)
# Microbiological diagnosis

(58 patients)

<table>
<thead>
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<th>Laboratory diagnosis</th>
<th>Nb of patients (n = 58 patients)</th>
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<tr>
<td>✓ Positive PCR assay</td>
<td>26</td>
</tr>
<tr>
<td>✓ Seroconversion or ≥ 4-fold ↑ of Ab titre</td>
<td>26</td>
</tr>
<tr>
<td>✗ Clinical features + presence of significant Ab titre</td>
<td>6</td>
</tr>
</tbody>
</table>

**Confirmed cases**

- Positive PCR assay
- Seroconversion or ≥ 4-fold ↑ of Ab titre

**Probable cases**

- Clinical features + presence of significant Ab titre
Diagnostic tools (1/3)

• Molecular detection:
  ▪ Most sensitive diagnostic test
  ▪ Within the 2 first weeks after onset of symptoms ++
  ▪ Acute phase blood (EDTA/citrate) or buffy coat, before antibiotic treatment

• Target genes:
  • Most frequent: 16S rDNA with sequence analysis
  • GroESL
  • msp2/p44: specific for Aph and multicopies (> 100 copies)
Diagnostic tools (2/3)

- Serologic testing:
  - Most commonly used diagnostic tool
  - IFA (IgM and IgG)
    - Human promyelocytic cell line (HL60) infected with tick/horse/human isolate
    - Commercial kits (Focus Technologies, MRL Diagnostics)
  - 2 sera: acute and convalescent phase
  - Often negative during the initial phase of disease!
  - No detectable antibodies in acute phase serum for ≥ 2/3 of the patients
Diagnostic tools (3/3)

- **Giemsa staining of peripheral blood smear:**
  - Early stage of infection only (first week++)
  - Before antibiotic treatment
  - Morulae: detected in 6 European cases only...
  - False-positive results: toxic granulations, Döhle bodies → Lacks sensitivity and specificity

- **Culture:**
  - Acute phase blood or buffy coat
  - Promyelocytic HL60 leukemia cell line
  - Development of cytopathic effects 5-12 days after inoculation
  - Almost never performed for diagnosis purpose
  - Europe: 2 cases in Czech Republic only (Hulinska et al. 2009)
Prospective study: Etiology of febrile illnesses after a tick bite
- Department of Infectious Diseases, Ljubljana, 1996 - 2004

Identified 24 adult patients with proven anaplasmosis

PCR assay was positive for 63% of patients between day 2 and 15 following onset of symptoms.

Specific antibodies were detected in only 25% of patients at initial presentation

Persistence of antibodies: IgG positive 2 years after diagnosis in 56% patients
• Eastern France: endemic area for Lyme borreliosis

• During summer 2009, anaplasmosis was investigated:
  ▪ in patients presenting a febrile syndrome
  ▪ with recent history of tick-bite or exposure to ticks

• 3 patients had a positive PCR assay in acute phase blood
  (molecular target: \textit{msp2/p44})

• Acute phase serum tested negative for \textit{A.ph} antibodies

• Specific antibodies were detected in convalescent serum

• Epidemiologic, clinical and biologic criteria from these
  3 patients were compatible with anaplasmosis
Sero-epidemiologic surveys

Prevalence of specific antibodies in healthy blood donors (%)

Prevalence of specific antibodies in tick-exposed populations (%)

- Limited recording and reporting?
- Underestimated disease?
- Asymptomatic / subclinical infection?
Infection rate in ticks

Prevalence of *A. phagocytophilum* in *I. ricinus* ticks

- **UK**: 1 - 2%
- **France**: 0.5 - 1.5%
- **Belg.**: 0.5%
- **NL**: 2 - 4%
- **Germany**: 1 - 2%
- **Austria**: 5 - 7%
- **Czech R.**: 10%
- **Switzerland**: 1 - 2%
- **Slovenia**: 3%
- **Croatia**: 3%
- **BH**: 14%
- **Serbia**: 8 - 13%
- **Slovakia**: 10%
- **Poland**: 8 - 14%
- **Estonia**: 1 - 3%
- **Latvia**: 3%
- **Lithuania**: 3%
- **Sweden**: 4.5%
- **Norway**: 3 - 9%
- **Denmark**: 0.5 - 1.5%
- **Swiss.**: 4.5%
- **Spain**: 8%
- **Italy**: 4 - 10%
- **UK**: 1 - 2%
- **Belg.**: 0.5%
- **NL**: 2 - 4%
- **Germany**: 1 - 2%
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- **Italy**: 4 - 10%
Conclusions

- Anaplasmosis should be investigated in patients presenting:
  - Undifferentiated febrile illness
  - Cytopenia and elevated rates of liver enzymes
  - Whose medical history reveals exposure to ticks.

- Clinical course:
  - Mild disease
  - Uneventful recovery in 1-2 weeks, even in the absence of specific antibiotic therapy
  - Situation ≠ in USA: opportunistic complications
Conclusions

- **Laboratory confirmation:**
  - **Acute phase:**
    - Blood smear examination: low sensitivity
    - PCR ++ (EDTA whole blood, before ATB treatment) = diagnostic test of choice
  - **Late infection/convalescence:** serologic testing

- **Limitations:**
  - Specific PCR assay is not widely available for routine use
  - Serologic tests: often negative in early stage of infection (> 2/3 patients)

- Doxycycline therapy leads to clinical improvement in 24-48 h
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