J Antimicrob Chemother 2015; **70**: 2129–2132
doi:10.1093/jac/dkv085 Advance Access publication 21 April 2015

Journal of Antimicrobial Chemotherapy

IFN-α2a or IFN-β1a in combination with ribavirin to treat Middle East respiratory syndrome coronavirus pneumonia: a retrospective study

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Received 29 December 2014; returned 31 January 2015; revised 4 March 2015; accepted 14 March 2015

Objectives: Middle East respiratory syndrome coronavirus (MERS-CoV) is associated with significant mortality. We examined the utility of plasma MERS-CoV PCR as a prognostic indicator and compared the efficacies of IFN-α2a and IFN-β1a when combined with ribavirin in reducing MERS-CoV-related mortality rates.

Methods: We retrospectively analysed 32 patients with confirmed MERS-CoV infection, admitted between April 2014 and June 2014, by positive respiratory sample RT–PCR. Plasma MERS-CoV RT–PCR was performed at the time of diagnosis for 19 patients.

Results: The overall mortality rate was 69% (22/32). Ninety percent (9/10) of patients with positive plasma MERS-CoV PCR died compared with 44% (4/9) of those with negative plasma MERS-CoV PCR. Mortality rate in patients who received IFN-α2a was 85% (11/13) compared with 64% (7/11) in those who received IFN-β1a ($P=0.24$). The mortality rate in patients with renal failure (14), including 8 on haemodialysis, was 100%. Age >50 years and diabetes mellitus were found to be significantly associated with mortality (OR=26.1; 95% CI 3.58–190.76; $P=0.001$ and OR=15.74; 95% CI 2.46–100.67; $P=0.004$, respectively). The median duration of viral shedding in patients who recovered was 11 days (range 6–38 days). Absence of fever was noted in 5/32 patients.

Conclusions: Plasma MERS-CoV RT–PCR may serve as an effective tool to predict MERS-CoV-associated mortality. Older age and comorbid conditions may have contributed to the lack of efficacy of IFN-α2a or IFN-β1a with ribavirin in treating MERS-CoV. Absence of fever should not exclude MERS-CoV.

Keywords: MERS-CoV, treatment, interferon

Introduction

Middle East respiratory syndrome coronavirus (MERS-CoV) pneumonia causes high mortality rates (67%).[1,2] While it reduces the production of IFN-α and IFN-β,[3–5] IFN-α inhibits the MERS-CoV-induced cytopathic response and viral replication,[6] particularly with ribavirin.[7] IFN-α2b and ribavirin were effective in macaques when administered 8 h after inoculation with MERS-CoV.[8] In humans, five patients who received IFN-α2b and ribavirin had 100% mortality,[9] but because administration was delayed in the study it is difficult to interpret the findings. A recent study showed a significant reduction in 14 day, but not 28 day, mortality rates between patients who received IFN-α2a with ribavirin and those who received standard supportive care.[10] Recent *in vitro* data demonstrated superiority of IFN-β over IFN-α2b and IFN-α2a, with IC_{50} values of 1.37, 21.4 and 160.8 U/mL, respectively.[11] This could have major implications for MERS-CoV treatment. In the absence of effective treatment, more data on the use of different types of IFNs to treat MERS-CoV infections are crucial. Moreover, data are needed to identify patients at highest risk of mortality. We aimed to explore the association of plasma MERS-CoV RT–PCR with mortality and to assess and compare the efficacy of IFN-α2a or IFN-β1a with ribavirin to treat MERS-CoV pneumonia.

Methods

Study design

A sequential retrospective cohort study was conducted on confirmed MERS-CoV-infected patients who were admitted to King Fahad Armed

Forces Hospital, Jeddah, Saudi Arabia, between 1 April 2014 and 30 June 2014. Institutional review board approval was obtained.

Case definition

MERS-CoV infection was confirmed when real-time MERS-CoV RT–PCR was positive from a respiratory tract sample that included nasopharyngeal (NP) swab, sputum, deep tracheal aspirate or bronchoalveolar lavage, in addition to the patient having respiratory tract symptoms including cough and shortness of breath.[12,13] Two real-time RT–PCR assays were used for MERS-CoV diagnosis confirmation; the target of one was upstream of the E gene and that of the other was the region within ORF1b.[14]

All confirmed cases were admitted in negative-pressure airborne isolation rooms and were followed until in-hospital death or hospital discharge.

