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DOXYCYLINE RESPONSE IN ACUTE FEBRILE ILLNESS AT UMPHANG HOSPITAL: A RETROSPECTIVE STUDY

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ABSTRACT

In southeast Asia doxycycline treatable infections represent up to 55% of non-malarial causes of fever but differential diagnosis is challenging so fever clearance is used as a proxy for diagnosis1. Our aim was to identify factors associated with time of fever clearance after the start doxycycline. Febrile in-patients who received doxycycline and had confirmed scrub typhus (CST) or a suspected rickettsial disease (SRD) were included retrospectively. Fever clearance time (FCT) was classified as failure (FCT>5 days) or cure (FCT ≤ 5 days), further divided into fast (FCT≤ 48 hours) and slow response (48 hours> FCT<5 days). Factors associated with fever response were investigated. 365 subjects, 134 in the CST and 231 in the SRD group were retrospectively included between 8/2015 and 9/2018 and analysed. Clinical failures had higher lymphocyte count and lower AST levels. Subjects with a slow response were found to have higher creatinine levels compared to fast responders. A similar trend was noted with transaminases but did not reach statistical significance.

INTRODUCTION

In rural areas of south and Southeast Asia doxycycline treatable infections represent up to 55 % of non-malarial causes of fever, the most recognised being leptospirosis, scrub typhus and murine typhus (Suttinont, *et al,* 2006; Thumnu, *et al,* 2017).

In addition, some seroprevalence and "cause of fever" studies suggest that diseases such as spotted-fever group rickettsioses, bartonellosis, ehrlichiosis and Q-fever may play a relevant role as well (Blacksell, *et al,* 2015; Kosoy, *et al,* 2010; Ellis, *et al,* 2006).

Because most of the above-mentioned diseases do not respond to typical empiric antibiotic regimens, which usually consist of beta-lactam drugs, it is extremely important to recognise them early in their course so to start adequate treatment and avoid serious complications. Unfortunately, clinical and laboratory features are often non-specific and rapid and reliable microbiological tests often inconvenient and not widely available(Saraswati, *et al,* 2018; Paris and Dumler 2016; Eldin, *et al,* 2017).

Consequently, fever response to antibiotic therapy is often used as a proxy for the diagnosis of rickettsial diseases in settings where reliable tests are not available. For scrub typhus, a fever clearance time of more than 48 hours after targeted antibiotic therapy is considered atypical and certain authors recommend seeking an alternative

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aetiology in such cases (WHO 1999; Brown, *et al,* 1984). Clinical trials with strict diagnostic criteria for rickettsial diseases have nonetheless shown that fever persistence beyond 48 hours is not uncommon, spanning between 10 and 54% for scrub typhus and being as high as 68% in a Mediterranean spotted fever case series in Greece (Gikas, *et al,* 2004), as summarised in table 1.

It is therefore essential that factors influencing fever clearance time (FCT) be sought to prevent clinicians from erroneously dismissing the diagnosis of a rickettsial disease. For scrub typhus, some studies have analysed factors associated with severe disease (which is associated with longer fever clearance time) and clinical failure (which is defined as fever clearance time longer than a particular threshold, usually 5 days) but, to our best knowledge (Sivarajan, *et al,* 2016; Kim, *et al,* 2019), none have directly explored which factors correlate with fever clearance time (Kim, Kim, Choi and Wie 2019; Wangrangsimakul, *et al,* 2020).

The aim of the present study was to identify factors positively or negatively associated with fever clearance time after the start doxycycline therapy, in patients with confirmed scrub typhus or the clinical suspicion of a rickettsial diseases.

METHODS

Ethical aspects

The study was approved by the ethics committee of the Faculty of Tropical Medicine of Mahidol University, Bangkok. Approval number: MUTM 2019-020-01.

Setting

This study was conducted at Umphang hospital in north-western Thailand on the border with Myanmar.

Inpatients of Umphang hospital, irrespective of age, who had received doxycycline and had fever (defined as a recorded temperature > 37.4°C or a history of fever) between 8/2015 and 09/2018 were screened retrospectively for inclusion in a doxycycline fever response database.

Patients with incomplete documentation regarding duration of fever or symptoms or for whom the time of doxycycline start was not documented were excluded from the database. Patients with an obvious primary focus of infection at admission (e.g., pneumonia, urinary tract infection or skin or soft tissue infection) or malaria were also excluded, if these diagnoses became apparent after doxycycline start, the patients were not excluded.

