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Rickettsial Infections among the Undifferentiated Febrile Patients Attending a Tertiary Care Teaching Hospital of Northern India: A Longitudinal Study

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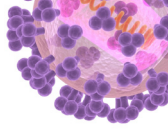[5]Independent Public Health Researcher, Dehradun, India
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Conflict of Interest
No conflicts of interest.

ABSTRACT

Background: Acute undifferentiated febrile illness (AUFI) is one of the most daunting challenges a physician faces in such settings. Among AUFI, rickettsial infections are most common and related infections (such as anaplasmosis, ehrlichiosis, and Q fever) which are caused by an unusual type of bacteria that can live only inside the cells of another organism. The present study was therefore planned with an objective to estimate the prevalence of rickettsial infection among patients of undifferentiated fever and to determine any association of socio-demographic characteristics with rickettsial disease.

Materials and Methods: Patients presenting with febrile illness and admitted or attending out-patient department of Sher-i-Kashmir Institute of Medical Sciences, Srinagar was approached and recruited in the study. Weil Felix Assay, enzyme-linked immunosorbent assay and indirect immunofluorescence assay were done to detect the anti-rickettsial antibodies. Serological evidence of a fourfold increase in IgG-specific antibody titer reactive with spotted fever group rickettsial antigen by indirect immunofluorescence antibody assays between paired serum specimens was considered a confirmatory diagnosis for the rickettsial disease.

Results: Most of the patients were males 61.6%, and most 46.2% were in the age group of 20 -39 years. Most of the patients, 80.8% belonged to rural areas, and 48% belonged to the upper middle (II) class of the socio-economic class according to modified Kuppuswamy scale. Of the studied participants, a majority, 47.0%, were determined undiagnosed, while 15.4% studied participants were diagnosed to have a rickettsial disease. In patients positive for typhus group, 67.8% were IgM positive, 28.5% were IgG positive, and only 3% were positive for IgM and IgG. In patients positive for Scrub Typhus Group, 32.7% were positive for IgM, and 62.0% were positive for IgG, and only 5.0% were positive for both IgM and IgG. In patients positive for spotted fever group, 36.1% were positive for IgM, and 58.5% were positive for IgG, and only 5.5% were positive for both IgM and IgG. The prevalence of rickettsial disease was found to be 11.3%.

Conclusion: Rickettsial diseases, typhoid and brucellosis, were the most prevalent diseased diagnosed among patients reporting to hospitals with undifferentiated febrile illness. Clinicians must consider rickettsial diseases as one of the differential diagnosis while treating patients with fever.

Keywords: Hyperthermia; Rickettsial infections; Spotted Fever Group Rickettsiosis; Typhus; Epidemic Louse-Borne

INTRODUCTION

In a country like India, infectious diseases are still considered the foremost reasons for disability and death in a tropical climate. Moreover, acute undifferentiated febrile illness AUFI is one of the most daunting challenges a physician faces in such settings. The term AUFI connotes fever of <14 days duration without any evidence of organ or system-specific aetiology [1]. In settings with limited resources, fever is usually self-treated or treated using empirical therapy due to the dearth of public health care facilities or limited access to diagnostic tests. In such circumstances, understanding the prevalence of local infections is a must to target the disease, carry out necessary clinical workup and follow-up treatment plans [2].

Several viruses, bacteria, protozoa, and rickettsia can cause AUFI, among which rickettsioses have emerged as significant culprits in recent times [3]. Clinical presentations of rickettsia infections vary with the causal agent and diseased patient; however, common symptoms that characteristically develop within 1 - 2 weeks of infection include raised body temperature, pain in the head, malaise, rash, nausea, and vomiting [4]. Many rickettsioses go together with a maculopapular, vesicular, or petechial rash or sometimes an eschar at the site of the tick bite. Serological tests remain an important tool in diagnosis, Weil-Felix test aids as a convenient and economic screening and diagnostic test for laboratory diagnosis of rickettsial disease [5]. Enzyme-linked immunosorbent assay (ELISA) is highly suitable as an alternative sensitive serological test being more flexible in the types of antigens that can be used than other techniques like immunofluorescence assay (IFA) or indirect hemagglutination antibody (IHA) technique.

