Sequential changes in hematologic and biochemical parameters in African tick bite fever

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Objective To evaluate the sequential changes and to estimate the frequencies of abnormalities in some commonly measured biological variables in patients with African tick bite fever (ATBF), an emerging spotted fever group (SFG) rickettsiosis in international travelers to rural sub-Saharan Africa.

Methods A study was done of hemoglobin, total leukocyte count, absolute lymphocyte count, blood platelet count and serum levels of C-reactive protein (S-CRP), alanine aminotransferase (S-ALAT), aspartate aminotransferase, lactic dehydrogenase, γ-glutamyl transferase, alkaline phosphatase, bilirubin, sodium and creatinine during the first two weeks of illness and prior to the institution of antirickettsial therapy in 108 patients with travel-associated ATBF.

Results There were significant falls in mean total leukocyte count, mean absolute lymphocyte count, and mean platelet count, and significant increases in mean S-CRP and S-ALAT. During the first ten days of illness, elevated S-CRP, lymphopenia and elevated S-ALAT were detected in 91.7%, 73.3% and 40.7% of patients, respectively. Most abnormalities were mild. For 55 patients who underwent both S-CRP and absolute lymphocyte count determination, at least one parameter was abnormal in 52 (94.5%) patients.

Conclusions The sequential changes in many biological parameters during the acute phase of ATBF mimic those reported in other SFG rickettsioses. Mild abnormalities are frequent, with increased S-CRP and lymphopenia being the two most consistent findings.

Keywords *Rickettsia africae*, C-reactive protein, aminotransferases, lymphocytes, rickettsial diseases

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INTRODUCTION

African tick bite fever (ATBF) is a flu-like illness frequently accompanied by multiple inoculation eschars, regional lymphadenitis, vesicular cutaneous rash and aphthous stomatitis. ATBF is caused by *Rickettsia africae*, a recently described spotted fever group (SFG) rickettsia, and is transmitted by ungulate ticks of the *Amblyomma* complex in rural sub-Saharan Africa and the West Indies [1]. Parallelling the rapid expansion of safari tourism to southern Africa, the number of imported ATBF cases has risen significantly in Europe and elsewhere during the last few years [2–4]. Characteristically, and in

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contrast to other SFG rickettsioses, most reported cases of travel-associated ATBF occur in clusters, frequently affecting 30% or more of exposed subjects [5–7]

The diagnosis of ATBF is not always straightforward. Important clinical signs such as inoculation eschars and cutaneous rash may be absent or overlooked, and seroconversion frequently occurs late [8]. The changes in commonly measured biological variables, and their possible diagnostic usefulness in ATBF, are largely unknown. Hematologic and biochemical data have been published for only a handful of cases, and discrepancies are notable, with some parameters reported as being either elevated, normal, or decreased [3–5,9]. We performed a study of the sequential changes in 13 biological variables determined during the first two weeks of illness and prior to the institution of antirickettsial therapy in 108 patients with travel-associated ATBF. Our aims were two-fold: (1) to describe the sequential changes in these parameters; and (2) to determine the proportion of cases who developed laboratory abnormalities during the first 10 days of illness, i.e. when most patients are likely to present.

MATERIALS AND METHODS

Patient selection

Medical records of patients diagnosed with travel-associated ATBF from 1994 through April 2002 in Norway, Denmark, Sweden, Italy and France were reviewed. The diagnostic criteria were as follows: a flu-like illness commencing no later than ten days after leaving rural sub-Saharan Africa; and either (1) direct evidence of *R. africae* infection by culture and/or PCR, (2) specific antibodies against *R. africae* detected by Western blot with or without cross-adsorption assays, or (3) serology specific for recent SFG rickettsial infection combined with clinical and epidemiologic features consistent with ATBF, such as multiple inoculation eschars, $\geq$1 eschar plus a vesicular cutaneous rash, and/or clustering of cases. To be included in the study, patients had to have at least one blood sample drawn for routine laboratory testing during the first 14 days of illness and before receiving antirickettsial chemotherapy.