Plasma MERS-CoV RT–PCR using the above-mentioned real-time RT–PCR assays was done for the confirmed MERS-CoV cases.[14]

Treatment protocols were IFN-α2a (180 μg subcutaneously once weekly) combined with ribavirin (loading dose of 2 g orally followed by 600 mg orally every 12 h) or IFN-β1a (44 μg subcutaneously three-times weekly) combined with ribavirin, dosed as above. Ribavirin doses were adjusted to calculated CL_{CR} and continued for the duration of treatment with both types of IFN.

Statistical analysis

Descriptive statistics were used for demographics. Categorical variables were compared using the χ^2 test or Fisher's exact test as appropriate. Mortality risk factors were assessed with a Cox proportional hazards model. Probability of survival in patients who received IFN-α2a, IFN-β1a or no IFN was estimated by the log-rank test. Two-sided *P* values of <0.05 were considered statistically significant. Data were analysed using IBM SPSS Statistics, version 19.0 (IBM Corp., USA).

Results

A total of 910 patients were tested with MERS-CoV RT–PCR for suspected MERS-CoV infections. There were 32 confirmed cases. Between 1 April and 30 April 2014, patients with MERS-CoV pneumonia received IFN-α2a with ribavirin. Upon mortality reviews on 30 April 2014 and based on data that demonstrated a favourable IC_{50} of IFN-β,[11] treatment regimens were switched to IFN-β1a and ribavirin on 1 May 2014. The distribution of patients according to their treatment regimens and outcomes is illustrated in Figure S1 (available as Supplementary data at *JAC* Online).

Demographics

Baseline characteristics of the MERS-CoV cohort are illustrated in Table S1. No significant differences were observed upon comparing the two treatment groups (Table 1). There were six healthcare workers diagnosed with MERS-CoV infection. One had not received IFN or ribavirin, given her stable clinical condition apart from upper respiratory tract symptoms. Another received IFN-α2a and ribavirin, while the remaining four received IFN-β1a and ribavirin. There were no deaths among them.

Clinical features

Clinical features upon diagnosis are presented in Table 1. Fever was absent on presentation and throughout the admission in five patients with confirmed MERS-CoV pneumonia (Table 1). Of these, three patients had diabetes mellitus, the fourth had haemochromatosis and the fifth had chronic renal impairment. All five patients died. Two of them presented with dyspnoea and increased oxygen requirement (>2 L/min) without cough.

Confirming diagnosis

Forty-seven percent (15/32) of confirmed MERS-CoV cases had initial negative NP swabs. Thirteen patients had subsequent positive sputum samples; the remaining two patients had a positive tracheal aspirate and a repeat NP swab, respectively.

Outcome

The overall mortality rate was 69% (22/32). Eighty-five percent (11/13) in the IFN-α2a group died and 64% (7/11) in the IFN-β1a group died (Figure S1). Patients received IFN-α2a for a median duration of 6 days (IQR 3–10.5 days) and IFN-β1a for a median duration of 6.5 days (IQR 5–8.5 days). IFN regimens were started within a median of 1 day following MERS-CoV diagnosis (ranging from 1 day before diagnosis to 1 day after diagnosis).

Plasma MERS-CoV RT–PCR

Plasma RT–PCR was done upon confirmation of MERS-CoV infection in 19/32 patients. It was positive in 10/19 and negative in 9/19. Ninety percent (9/10) of patients with positive plasma MERS-CoV PCR results died compared with 44% (4/9) of those with negative results [positive predictive value (PPV) for mortality was 90% (95% CI 55.46%–98.34%)].

Duration of MERS-CoV shedding

MERS-CoV RT–PCR of respiratory samples was repeated at 5 day intervals. While negative MERS-CoV RT–PCR results were documented in all survivors, none of the patients who died had a negative MERS-CoV RT–PCR result prior to death. Clearance of MERS-CoV virus was defined as two negative RT–PCR samples collected on two consecutive days. The median duration of viral shedding in survivors was 11 days (range 6–38 days).