For the present study a subgroup of subjects was included. The subgroup consisted of:

1) Patients with documented fever (temperature ≥ 37.5°C) or reporting fever as a current symptom, AND a confirmed scrub typhus (CST), defined as a positive scrub typhus RDT (SD BIOLINE™ Tsutsugamushi Assay, Standard Diagnostics, Inc., Yongin, South Korea) OR eschar.
2) Those with a suspected rickettsial disease (SRD), defined as documented fever or fever history not matching the criteria for CST, not having another confirmed cause of fever or clear infection focus at the time of doxycycline start and having at least one of the following symptoms or signs: headache, rash, lymphadenopathy, cough, conjunctival injection; as adapted from the WHO surveillance standards(WHO 1999).

Patients with both CST and another cause for fever (e.g. CST and dengue) were excluded. CST and SRD patients were grouped and analysed together.

Clinical, anamnestic and laboratory values at presentation were recorded. If present, or if they developed during hospital stay, complications (sepsis, acute kidney injury, respiratory insufficiency/pneumonia, central nervous system complications or transaminitis/liver failure) were recorded.

Outcome measures:

The main outcome measure was fever clearance time (FCT), defined as the time between the first dose of doxycycline and the first time a body temperature below 37.5°C and remained below

37.5°C for at least 24 hours or until discharge. Subjects whose FCT was longer than 5 days were classified as clinical failures, those with a FCT of less than 5 days were classified as clinical cures and further divided into fast responders (FCT<=48 hours) and slow responders (48 hours> FCT<5 days).

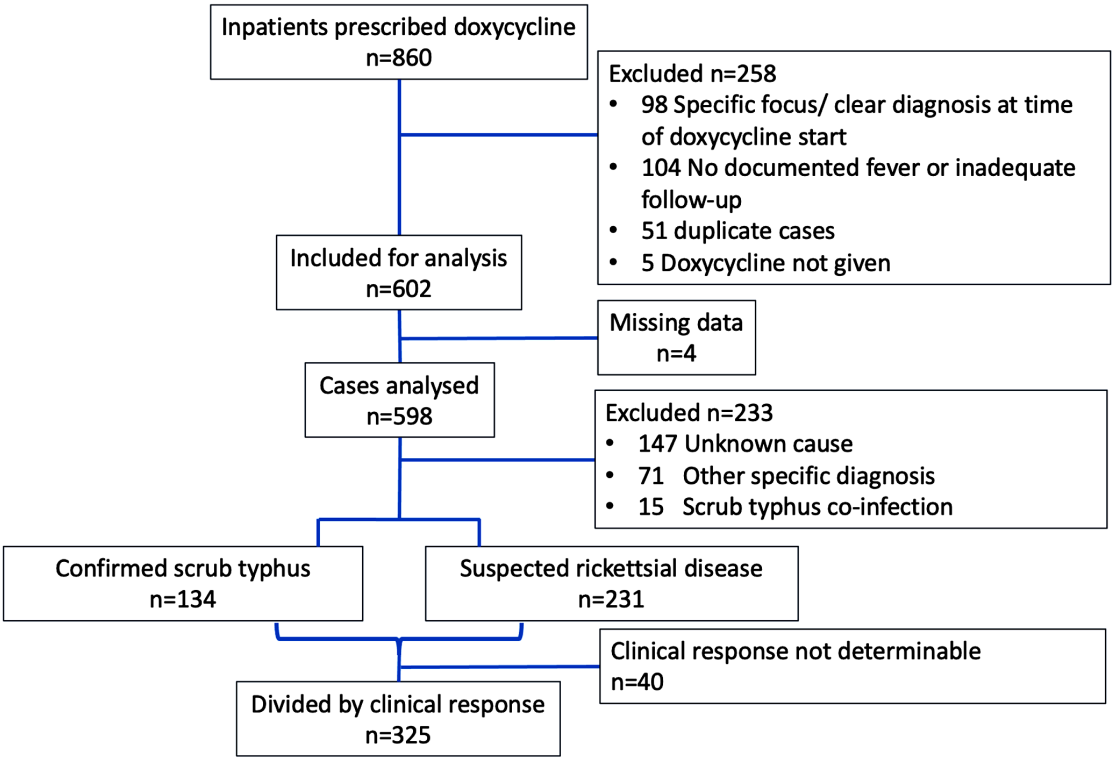
Statistical analyses were conducted using STATA® version 15 or older.