Laboratory data

The following hematologic and biochemical parameters were studied: hemoglobin, total leukocyte count, absolute lymphocyte count, blood platelet count, serum C-reactive protein (S-CRP), serum alanine aminotransferase (S-ALAT), serum aspartate aminotransferase (S-ASAT), serum lactic dehydrogenase (S-LDH), serum γ-glutamyl transferase (S-GGT), serum alkaline phosphatase (S-ALP), serum bilirubin, serum sodium (S-Na), and serum creatinine. For hematologic parameters, assays were performed according to standard procedures (reference ranges in parentheses): hemoglobin (males, 12.5–16.5 g/dL; females, 11.5–15.5 g/dL), total leukocyte count (3.0–11.0 $\times$ 10^9/L), absolute lymphocyte count (1.5–4.0 $\times$ 10^9/L), and blood platelet count (150–450 $\times$ 10^9/L). For biochemical parameters, different laboratory methods (e.g. dry chemistry versus wet chemistry) impeded direct comparison of data. Therefore, before analysis, biochemical data were transformed and expressed as proportions of the upper (or lower, if appropriate) age- and gender-dependent reference limits used at the laboratory in question.

Days of illness were counted, starting with the day of onset of flu-like symptoms (day 1). Laboratory test results were tabulated for each day of illness until, and including, the day of initiation of antirickettsial therapy. Data collected during the first ten days after symptom onset were then grouped in pairs (i.e. as day 1–2, day 3–4, etc.), whereas data for day 11–14 were collected into one group. Finally, computerized analyses of the grouped data were performed to determine the kinetics, as well as the percentage of abnormal values, of each variable.

Nucleic acid amplification

For molecular detection and identification of *R. africae*, DNA was extracted from 200 µL of serum with the QIAmp blood kit (QIAGEN GmbH, Hilden, Germany), according to the manufacturer's recommendations. These DNA extracts were used as templates in a suicide-PCR assay, as previously described [2]. All positive PCR products were sequenced as described elsewhere for identification of the infecting rickettsial species [10].

Culture of *R. africae*

Isolation of the microorganism was achieved using skin biopsies and heparinized blood samples, which were inoculated and cultivated as previously reported [5].

Serology

Microimmunofluorescence assay (MIF), the serological reference method, was carried out as previously reported, using both *R. conorii* strain Seven (Malish, ATCC VR-613T) and *R. africae* strain ESF-5 (provided by Dr G. Dasch) as antigens. Titers of 1 : 64 for IgG and/or 1 : 32 for IgM were considered to constitute evidence of recent infection by a *Rickettsia* species. Western blotting procedures were performed as described elsewhere [11]. Twenty micrograms of *R. africae* or *R. conorii* antigen was used per lane. Cross-adsorption for serologic testing was performed as previously described [12]. We considered as definite serologic evidence of *R. africae* infection IgG plus IgM MIF titers at least two dilutions higher to *R. africae* than to *R. conorii*, a Western blot profile that revealed only *R. africae*-specific antibodies, and/or cross-adsorption studies demonstrating that the homologous antibodies were directed against *R. africae*. As serologic evidence of recent SFG rickettsiosis, we considered titers of IgG $\geq$ 1 : 64 and IgM $\geq$ 1 : 32 as not fulfilling the above criterion, and a Western blot showing antibodies directed to both high-molecular-weight surface proteins (rOmpA and rOmpB) and the lipopolysaccharide of more than one rickettsial species.

Statistical analysis

All data were analyzed using a database software program (SPSS version 11.0; SPSS, Chicago, IL, US). Differences were compared with the Mann–Whitney *U* rank sum test for unpaired data. $P < 0.05$ in two-tailed tests was considered to be statistically significant.