Many states like West Bengal, Tamil Nadu, Himachal Pradesh, Rajasthan, Jammu and Kashmir, and Uttar Pradesh have reported Rickettsial disease cases. The reported numbers are an underestimate due to a lack of surveillance data [6-9]. Limited studies have been done regarding the rickettsial diseases in India, and as far as the state of Jammu and Kashmir is concerned, only a few case reports are available. Therefore, the present study was planned to estimate the prevalence of rickettsial infection among patients of AUFI who reported to a tertiary care medical centre for treatment and to determine any association of socio-demographic characteristics like gender, age, place of residence and socio-economic status with the rickettsial disease among the studied sample. Such surveillance data would help promote awareness of rickettsioses amongst the medical fraternity and upsurge the likelihood that individual patients with rickettsioses would be identified promptly and receive appropriate therapy early in their illness.

MATERIAL AND METHODS

This longitudinal study was conducted in the Post Graduate Department of Microbiology in collaboration with the Post Graduate Department of Medicine and Department of Pediatrics Sher-i-Kashmir Institute of Medical Sciences, Srinagar. The study was conducted from June 2016 to December 2017. Patients presenting with febrile illness and admitted or attending the outpatient department (OPD) of Sher-i-Kashmir Institute of Medical Sciences, Srinagar was approached and recruited in the study after taking proper consent/assent whichever was applicable.

Inclusion criteria: All patients presenting with undifferentiated fever of >38°C of fewer than 2 weeks duration history without any specific localizing signs or symptoms were enrolled for the study during the study period. *Exclusion criteria:* Those patients who developed a new-onset fever after 72 hrs. of hospital admission were not included. Additionally, we excluded febrile patients with confirmed pneumonia, skin or soft tissue infections, urinary tract infections, or any laboratory-confirmed cases of an infection other than the pathogen of interest. The patients were followed up till discharge from the hospital following the initial acute specimen collection when they were declared treated by the consulting physician.

The investigator first looked for cases in the medical and pediatric In-patient departments and recruited those who met the case definition using a convenient sampling method. After obtaining the required consent/assents from the participants. If the investigator was unable to enroll samples, then s/he collected the rest of the samples from the medical and pediatric outpatient departments using the same case definition. The study investigator collected all the study related socio-demographic details and clinical details in a standardized assessment form, which was pre-tested before the commencement of the actual study. The studied population's socio-economic status was calculated using Kuppuswamy socio-economic scale update [10], while the participants' residence was classified based on rural and urban areas. Laboratory related data was also collected on an appropriate laboratory form. All the data was kept confidential and was used for this study purpose only.

Case definitions:
1. AUFI: All patients presenting with undifferentiated fever of >38ºC of fewer than 3 weeks duration history without any specific localizing signs or symptoms [11].
2. Laboratory criteria of rickettsial infection: Serological evidence of a fourfold increase in IgG-specific antibody titer reactive with spotted fever group rickettsioses antigen by indirect immunofluorescence antibody assays (IFA) between paired serum specimens (one taken in the first two weeks after illness onset and a second was taken two to ten weeks after acute specimen collection) [12].
3. The sampling procedure from AUFI patients based on the case definition adapted for the study: Three ml of blood was collected in a plain vial. The serum was pipetted out after blood was centrifuged at 2,000 RPM for 10 minutes. The serum was separated and kept at - 80ºC until further use as described elsewhere [13]. Weil Felix Assay, ELISA, and indirect IFA were done to detect the anti-rickettsial antibodies. For the Weil Felix test, the criterion for a positive result is either one determination of a titre of 1:320 or greater or a fourfold rise in titre. However, group-specific IgM/IgG determined by ELISA is highly sensitive tests available for rickettsial disease diagnosis, and the presence of IgM antibodies indicates recent infection with the rickettsial disease. In scrub typhus infection, a significant IgM antibody level was observed by the end of the first week by the ELISA test, which aided in early diagnosis. Nevertheless, the group-specific

prevalence of rickettsial positive patients was determined using IFA during the second week of illness. To achieve the test's best sensitivity, paired serum samples were tested 2 - 3 weeks apart to demonstrate a rising IgG or IgM antibody titre following the study protocol [14]. To meet the other objectives of the study, other related tests to determine the cause of AUFI were also done, which included ELISA test to detect IgM antibodies against leptospira and Chikungunya, Widal test for Salmonella, detection of antibodies against brucella, ELISA test to detect viral capsid antigen (VCA) of Epstein-Barr Virus, card test for malarial antigen, NS1 antigen testing for dengue and blood culture to find our other related organisms. ELISA for chikungunya and NS1 antigen testing for dengue was done only for those with recent travel history (<1 months) outside the study area (Kashmir valley). The detailed procedure of the tests can be found in the **Supplementary material** attached to this manuscript.