To compare continuous variables, including FCT, between only two groups (fast versus slow responders or clinical cure versus clinical failure) medians were compared using Wilcoxon signed-rank test (Mann-Whitney U test). For comparisons among more than two groups Kruskall-Wallis test was used, followed by Wilcoxon signed-rank test with Bonferroni correction for post-hoc analysis. Factors with a p-value of 0.1 or less on univariate analysis were included in a multivariate logistic regression analysis model.

Clinical signs and symptoms that were not documented were analysed as absent in statistical analysis. Local reference ranges for biochemical values were used with the exception of haematological values for children where UK NHS reference ranges were used instead, as no local reference ranges for children were available.

RESULTS

860 IPD patients had received doxycycline between August 2015 and September 2018, 258 did not fit the inclusion/exclusion criteria and an additional 4 were excluded during analysis because of undeterminable FCT. 598 patients were included in the doxycycline fever database, 365 of which had a study diagnosis of either CST or SRD and were included, in 40 of these fever clearance time was not determinable so they were excluded.



Clinical response to doxycycline

Fig 1 Study flow chart.

All patients

When all patients (CST and SRD) were grouped together, white blood cell counts, neutrophil and lymphocyte differential percentages, lymphocyte count, transaminases and creatinine levels were found to differ among fast responders, slow responders and clinical failures as shown in Table 1. None of the clinical symptoms and signs (including rash and eschar) were found to be more common or less common in any of the three groups at a statistically significant level except for headache.

Table 1 Haematologic and biochemical values in fast responders, slow responders and clinical failures.

	Clinical cure		Clinical Failure	*p* value
	Fast	Slow		
Hematocrit, %, median (IQR)	38 (33-42)	37 (34-42.5)	36 (30-39)	0.265
	n=233	n=76	n=15	
Platelet count, *10^3/µl, median (IQR)	156.5 (111-223)	142 (85-194.5)	168 (127-191)	0.122
	n=234	n=76	n=15	
WBC count, *10^3/µl, median (IQR)	7.9 (5.2-12.4)	7.4 (5.4-10.6)	10.2 (7.8-13.2)	**0.049**
	n=234	n=76	n=15	
Neutrophil percentage, median (IQR)	71 (61.4-81.6)	75 (67-82)	81 (73-84)	**0.043**
	n=234	n=76	n=15	
Lymphocyte percentage, median (IQR)	22 (12.8-32)	17 (11-25)	16 (10-23)	**0.014**
	n=233	n=76	n=15	
Neutrophil count, *10^3/µl, median (IQR)	5.67 (3.4-8.9)	5.32 (4.0-7.2)	7.59 (4.7-10.8)	0.079
	n=234	n=76	n=15	
Lymphocyte count, *10^3/µl, median (IQR)	1.4 (0.9-2.4)	1.06 (0.6-1.8)	1.53 (1.1-2.1)	**0.025**
	n=233	n=76	n=15	
BUN, median (IQR)	13 (10-17)	15 (12-20)	14 (12-16)	0.262
	n=162	n=61	n=13	
Creatinine, mg/dl, median (IQR)	0.80 (0.61-1.01)	0.91 (0.74-1.08)	0.73 (0.57-0.84)	**0.026**
	n=163	n=62	n=13	
AST, IU/L, median (IQR)	87 (40-197)	147 (87-243)	35 (33-87)	**0.013**
	n=74	n=19	n=8	
ALT, IU/L, median (IQR)	68 (34-117)	120 (60-166)	31 (23-92)	**0.021**
	n=74	n=19	n=8	
Platelet count <100* 103/µl	40/234 (17.1%)	24/76 (31.6%)	2/15 (13%)	**0.021**

Since this might be more practical for clinical use, the proportion of subjects with pathological values (above or below normal ranges) was also compared among the three response groups, local thresholds adjusted for age/and sex as appropriate were used. Some thresholds, values and variables that have been found to be associated with severe disease, clinical failure or increased mortality in the literature were also included (thrombocytes <100'000/µl (Varghese 2006), Transaminases>5x upper limit (Wangrangsimakul), age>60(Kim 2010).

Thrombocytes below 100'000/µl), low lymphocyte count and elevated transaminases were found to differ at a statistically significant level among the three groups.