RESULTS

One hundred and eight patients were included in the study: 64 from Norway, 26 from France, eight from Sweden, six from Italy, and four from Denmark. There were 74 (68.5%) males and 34 females, and the median age was 48 years (range 16–72 years). The four most common countries of disease acquisition were South Africa (69.4% of the cases), Lesotho (11.1%), Botswana (6.5%), and Zimbabwe (5.6%). Seventy-nine (73.1%) patients received antirickettsial therapy, and 42 (38.9%) were hospitalized. Except for two cases of reactive arthritis, no complications were documented. There was direct evidence of *R. africae* infection in 12 patients, serology specific for recent *R. africae* infection in 57 cases, and serologic evidence of recent SFG rickettsiosis combined with epidemiologic and/or clinical features consistent with ATBF in 39 cases.

Hematologic parameters

Hemoglobin levels were measured 93 times in 74 patients. No major variation in hemoglobin was noted. Two cases of mild anemia were recorded.

Total leukocyte counts were performed 135 times in the 93 patients. Mean levels reached a nadir on day 5–6, and then increased until day 11–14 ($P = 0.007$) (Figure 1). During the first ten days of illness, leukopenia was documented in eight of 91 (8.8%) patients and leukocytosis in three of 91 (3.2%) patients.

Absolute lymphocyte counts were measured 78 times in 65 patients. The mean count was at its lowest at day 1–2, and stayed below the lower reference limit until day 11–14 ($P = 0.011$) (Figure 1). During the first ten days of illness, lymphopenia was observed in 44 of 60 (73.3%) patients and lymphocytosis in no patients.

Blood platelet counts were performed 124 times in 90 patients. The mean levels reached a nadir on day 3–4, and rose thereafter until day 11–14 ($P = 0.003$) (Figure 1). During the first ten days of illness, 18 of 89 (20.2%) patients had documented thrombocytopenia, whereas none had detectable thrombocytosis.

Biochemical parameters

S-CRP levels were measured 135 times in 88 patients. The mean value peaked on day 7–8 and returned to almost normal on day 11–14 ($P = 0.004$) (Figure 2). During the first ten days of illness, elevated S-CRP levels were documented in 77 of 84 (91.7%) patients.

S-ALAT and S-ASAT were measured 128 times each in 96 patients, S-LDH was measured 94 times in 69 patients, S-GGT was measured 83 times in 65 patients, and S-ALP was determined 84 times in 68 patients. The mean values of all five parameters increased gradually from day 1–2 to day 9–10; however, this rise was statistically significant only for S-ALAT ($P = 0.021$) (Figure 2). During the first ten days of illness, elevated levels of S-ALAT were documented in 37 of 91 (40.7%) patients, elevated