Mortality risk factors

All renal failure patients on haemodialysis (8/8) and those with chronic renal impairment (14/14) died. Age >50 years and diabetes mellitus were significantly associated with mortality (OR=26.1; 95% CI 3.58–190.76; $P=0.001$ and OR=15.74; 95% CI 2.46–100.67; $P=0.004$, respectively) (Table 2). There was no difference in time to death among patients who received IFN-α2a, IFN-β1a or no IFN (Figure S2).

Discussion

MERS-CoV pneumonia is associated with high mortality rates. The role of positive plasma MERS-CoV RT–PCR in predicting poor outcome has not been previously explored. Although not statistically significant, possibly due to the small number of patients, there was a trend towards a higher mortality rate in patients with a positive MERS-CoV RT–PCR. Whether positive plasma MERS-CoV

Table 1. Baseline characteristics and clinical and laboratory features upon MERS-CoV diagnosis

Variable	IFN-α ($n=13$)	IFN-β ($n=11$)	P
Age (years), median (IQR)	65 (33–84)	67 (25–88)	0.96
Male, *n* (%)	10 (77)	4 (36)	0.045
Diabetes mellitus, *n* (%)	10 (77)	5 (45)	0.12
Hypertension, *n* (%)	11 (85)	7 (64)	0.24
Chronic renal impairment[a], *n* (%)	7 (54)	3 (27)	0.19
Renal failure on haemodialysis, *n* (%)	4 (31)	2 (18)	0.41
Low ejection fraction, *n* (%)	5 (38)	2 (18)	0.26
Recent hospital admission, *n* (%)	6 (46)	3 (27)	0.7
Healthcare worker, *n* (%)	1 (7.7)	4 (36)	0.11
Contact with camels, *n* (%)	1 (7.7)	2 (18)	0.44
Fever[b], *n* (%)	11 (85)	8 (73)	0.42
Cough, *n* (%)	11 (85)	10 (91)	0.57
Sputum production, *n* (%)	9 (69)	8 (73)	0.61
Shortness of breath, *n* (%)	12 (92)	8 (73)	0.11
Diarrhoea, *n* (%)	2 (15)	1 (9)	0.57
Abdominal pain, *n* (%)	0 (0)	2 (18)	0.2
Confusion, *n* (%)	0 (0)	1 (9)	0.46
Vomiting, *n* (%)	2 (15)	2 (18)	0.2
Oxygen requirement (>2 L/min), *n* (%)	5 (38)	4 (36)	0.24
Mechanical ventilation, *n* (%)	10 (77)	6 (55)	0.24
Acute renal failure, *n* (%)	5 (38)	3 (27)	0.67
Laboratory variable			
white blood cells ($\times10^9$ cells/L), median (IQR)	7 (2.4–18.3)	6.7 (2.9–21.8)	0.65
ANC ($\times10^9$ cells/L), median (IQR)	5.7 (1.7–16.9)	4.2 (2.1–16.2)	0.57
ANC nadir ($\times10^9$ cells/L), median (IQR)	0.4 (0.1–2.53)	0.6 (0.4–2.8)	0.21
ALC ($\times10^9$ cells/L), median (IQR)	0.9 (0.5–6.9)	0.7 (0.4–4.3)	0.91
ALC nadir ($\times10^9$ cells/L), median (IQR)	0.4 (0.1–2.5)	0.6 (0.4–2.8)	0.21
LDH (U/L), median (IQR)	437 (150–1145)	329 (170–4790)	0.61
C-reactive protein (mg/L), median (IQR)	86.5 (25–226)	80 (19.3–346)	0.61
bilirubin (μM), median (IQR)	9 (1.5–243)	6 (2.4–20)	0.047
ALT (U/L), median (IQR)	23 (12–222)	30 (20–881)	0.15
platelets ($\times10^9$ cells/L), median (IQR)	169 (24–467)	185 (96–323)	0.46
haemoglobin (g/dL), median (IQR)	9.9 (7.1–14.1)	10.9 (7.5–14.4)	0.73
creatinine (μM), median (IQR)	111 (50–605)	85 (44–631)	0.57
APACHE II score, median (IQR)	18.0 (15.5–24)	15.0 (3.0–20.0)	0.15
positive plasma MERS-CoV RT–PCR, *n*/*n* (%)	5/11 (45)	5/8 (63)	0.65

ANC, absolute neutrophil count; ALC, absolute lymphocyte count; LDH, lactate dehydrogenase.
[a]CL_{CR} <50 mL/min, calculated by MDRD formula.
[b]Temperature >38°C.