Data analysis: The study's continuous variable is shown in terms of descriptive analysis and categorical variables in frequency and percentage. The recorded data was compiled and entered in a spreadsheet (Microsoft Excel 2007 spreadsheet) and then exported to the data editor of SPSS Version 26.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were summarized in the form of means, and standard deviations and categorical variables were summarized as percentages. Chi-square test or Fisher's exact test, whichever appropriate, was applied for comparing categorical variables. A *P*-value of less than 0.05 was considered statistically significant at 95% confidence interval.

The investigator obtained written informed consent/assent from all the study participants (whichever was applicable). The Ethics Review Committee of Sher-i-Kashmir Institute of Medical Sciences, Srinagar, reviewed and approved the study protocol before the study's commencement. The IRB approval for the study, as mentioned in the institutional records, is IEC/SKIMS Protocol #85/2016.

RESULTS

A total of 525 patients were admitted during the study period. Out of these, 344 were enrolled in the study and 181 were excluded as per exclusion criteria. Among the enrolled patients, 17 had a history of travel. The socio-demographic information of the studied patients is shown in **Table 1**. In **Table 2**, the determining cause and estimated prevalence of undifferentiated fever among the studied population is described. We could not diagnose most of the study

Table 1. Sociodemographic characteristics of the studied patients

Characteristics	Variable	n (%)
Gender	Male	212 (61.6)
	Female	132 (38.4)
Age	0 - 19	74 (21.5)
	20 - 39	159 (46.2)
	40 - 59	88 (25.6)
	≥60	23 (6.7)
Residence	Rural	278 (80.8)
	Urban	66 (19.2)
Socioeconomic Status	Upper (I)	58 (16.8)
	Upper Middle (II)	165 (48.0)
	Lower Middle (III)	32 (9.2)
	Upper Lower (IV)	40 (11.6)
	Lower (V)	49 (14.4)

Table 2. Cause of the undifferentiated fever determined during the study among the studied patients

Diagnosis	n (%)
Rickettsial disease	53 (15.4)
Leptospirosis	22 (6.3)
Salmonellosis	46 (13.3)
Brucellosis	36 (10.4)
EBV infection	2 (0.5)
Malaria	5 (1.4)
Dengue fever	1 (0.2)
Others	17 (4.9)
Undiagnosed	162 (47.0)
Total	344 (100.0)

EBV, Epstein-Barr virus.

Table 3. Distribution of Weil Felix positive samples among the studied patients done after 2 - 3 weeks

Reactivity	Antigen	n (%)
Positive	OX-2 (Spotted Fever Group) (8) OX-19 (Typhus Group) (4) OX-K (Scrub Typhus) (12)	24 (7.0)
Negative	-	320 (93.0)
Total		344 (100)

participants even after applying the available tests, even though 15% were diagnosed to have a rickettsial infection, 13.0% salmonellosis, and further 10% brucellosis, respectively. Although very rare in our region, the prevalence of mosquito-borne disease was also found among some participants. Out of a total of 344 samples analyzed by Weil Felix, 8 were positive for OX-2 (spotted fever group), 4 were positive for OX-19 (typhus group), and 12 were positive for OX-K (scrub typhus), as shown in **Table 3**. For the Weil Felix test, the criterion for a positive result was either one determination of a titre of 1: 320 or greater or a fourfold rise in titre on subsequent testing.

