Quantitative bacteriology of Typhoid fever in Vietnam

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Abstrak

Terdapat kekurangan informasi mengenai jumlah bakteria di dalam darah dan sumsum tulang pada pasien demam tifoid dan hubungan antara jumlah bakteria dan gambaran klinis. Telah dilakukan penghitungan jumlah bakteria di dalam darah, buffy coat, buffy coat *yang dilisiskan, aspirat sumsum tulang dari pasien demam enterik dengan kultur positif menggunakan metode plat tuang. Median jumlah bakteri di dalam darah dari 375 pasien adalah 1 cfu/ml (kisaran IQ <0,3-5). Pada 29% pasien, terdapat 1 cfu/5 ml darah, maka diperlukan 13 ml darah untuk 95% kultur positif rate pada infeksi* S. typhi. *Pada 187/375, 100* µl *lapisan* buffy coat *dipisahkan dari 1 ml darah. Sebanyak 37,5% (95% CI 32,9-42,1) dari total bakteria terkonsentrasi pada lapisan ini. Pada 81/375 pasien, median jumlah bakteria per sel terinfeksi yang terdapat pada lapisan* buffy coat *yang dilisiskan dengan digitonin adalah 1,3 cfu per sel. Di sumsum tulang 78 pasien terdapat rata-rata 4 kali lebih banyak bakteria daripada di dalam darah pada pasien yang sama pada volume yang sama. Terdapat korelasi negatif yang bermakna (p<0,05) antara jumlah bakteria dan umur, dan lamanya demam sebelum masuk rumah sakit. Terdapat korelasi positif yang bermakna (p=0,05) antara jumlah bakteria dan suhu, waktu demam turun setelah pengobatan, dan resistensi multipel dari setiap isolat bakteria, walaupun terdapat riwayat pengobatan yang sama sebelumnya. Sebagai ringkasan, apabila dilakukan kultur darah untuk menegakkan diagnosis, maka paling sedikit dibutuhkan 13 ml darah. Kuman* S. typhi *terkonsentrasi di dalam lapisan* buffycoat *sehingga lapisan ini merupakan spesimen yang baik untuk isolasi* S. typhi. *Sumsum tulang merupakan spesimen terbaik untuk kultur* S. typhi. S. typhi *yang multi resisten berada dalam jumlah lebih banyak di dalam darah daripada strain yang masih sensitif. Terdapat korelasi langsung antara jumlah bakteria dan perubahan klinis saat pengobatan.*

Abstract

There is paucity of information on the number of bacteria in the blood and bone marrow of typhoid patients, and the relationship between bacterial load and clinical outcome. We have counted the bacteria in whole blood, buffy coats, lysed buffy coats, and bone marrow aspirates from culture positive enteric fever patients using the pour plate method. The median number of bacteria in whole blood from 375 patients was 1cfu/ml (IQ range<0.3-5). In 29% of patients there was 1cfu/5mls blood and so 13mls of blood is required for a 95% culture positive rate in S. typhi *infections. In 187/375, 100* µl *comprising the buffy coat layer, was separated from 1ml of whole blood. 37.5% (95% CI 32.9-42.1) of the total bacteria were concentrated in this layer. In 81/375 patients for the median numbers of bacteria per infected cell were found, by digitonin lysis of buffy coats, to be 1.3 cfu per cell. In the bone marrow of 78 patients there were an averange of four times as many bacteria as from the blood of the same patients for equal volumes. There was a negative, significant correlation (p<0.005) between counts and age, and duration of fever preceeding hospital admission. There was a positive significant correlation (p=0.05) between counts and temperature, fever clearance time after treatment, and multi-drug resistance of the bacterial isolate, despite similar pretreatment histories. In summary if blood culture is used to establish a diagnosis at least 13mls must be cultured. The* S. typhi *is concentrated in the buffy coat layer which provides a reliable specimen for the isolation of* S. typhi. *Bone marrow is the best specimen from which to reliably culture of* S. typhi. *Multidrug resistant* S. typhi *is present in higher numbers in the blood than is found with fully sensitive strains. There is a direct correlation between bacterial load and clinical response to treatment.*

INTRODUCTION

Typhoid fever is characterised by bacteraemia with *Salmonella typhi* a highly evolved parasite bacterium that infects only man. Although blood culture is the mainstay of laboratory diagnosis, there is little information on the numbers of bacteria circulating in the blood or their distribution between plasma and phagocytic cells. Previous studies have shown that *S. typhi* is present only in the white cell and platelet fraction of the blood[1]. Using standard broth cultures *S. typhi* is found in the blood or bone marrow of 30-90% of clinically suspected cases of enteric fever. The propotion of positive cultures depends on the specimen collected and duration of preceding illness [2], and the laboratory methods used[3]. Although a reduction in isolation rate, with increasing duration of fever, is described[3-5] and is attributed to a reduction

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in the number of bacteria in the blood, this has not been quantitated. We have performed quantitative bacteriological cultures on the blood from more than 400 and the bone marrow from 122 patients with clinically diagnosed uncomplicated typhoid fever to determine the clinical and diagnostic relevance of bacterial counts.

MATERIALS AND METHODS

Patients were recruited at the Center for Tropical Diseases, Ho Chi Minh City and the Friendship Provincial Hospital, Dong Thap Province, Vietnam. Those diagnosed on admission as having enteric fever, were investigated by quantitative blood culture. The criteria for a clinical diagnosis of typhoid fever were usually fever for ≥ 7 days, no obvious focus of infection, negative malaria blood smear, abdominal discomfort with change of bowel habit, and in some cases characteristic changes in affect. All patients were treated with ofloxacin (Oflocet Rousel, Paris, France). Fever clearance times were calculated as the time from the beginning of treatment until the time at which the fever became 37.5°C, or less and then remained so for 48 hrs.