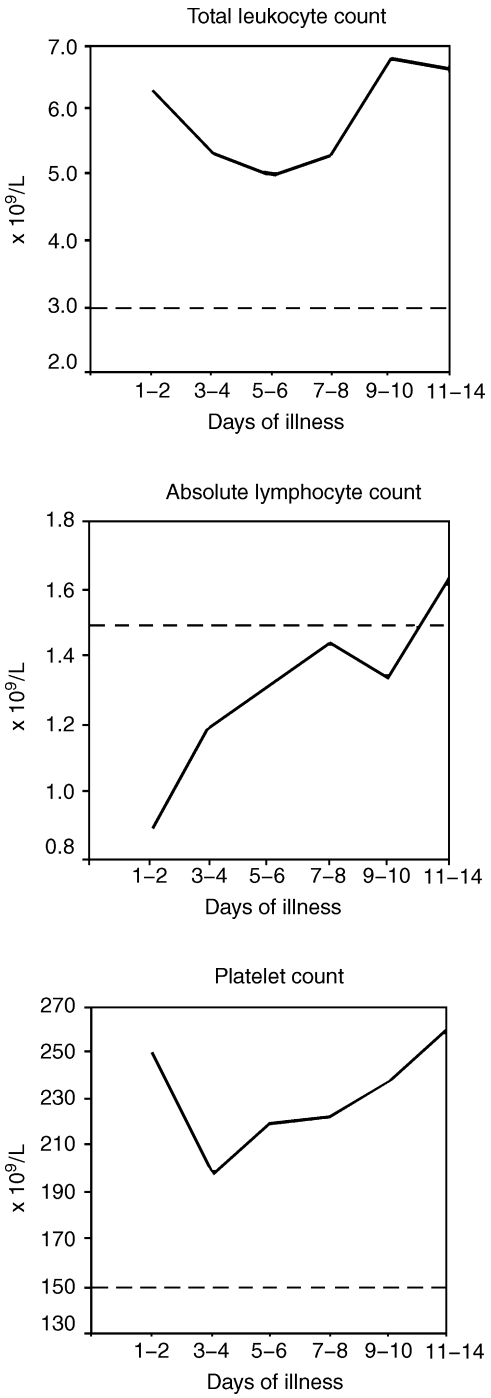

Figure 1 Sequential changes in mean levels of leukocyte count, absolute lymphocyte count and blood platelet count in 108 patients with African tick bite fever. Dotted lines denote lower reference limits.

S-ASAT in 25 of 91 patients (27.5%), elevated S-LDH in 23 of 63 patients (36.5%), elevated S-GGT in 19 of 62 (30.6%) patients, and elevated S-ALP in 10 of 64 (15.6%) patients.

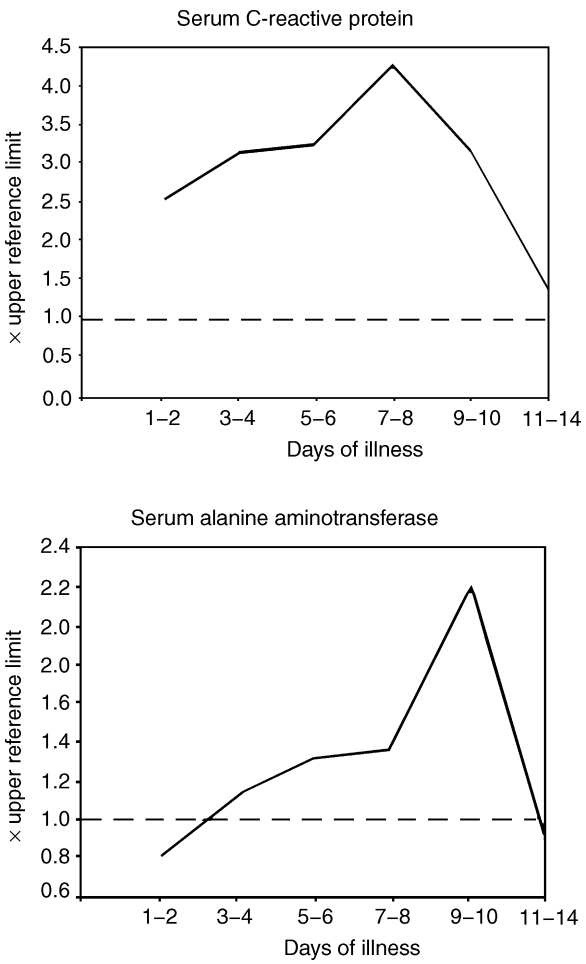

Figure 2 Sequential changes in mean levels of serum C-reactive protein and serum alanine aminotransferase in 108 patients with African tick bite fever. All data are expressed as proportions of upper reference limits (=1.0; denoted by dotted lines).

Serum bilirubin was measured 38 times in 33 patients, serum creatinine 64 times in 55 patients, and S-Na 36 times in 33 patients. No specific patterns were detected for the mean levels of any of these three parameters. Except for two cases with slightly elevated serum creatinine and one case of mild hyponatremia, no abnormal values were documented.