In patients positive for typhus group, 67.8% were IgM positive, 28.5% were IgG positive, and only 3.0% were positive for IgM and IgG. In patients positive for scrub typhus group, 32.7% were positive for IgM, and 62.0% were positive for IgG, and only 5.0% were positive for both IgM and IgG. In patients positive for spotted fever group, 36.1% were positive for IgM, and 58.5% were positive for IgG, and only 5.5% were positive for both IgM and IgG, as shown in **Table 4**. However, group-specific IgM/IgG determined by ELISA is considered highly sensitive

Table 4. Distribution of group specific IgM/IgG positive patients by ELISA done after 2 - 3 weeks

Parameter	n (%)
Typhus Group (28)	
IgM	19 (67.8)
IgG	8 (28.5)
Both	1 (3.5)
Scrub Typhus (58)	
IgM	36 (62.0)
IgG	19 (32.7)
Both	3 (5.1)
Spotted Fever (36)	
IgM	21 (58.3)
IgG	13 (36.1)
Both	2 (5.5)
Total (344)	122 (35.4)

Table 5. Distribution of group specific prevalence of rickettsial positive patients by IFA done after 2 - 3 weeks

Reactivity	Antigen	Frequency
Positive	Spotted Fever Group (16)	53 (15.4)
	Typhus group (12)	
	Scrub typhus (25)	
Negative	-	291 (84.6)
Total		344 (100.0)

tests available for rickettsial disease diagnosis. The presence of IgM antibodies indicates a recent infection with the rickettsial disease. In scrub typhus infection, a significant IgM antibody level was observed by the end of the first week by the ELISA test, which aided in early diagnosis. **Table 5** shows the group-specific prevalence of rickettsial positive patients by IFA during the second week of illness. To achieve the test's best sensitivity, paired serum samples were tested 2-3 weeks apart to demonstrate a rising IgG or IgM antibody titre. The most commonly detected group was found out to be scrub typhus (7.2%), followed by spotted fever group (4.6%) and typhus group (3.4%).

Table 6 describes the association of socio-demographic characteristics of the patients with the prevalence of the rickettsial infection. The prevalence of rickettsial disease was found to be 11.3% among males, and the association was found to be statistically significant (*P*-value = 0.008). Furthermore, the prevalence of rickettsial disease was higher with increasing age, but the association of age and the disease was not statistically significant. Among the patients who belonged to the rural areas, the prevalence of rickettsial disease was 17.6%, and the association was statistically significant. (*P*-value = 0.019). However, we found an association between the socio-economic class determined by the Kuppuswamy socio-economic scale [10] and the prevalence of the rickettsial disease in the studied population, which was statistically significant. (*P*-value = 0.001) .

DISCUSSION

Rickettsial diseases are some of the most covert re-emerging infection burdens of the present times. They are generally incapacitating and notoriously difficult to diagnose; untreated cases

Table 6. Association of sociodemographic characteristics and rickettsial disease among the studied patients

Characteristics	Variable	Rickettsial serology positive	Rickettsial serology negative	Prevalence	*P*-value
Gender	Male	24	188	11.3	0.008[a]
	Female	29	103	22.0	
Age (years)	0 - 19	9	65	12.2	0.753
	20 - 39	24	135	15.1	
	40 - 59	16	72	18.2	
	≥60	4	19	17.4	
Residence	Rural	49	229	17.6	0.019[a]
	Urban	4	62	6.1	
Socioeconomic Status	Upper (I)	4	54	6.8	0.001[a]
	Upper Middle (II)	10	155	6.0	
	Lower Middle (III)	9	23	28.1	
	Upper Lower (IV)	13	27	32.5	
	Lower (V)	17	32	34.6	

[a]*P*-value ‹0.05 considered significance at 95% confidence interval.

can have fatality rates as high as 30 - 35%, but they are often easily treated when appropriately diagnosed. The present study was undertaken to determine the prevalence of rickettsial infections in patients of undifferentiated febrile illness among patients attending a tertiary care hospital of Kashmir Valley.

Three hundred forty-four patients were recruited who satisfied the inclusion criteria, and the patient age ranged from 6 - 65 years. Most of the patients were adults in the age group of 20 - 39 years, and the average age of the patients was 32.5 ± 0.77 years. The study population comprised the majority of males. Gowri Veligandla et al. (2016), in their study, reported 33.6% were children, 63.7% were adults, and 3.4% were >60 years [15]. Furthermore, Salim Mattar et al. (2015), in a study reported, patient age ranged from 1 - 79 years, with the mean age of the population being 27 years. Forty patients were from the pediatric age group, and 60 were adults [16].