While thrombocytopenia as defined by local cut-off (150'000/µl) did not differ significantly among the groups, when a cut-off of 100'000 cells/µl was used, there was a statistically significant difference, such levels have been found to be associated with scrub typhus mortality in the literature(Varghese, *et al,* 2006).

When fast and slow responders were grouped together as "clinical cure" and compared to clinical failures, median WBC count, neutrophil count and AST were found to differ at a statistically significant level, as was the proportion of subjects with elevated transaminases and of those with cough. Due to the non-linear distribution of most variables when all three categories were compared, multivariate logistic regression was only performed comparing the clinical cure and clinical failure categories, as summarised in table 2. Only elevated transaminases were found to differ, with higher values being more common in clinical response.

In multivariate logistic regression analysis, the proportion of subjects with elevated ALT levels was found to be higher in clinical cures at a statistically significant level.

Suspected rickettsial disease compared to confirmed scrub typhus

Overall 134 subjects were included in the CST group and 231 in the SRD group. Of those in the SRD group, 116 had tested negative for scrub typhus IgM and 115 had not been tested.

Median fever clearance time was 34.25 hours (IQR 17.75-53.5) in the CST group and 31 hours (12.25-53.5) in the SRD group, this difference was not statistically significant (p>z=0.076). The difference in the proportion of fast, slow responders and clinical failures in the CST groups was also not statistically significant (p=0.141). Demographic factors did not differ significantly in the two groups nor did most laboratory variables.

Relevant differences and demographic factors are summarised in tables 3 and 4.

Importantly, the proportion of subjects with elevated transaminases was higher in CST subjects. Median symptom duration was also one day longer in CST patients compared to SRD, at a statistically significant level.

Table 2 Variables compared between clinical cure and clinical failure.

	Clinical response	**Clinical failure**	***p*-value UV**	***p*-value MV**
ALT, >50 IU/L, *n* (%)	69/93 (74)	2/8 (25)	**0.008**	**0.011**
AST >50 IU/L, *n* (%)	72/93 (77)	3/8 (38)	**0.025**	NA
Reduced lymphocyte count (age specific)	123/310 (40)	2/15 (13)	**0.041**	0.279
Cough (symptom)	100/310 (32)	9/15 (60)	**0.026**	0.472

UV=univariate analysis, MV=multivariate logistic regression

Table 3 Clinical response in confirmed scrub typhus and suspected rickettsial disease subjects.

	Fast response	Slow response	Clinical failure
Confirmed scrub typhus (*N*=121)	84 (69.4%)	34 (28.1%)	3 (2.5%)
Suspected rickettsial disease (*N*=204)	150 (73.5%)	42 (20.6%)	12 (5.9%)

Table 4 Selected variables in confirmed scrub typhus and suspected rickettsial disease subjects compared.

	Confirmed scrub typhus (*n*=134)	Suspected rickettsial disease (*n*=231)	*p*-value1
Female, n (%)	52 (38.8%)	96 (41.6%)	0.61
Age Median (IQR)	24 (10.25-44)	31 (18-44.5)	0.0442
Thai ethnicity, *n* (%)	66 (49.3%)	111 (48.1%)	0.83
Farmer, n (%)	37 (27.6%)	67 (29.0%)	0.78
FCT, days Median (IQR)	1.31 (0.60-2.21)	1 (0.38-2.08)	0.1062
ALT, >50 IU/L, *n* (%)	41 (89.1%)	37 (55.22%)	<0.001
AST, >50 IU/L, *n* (%)	40 (87.0%)	42 (62.7%)	<0.001
Symptoms before doxycycline start (days) median (IQR)	4 (3-7)	3 (2-5)	0.006

[1]Chi-squared test, [2]Kruskall-Wallis test.

DISCUSSION

The variables that most consistently differed among clinical response categories (fast response, slow response and clinical failure) were transaminases and haematological values, with the proportion of lymphocyte and neutrophils probably being the most useful. Neutrophils and WBC were highest among clinical failures but post-hoc analysis with Bonferroni correction couldn't show a statistically significant difference among WBC in any particular pair of groups. The data seems to point out that those with a more "septic" response with higher WBC counts and left shift were more likely not to respond to doxycycline therapy (and probably have a non-rickettsial cause for their febrile illness).