For 55 patients for whom both S-CRP and absolute lymphocyte count were analyzed, at least one parameter was abnormal in 52 (94.5%) patients.

DISCUSSION

This first study of biological variables in ATBF adds new information on an emerging problem in today's travel medicine. Our data show that the

sequential changes in many hematologic and biochemical parameters in ATBF, although less pronounced, mimic those of potentially more severe SFG rickettsioses. For instance, falls in total leukocyte and platelet counts, with nadir levels during the first week of illness, are also common in Mediterranean spotted fever (MSF) caused by *R. conorii* and Rocky Mountain spotted fever (RMSF) caused by *R. rickettsii* [13,14]. In MSF, where 20% of hospitalized patients develop leukopenia [14], the fall in total leukocyte counts has been attributed to increased diapedesis secondary to increased vascular permeability or attachment of polymorphonuclear cells to endothelial cells under the influence of tumor necrosis factor alpha (TNF-α). Similarly, thrombocytopenia, which commonly evolves in hospitalized cases of MSF and RMSF [14,15], is postulated to be caused by adherence to infected endothelial cells [16] or immune-mediated consumption. It is noteworthy that lymphopenia, which was detected in as many as 73% of our patients, has not been systematically reported in other SFG rickettsioses. The cause of lymphopenia in ATBF is unknown, but the phenomenon might be secondary to increased activation of TNF-α during the acute phase (M. Jensenius et al, unpublished data). A depression of $CD4^+$ T-cell subset profile has been described in MSF [17,18], but whether this occurs also in ATBF has yet to be determined.

Elevated serum liver enzymes, which were detected in more than one-third of our patients with ATBF, are common in many SFG rickettsioses [13]. The underlying pathophysiologic liver process is probably non-specific. Hyperbilirubinemia is uncommon, and liver biopsies in cases of MSF usually only reveal scattered foci of hepatocellular necrosis with mononuclear infiltrations, or, occasionally, granulomas without giant cells or epitheloid cells [19]. Furthermore, the elevations of S-ASAT and S-ALAT may also have extrahepatic sources, as both enzymes are secreted in vitro by human endothelial cells infected by *R. conorii* [20].

Other commonly measured biological parameters, such as hemoglobin, S-Na, and serum creatinine, are apparently little affected during ATBF. This is in contrast to MSF and RMSF, where severe cases frequently develop anemia, hyponatremia, and impaired renal function, especially during the later stages of the disease [13–15].

Could routine laboratory tests provide any diagnostic help for the physician considering the possibility of ATBF in a febrile returnee from endemic areas? It is noteworthy that ~95% of 55 eligible cases in the present series had elevated S-CRP or/and lymphopenia during the first ten days of illness, i.e. when most patients are likely to present. Our data thus suggest that normal levels of both S-CRP and absolute lymphocyte count in a symptomatic and recently returned traveler from endemic areas would make ATBF a less plausible diagnostic alternative. On the other hand, since cytopenia and elevated liver function tests frequently occur in malaria and many other tropical fevers [21], the detection of such abnormalities in a febrile returnee is probably of limited differential diagnostic value. However, to fully evaluate the diagnostic usefulness of routine laboratory tests in ATBF, prospective studies are warranted.

In conclusion, during the early stage of ATBF, mild abnormalities in many commonly measured hematologic and biochemical variables are frequent, with increased S-CRP and lymphopenia being the two most consistent findings.