Another study by Abraham et al. documented a male preponderance with most of the patients in the productive phase of life, which correlated with the present study. The predominance of males in our study may be due to increased exposure to vectors and contaminated water due to their work nature [17, 18].

Our study population comprises the majority of rural dwellers, and the majority among the participants belonged to the upper-middle class of socioeconomic status. In a study conducted by Rwituja Thomas et al., out of a total of 262 patients, most were from the rural areas of Karnataka, Andhra Pradesh, and Tamil Nadu and belonged to an upper middle socioeconomic class, which corroborates with the finding of our study [19]. Our study population's rural predominance may be due to the agrarian lifestyle, poor hygiene practices, greater exposure to contaminated water of people living in villages.

IgM ELISA was carried out to determine the prevalence of leptospirosis in our study population, which revealed a positivity rate of 6.4%. In a study conducted by Salim et al. in Columbia, it was found that out of a total of 69 patients of undifferentiated febrile illness, 39.0% were found to have leptospirosis [20]. The results of this study were not following the present study. Another study by H. Sahira et al. in Kerala revealed that, out of a total of 1924 patients presenting with undifferentiated febrile illness, 11.2% tested positive for leptospirosis by IgM- ELISA [21].

Our study demonstrated the presence of Salmonella antibodies by the Widal agglutination test. Only 8.7% tested positive. Nsutebu EF et al. reported in their study detection of Salmonella by Widal agglutination to be 2.5% among those with febrile illness [22]. In contrast to our study, Ramyil MS et al. found that the prevalence of Salmonella to be 62.0% by the Widal agglutination test [23].

The prevalence of antibodies to Brucella abortus and mellitensis by agglutination in our study was found to be 7.3%. Bouley AJ et al. carried a study in which the prevalence of brucellosis in febrile patients was 16.0%, much higher than the present study [24].

By performing IgM antibody ELISA to VCA of Epstein-Barr virus (EBV), we determined the prevalence of EBV, and only 2.3% of samples tested positive. In a study conducted by Moeini et al., it was found that out of 346 patients, 30.0% (104) were positive for EBV VCA-IgM [25].

Malarial antigens were also detected in our studied population by malarial card test. Seventeen patients were clinically suspected of having malaria and had a history of travel outside the state. Out of these 17 patients, five were positive (1.4%). In a study conducted by Oyetunde T et al., the overall prevalence of malarial parasites obtained through rapid diagnostic tests was 36.8% [26]. In patients with a history of travel outside the state, we also carried out the Dengue card test in which two samples tested positive (0.5%). Faruque LI et al. found that 9.6% of patients had IgM antibodies against the dengue virus. The prevalence of patients with antibodies against the dengue virus was 8.6% in government hospitals and 11% in private hospitals [27].

Mosquitoes and malaria transmission are sensitive to altitude Temperature are inversely related to altitude, dropping by approximately 0.98°C for every 100-meter increase above absolute sea level [28].

This explains the low prevalence of malaria and dengue in our study as altitude limits malaria transmission and its distribution variably. It influences the rainfall and thus the breeding conditions of the main anopheles' vectors. Temperature variations have an even more significant effect on both the vector density and the plasmodium species' infectivity undergoing its development in the female anopheles [29].

In our study, the etiological agent could not be established in approximately 47.0% of patients. We did not extensively look for other agents responsible for acute undifferentiated fever, including viruses, because of the high percentage of undiagnosed patients in our study. According to a study by Gowri Veligandla e al., in which 100 patients with acute febrile illness were recruited, the undiagnosed cases were 63.0%. A review article by Susilawati TN et al. states that the aetiology of AUFI remains undiagnosed in 8-80% of cases [3].

We detected antibodies to Rickettsial antigens by various serological methods, namely Weil Felix, ELISA, and indirect immunofluorescence assay. The Weil Felix test revealed that out of a total of 344 samples, 7.0% tested positive. The low prevalence can be explained by the low sensitivity and low specificity of the Weil Felix test. In a study conducted by SK. Mahajan et al. [30], in Shimla, it was found that 9.0% had Weil Felix test positive showing titer of > 1:80. E. Mathai et al. [31], in a study, found that 5.0% of the 132, 3.0% of the 132, and 10.0% of the 211 serum samples investigated using the WF test gave titers of at least 1:160. Detection of anti Rickettsial antibodies by ELISA was also carried out in our study. Out of a total of 344 samples, 35.4% tested positive. The high prevalence by ELISA may be due to false-positive results as compare to IFA.