Median transaminase levels were found to be slightly elevated (AST 87, IQR 40-197, and ALT 67.5 IU/L, IQR 34-117), moderately elevated (AST 147, IQR 87-243; ALT 120, IQR 60-166) and normal (AST 35, IQR 33-87; ALT 31, IQR 22.5-92) in fast responders, slow responders and clinical failures, respectively. Similarly, the proportion of subjects with elevated transaminases was highest in slow responders and lowest in clinical failures, both fast and slow responders differing at a statistically significant level from clinical failures when ALT was considered. This could point to subjects having scrub typhus or

other rickettsial diseases showing transaminase levels proportional to the severity of their disease and therefore proportional to their recovery speed while those with normal transaminases and fever in the study area are less likely to have a disease that is treatable with doxycycline. Other values (e.g. serum creatinine and platelet counts) that are indicative of systemic involvement and severity in rickettsial diseases seemed to have similar distributions in the three categories, with levels more overtly pathological in slow responders than in fast responders and the least pathological or normal in clinical failures. However, these other differences were not found to be statistically significant.

Importantly 80% (12/15) of subjects with clinical failures had received antibiotics in addition to doxycycline, for 8/12 (75%) at least one other antibiotic was given 48 hours or less before doxycycline, in 1/12 less than 48h after doxycycline, in 2/12 more than 48h after and in the remaining subject more than 48h after. This compares to 41% of fast responders and 36% of slow responders who received additional antibiotics, this difference was statistically significant (p=0.009). The timepoint relative to doxycycline among the ones receiving other antibiotics did not differ significantly among the three response groups.

Although most variables were similar, CST patients had higher transaminase levels and longer symptoms before doxycycline start, which could mean that they were clinically more severe, but this is only speculative. Clinical response did not markedly differ between the two categories.

LIMITATIONS

The study was retrospective in design and consequently prone to selection bias, it is possible that subjects with a higher a-priori chance of having a clinical response were preferentially given doxycycline. In particular, the response observed in the SRD category might be faster than that of a real-life cohort of unselected patients. Additionally, which tests a particular patient received was at the discretion of the treating physician. Relevant values that were not performed consistently include transaminases and creatinine that were reported only for

31% and 73% of subjects, respectively. Outpatients were also not included, so subjects with milder disease were probably underrepresented.

Scrub typhus diagnosis in this study relied on a scrub typhus RDT, which has been shown to have sensitivity and specificity issues(Saraswati, Day, Mukaka and Blacksell 2018). One study conducted on children showed a positive predictive value of only 58%(Wangrangsimakul, Greer, Chanta, Nedsuwan, Blacksell, Day and Paris 2020).

Coadministration of antibiotics other than doxycycline is also a limitation, with 42% of patients receiving one or more. Importantly, the proportion of subjects receiving other antibiotics did not differ significantly between subjects with fast or slow response rates.

CONCLUSION

The results are compatible with the hypothesis that subjects with more severe forms of scrub typhus or other rickettsial diseases may have fever clearance times above 48 h and that lower levels of biochemical markers, in particular transaminases and creatinine, are more commonly found in febrile patients that do not respond to doxycycline in settings with high scrub typhus endemicity.

Median leukocyte counts were also found to be significantly higher in subjects who did not respond to doxycycline therapy.

Prospective trials are warranted to verify these findings.

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Operational diagnoses definitions:

Dengue: Positive Dengue IgM or NS1Ag. If NS1Ag and Dengue IgM were not tested, two or more of: headache, retroorbital pain, rash, haemorrhagic manifestation, leucopenia, plus thrombocytes <100 or Hct increase >20% or bleeding.

Leptospirosis: Positive serology (single or paired sera)

Melioidosis: Positive blood culture or serology (single or paired sera)

Operational definitions, complications:

Sepsis: Clinical diagnosis by attending physician plus two or more SIRS criteria (Temperature higher than 38°C or lower than 36°C, RR>20, WBC>12000/mm^3 or <4000/mm^3.

AKI: Increase of creatinine of at least 1.5 times baseline value (at discharge or admission)

Respiratory insufficiency: Oxygen saturation <90% in room air and/or oxygen supplementation and/or mechanical ventilation, as noted on the patient chart.

Central nervous system complications (coma, encephalitis): As noted in the patient chart or increased CSF cellularity.

Liver failure: Increased transaminases associated with coagulopathy (clinical bleeding or INR>1.5 with no other explanation) and alteration of consciousness.

Hepatitis/transaminitis: Increase in transaminases without alteration of consciousness, with or without coagulopathy.