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REFERENCES

1. Kelly PJ, Beati L, Mason PR, Matthewman LA, Roux V, Raoult D. *Rickettsia africae* sp. nov., the etiological agent of African tick bite fever. *Int J Syst Bacteriol* 1996; 46: 611–14.
2. Raoult D, Fournier PE, Fenollar F *et al*. *Rickettsia africae*, a tick-borne pathogen in travelers to Sub-Saharan Africa. *N Engl J Med* 2001; 344: 1504–10.
3. Brouqui P, Harle JR, Delmont J, Frances C, Weiller PJ, Raoult D. African tick-bite fever. An imported spot- less rickettsiosis. *Arch Intern Med* 1997; 157: 119–24.
4. Jensenius M, Hasle G, Henriksen AZ *et al.* African tick-bite fever imported into Norway: presentation of 8 cases. *Scand J Infect Dis* 1999; 31: 131–3.
5. Fournier PE, Roux V, Caumes E, Donzel M, Raoult D. Outbreak of *Rickettsia africae* infections in participants of an adventure race in South Africa. *Clin Infect Dis* 1998; 27: 316–23.

6. Neal S, Cleslak P, Hedberg K, Fleming D. African tick-bite fever among international travelers—Oregon, 1998. *MMWR* 1998; 47: 950–2.
7. Smoak BL, McClain JB, Brundage JF *et al.* An outbreak of spotted fever rickettsiosis in US Army troops deployed to Botswana. *Emerg Infect Dis* 1996; 2: 217–21.
8. Fournier PE, Jensenius M, Laferl H, Vene S, Raoult D. Kinetics of antibody responses in *Rickettsia africae* and *Rickettsia conorii* infections. *Clin Diagn Lab Immunol* 2002; 9: 324–8.
9. Fournier PE, Beytout J, Raoult D. Tick-transmitted infections in Transvaal: consider *Rickettsia africae*. *Emerg Infect Dis* 1999; 5: 178–81.
10. Fournier PE, Roux V, Raoult D. Phylogenetic analysis of spotted fever group rickettsiae by study of the outer surface protein rOmpA. *Int J Syst Bacteriol* 1998; 48: 839–49.
11. Raoult D, Dasch GA. Line blot and western blot immunoassays for the diagnosis of Mediterranean spotted fever. *J Clin Microbiol* 1989; 27: 2073–9.
12. Hechemy KE, Anacker RL, Carlo NL, Fox JA, Gaafar HA. Absorption of *Rickettsia rickettsii* antibodies by *Rickettsia rickettsii* antigens in four diagnostic tests. *J Clin Microbiol* 1983; 17: 445–9.
13. Drancourt M, Raoult D, Harle JR *et al.* Biological variations in 412 patients with Mediterranean spotted fever. *Ann NY Acad Sci* 1990; 590: 39–50.
14. Raoult D, Weiller PJ, Chagnon A, Chaudet H, Gallais H, Casanova P. Mediterranean spotted fever: clinical, laboratory and epidemiological features of 199 cases. *Am J Trop Med* 1986; 35: 845–50.
15. Helmick CG, Bernard KW, D'Angelo LJ. Rocky Mountain spotted fever: clinical, laboratory, and epidemiological features of 262 cases. *J Infect Dis* 1984; 150: 480–8.
16. Silverman DJ. Adherence of platelets to human endothelial cells infected by Rickettsia rickettsiae. *J Infect Dis* 1986; 153: 694–700.
17. Cillari E, Milano S, D'Agostino P *et al.* Depression of CD4 T cell subsets and alteration in cytokine profile in boutonneuse fever. *J Infect Dis* 1996; 174: 1051–7.
18. Feng H, Popov VL, Yuoh G, Walker DH. Role of T lymphocyte subsets in immunity to spotted fever group Rickettsiae. *J Immunol* 1997; 158: 5314–20.
19. Walker DH, Staiti A, Mansueto S, Tringali G. Frequent occurrence of hepatic lesions in boutonneuse fever. *Acta Tropica* 1986; 43: 175–81.
20. Raoult D, Arnoux D, Drancourt M, Ardissone JP. Enzyme secretion by human endothelial cells infected by Rickettsia conorii. *Acta Virol* 1987; 31: 352–6.
21. Humar A, Keystone J. Evaluating fever in travellers returning from tropical countries. *BMJ* 1996; 312: 953–6.