Brain heart infusion broth (Oxoid, UK) containing 0.05% sodium polyanethosulphonate (Sigma, UK) (sps) was used for broth culture with a minimum specimen to broth ratio of 1 to 5. After the diagnosis of typhoid had been made either clinically or when laboratory results were available, specimens for quantitative culture were collected into a sterile heparinised tube and transported immediately to the laboratory. Quantitative cultures were performed using a pour plate method, three 1 ml aliquots of blood were each mixed with 19 mls of molten (50°C) Columbia agar (Unipath, Basingstoke, UK) containing sps in a sterile petri dish. After allowing the agar to set, the plates were incubated at 37°C. After 4 days colonies were counted and recorded as colony forming units (cfu) per ml. Identification of *S. typhi* was carried out by standard biochemicals tests and agglutination with specific antisera (Wellcome diagnostics UK). For the buffy coat counts heparinised whole blood (2.5 or 5 mls) was centrifuged at 3,000 rpm for 10 minutes. The plasma was carefully removed using a sterile plastic pipette and the buffy coat layer (0.1 ml) was collected using a sterile 1 ml syringe. In order to lyse the phagocytic cells equal volumes of 0.1% digitonin (Sigma, UK) and buffy coat or bone marrow were mixed and incubated for 10 minutes at 37°C.

Extra cellular bacterial counts (Ex-cell) were calculated by subtracting the buffy coat cfu/ml from the whole blood cfu/ml. The average number of viable bacteria per infected cell (cfu/ml) was estimated by dividing the cfu/ml from the lysed buffy coat culture, by the number of cfu/ml in the buffy coat. Mann-Whitney U tests were used to compare fever duration periods between groups. Spearman's rank correlation coefficients were calculted to measure associations between clinical and bacterial counts. Confounding factors, were controlled for during investigation of the relationship between QBC and clinical factors using partial correlation.

RESULTS AND DISCUSSION

All patients were considered clinically to have mild or moderate typhoid fever. There were no complications and no manifestations of severe typhoid. All patients made an uncomplicated recovery. Quantitative culture was performed on blood taken from 426 patients with clinically suspected enteric fever of which 383 were subsequently broth culture positive (89.9%). In four cases pour-plates were positive while broth culture was negative. This allowed estimation of bacterial numbers in the blood from 387 patients (Figure 1). For a subgroup of 187 patients the number of bacteria in the buffy coat layer was shown to have a mean of 37.5% and from 81 patients

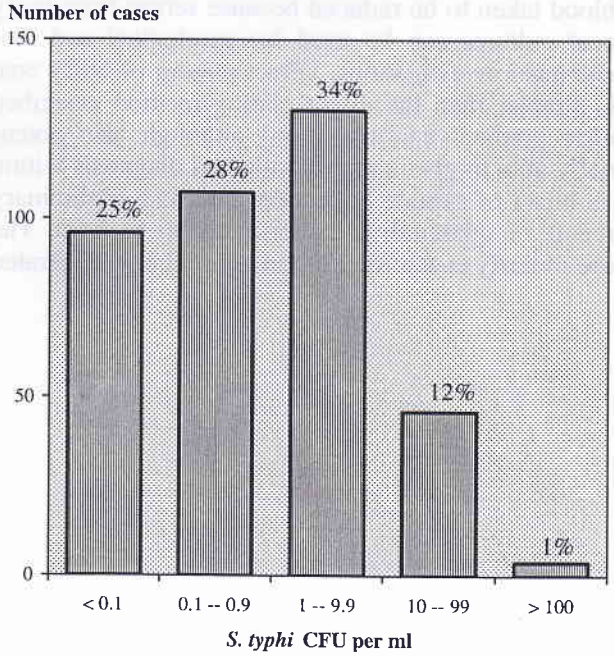

Figure 1. Quantitative blood culture from typhoid patients N = 376

the median number of bacteria per cell was shown to be 1.3. Quantitative culture was performed on bone marrow aspirates from 84 patients. The median count was 8 cfu/ml. The same group of patients had a median of 1 cfu/ml of blood. There was a negative significant correlation between duration of illness and QBC (Table 1) and between age and QBC and a positive correlation between multi-drug resistance and QBC ($p<0.05$) suggesting a connection between drug resistance and virulence. There was a positive significant correlation between QBC and fever clearence time ($p<0.005$).

Table 1: Quantitative blood culture (qbc) and broth culture by week of illness

Days of fever	0-7 days (n=121)	8-14 days (n=181)	15-21 days (n=55)	>21 days (n=17)
Whole blood qbc: cfu/ml (median IQ range)	1.7 (0.3-9.5)	1 (<0.2-3.5)	1 (<0.2-3)	0.3 (<0.2-3.2)
% culture positives by broth culture	89 (n=154)	89 (n=195)	93 (n=60)	94 (n=17)

Buffy coat cultures were positive by direct culture in 190/191 (99.5%) cases positive by broth blood culture. Buffy coat culture is therefore valuable for the isolation of *S. typhi* and allows the total volume of blood taken to be reduced because serum from buffy coat cultures can be used for serological and biochemical investigations. Direct plating of buffy coat is simpler than the direct plating method described after gradient centrifugation[1] although also potentially able to give a microbiological diagnosis within 18 hours of sample collection. This is a preliminary report of quantitative bacteriology in typhoid. The use of buffy coat allows organisms to be concentrated from larger volumes of blood and removes the need for expensive culture broth. Buffy coat methods are easy to perform, cheaper, and more rapid than ox bile broth or standard blood culture followed by plating on solid media.

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