Table 2. Risk factors for mortality in patients with MERS-CoV infection

Variable	OR	95% CI	P
Age (≥50 versus <50 years)	26.1	3.58–190.76	0.001
Diabetes mellitus	15.74	2.46–100.67	0.004
Oxygen requirement[a] (yes versus no)	0.32	0.9–1.1	0.07
Creatinine (>110 versus ≤110 μM)[b]	2.33	0.73–7.4	0.15
IFN-α treatment	0.16	0.02–1.38	0.09
IFN-β treatment	0.28	0.03–2.33	0.24

[a]Requirement of >2 L/min.
[b]Equivalent to 1.24 mg/dL.

RT–PCR at the time of MERS-CoV diagnosis reflects worse prognosis is worth investigating in a larger cohort. Confirming such an association could serve to identify high-risk patients who could be targeted with therapy such as convalescent sera.[15]

The lack of effective MERS-CoV treatment has become an impediment. IFN-α2a was chosen due to unavailability of IFN-α2b. This may have played a role in the ineffectiveness of IFN treatment in this cohort, particularly with the lower IC_{50} of IFN-α2b for MERS-CoV.[11] A possible reason for the high mortality rate is the old age (≥50 years) of the majority of patients (20/32), which was associated with mortality in this and a previous cohort.[2] Moreover, 8/32 had end-stage renal disease and were on haemodialysis, all of whom died. Recently, IFN-α2a and

ribavirin significantly reduced MERS-CoV-associated mortality at 14 days, but not 28 days, when compared with standard supportive care.[10] However, there were no patients with end-stage renal disease in that study.

There was no difference in survival between the IFN-α2a and the IFN-β1a groups in our cohort (Figure S2). Additionally, the absence of fever in 5/32 patients with MERS-CoV pneumonia questions the validity of the WHO and CDC definitions of probable MERS-CoV infections, which include fever.[12,13] These patients' inability to mount a fever could signal an impaired immune response, and they represent a high-risk group, evidenced by their poor outcome. Therefore, absence of fever should not preclude testing for MERS-CoV once suspected. Six healthcare workers were infected with MERS-CoV; the absence of mortality among them is probably related to the lack of risk factors in these patients. They were all <50 years of age and none had impaired renal function. The observed high number of initial negative NP swabs may be related to poor collection technique. However, lower viral loads in NP swabs compared with sputum and tracheal aspirate samples on quantitative RT–PCR testing remains the most likely explanation.[16] Therefore, negative NP swabs should not rule out MERS-CoV infections without testing lower respiratory tract samples. Finally, determining the duration of viral shedding in survivors of MERS-CoV infections has valuable implications for infection control.

Our study is limited primarily by its retrospective nature and the small number of patients. However, this is the largest cohort that assessed the use of two different IFN regimens to treat MERS-CoV to date.

In conclusion, the role of plasma MERS-CoV RT–PCR as a mortality predictor would have major implications and should be assessed in larger cohorts. Additionally, neither IFN-α2a nor IFN-β1a in combination with ribavirin was effective in reducing MERS-CoV mortality in this cohort. These results should be verified in a larger prospective randomized setting.

Acknowledgements

We would like to thank Abdulwahab AlZahrani, Sultan Fallatah, Abdulmohsen Jamal, Waleed Al-Taweli and Danya Abu-AlSaod for their great technical assistance with the samples. We are grateful to Shahid Husain MD for his critical review of the manuscript prior to submission.

Funding

This study was supported by internal funding.

Transparency declarations

None to declare.

Supplementary data

Figure S1, Figure S2 and Table S1 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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