The study population was distributed into different rickettsial groups. The most common group in our study was the Scrub typhus group (23.0%), followed by spotted fever (17.0%) and typhus group (8.2%). In a study by Tanveer Nawab et al., out of 30 patients with possible rickettsioses, scrub typhus was diagnosed in (46.7%), spotted fever in (26.7%), typhus in (6.6%), and mixed features in (20.0%) [32].

We carried out the detection of group-specific IgM/IgG in the study population. Typhus group, scrub typhus, and spotted fever specific IgM was detected in 67.8%, 62.0%, and 58.3% of the samples, followed by IgG detected in 28.5%, 32.7%, and 5.5% of the samples, respectively. In a study conducted by Faruque LI et al. [27], it was observed that out of 360 febrile patients, scrub typhus IgM ELISA was positive in 107 patients, the results of which are following our study.

The detection of rickettsial infections in the studied population was done by IFA, and it was found that 15.4% tested positive, and 84.6% tested negative. The group most commonly detected was the scrub typhus (17.0%), followed by the spotted fever group (10.6%). This contrasts with a study conducted by Salim et al. in Columbia, wherein out of 100 patients, 69 demonstrated AUFI. Out of these, 2.8% were confirmed rickettsioses by IFA [20].

The highest prevalence of rickettsial infections was found in the age group 40 - 59 years, and their association was found to be statistically insignificant (P-value = 0.753). This shows that the rickettsial infection does not affect any specific age and all age groups are vulnerable. However, the association between the prevalence of rickettsial disease and gender was statistically significant (P-value = 0.008). Similar findings were reported by Bithu R et al. [33], where Scrub typhus IgM antibodies by ELISA were detected in 49.1% of patients, scrub typhus positivity was significantly higher among females in comparison to males (P <0.05). The female predominance of rickettsial infection may be attributed to the women working in paddy fields during the rice harvesting season. Simultaneously, the association of prevalence of rickettsial disease and residence of the study population demonstrated a statistically significant association with a higher prevalence of rickettsial infections found in patients belonging to the rural areas and those with the more impoverished socioeconomic background (P-value = 0.001). This finding is similar to those of other studies, where the rural population and low socioeconomic strata appear to be more susceptible to rickettsial infections. The more significant predominance among the rural population may be attributed to rural dwellers' greater agricultural and plantation activities and the greater probability of contact with rodents and other animals in these areas.

In our study, all the three serological findings corroborate each other as all the methods indicate scrub typhus to be the most common rickettsial infection in the valley, which is an expected finding considering Jammu and Kashmir falls within what is called the "tsutsugamushi triangle" with Japan in the east, Afghanistan and Middle-East region in the west and the pacific islands area, north Australia, Indonesia, south-east Asia, China, Korea in between [34].

To the best of our expertise, we diagnosed patients with undifferentiated fever with the available tests like the Weil-Felix test, ELISA, IFA, etc. We couldn't perform diagnosis at the molecular level based on reverse transcription polymerase chain reaction due to funding constraints. We could not report on the possibility of cross-reactivity in serologic studies of rickettsial infection and other diseases. Moreover, 47% of the patients were not being diagnosed even after applying all the available tests, which we consider one of this study's limitations.

We could only find an association with some of the rickettsial infection factors in our study. More evidence-based research is warranted in this field with a larger sample size, longer study duration, and robust study design in the future.

We recommend that clinicians and primary care physicians consider rickettsial diseases as a differential diagnosis while treating patients with fever.

In conclusion, rickettsial diseases, typhoid and brucellosis, were the most prevalent diseased diagnosed among patients reporting to hospitals with AUFI. IFA is the recommended test to be done during the second week of disease among those suspected of rickettsial infection. To achieve the test's best sensitivity, paired serum samples need to be tested 2 - 3 weeks apart to demonstrate a rising IgG or IgM antibody titre. Clinicians must consider rickettsial diseases as one of the differential diagnosis while treating patients with fever.

ACKNOWLEDGEMENT

SUPPLEMENTARY MATERIAL

Supplementary Material

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