Comparison of serological assays in human Middle East respiratory syndrome (MERS)-coronavirus infection

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Plaque reduction neutralisation tests (PRNT), microneutralisation (MN), Middle East respiratory syndrome (MERS)-spike pseudoparticle neutralisation (ppNT) and MERS S1-enzyme-linked immunosorbent assay (ELISA) antibody titres were compared using 95 sera from 17 patients with MERS, collected two to 46 days after symptom onset. Neutralisation tests correlated well with each other and moderately well with S1 ELISA. Moreover to compare antigenic similarity of genetically diverse MERS-CoV clades, the response of four sera from two patients sampled at two time periods during the course of illness were tested by 90% PRNT. Genetically diverse MERS-CoV clades were antigenically homogenous.

Introduction

Middle East respiratory syndrome (MERS) poses a major threat to global public health [1]. Validated serological assays are important for diagnosis and for seroepidemiology to define prevalence and risk factors [2,3]. Serological assays for detecting antibody for MERS-coronavirus (CoV) infection include antibody arrays, enzyme-linked immunosorbent assay (ELISA), immune-fluorescence, microneutralisation (MN), plaque reduction neutralisation (PRNT) and MERS-spike pseudoparticle neutralisation tests (ppNT) [2,4-6]. While data from individual case reports exist [6], there are limited comparative data on serological methods for detecting MERS-CoV antibody in humans, because of a lack of well-characterised sera [7]. We used 95 sera from 17 patients with real-time reverse transcription polymerase chain reaction (RT-PCR) confirmed MERS-CoV infection diagnosed during an outbreak of MERS in South Korea [8,9] to compare PRNT antibody titres using 90% ($PRNT_{90}$) and 50% ($PRNT_{50}$) plaque reduction end points, MN, MERS-spike ppNT and S1-ELISA tests. The sera were also used to investigate the antigenic similarity of three genetically diverse strains of MERS-CoVs [10]. We had previously reported that early $PRNT_{50}$ and S1-ELISA antibody responses in this patient-cohort were associated with improved clinical outcome [9].

Methods

Patients

Patients with RT-PCR confirmed MERS-CoV infections admitted to Seoul National University (SNU) Hospital, SNU Boramae Medical Center and SNU Bundang Hospital within the first 14 days after onset of illness during the outbreak of MERS-CoV between May and June 2015 in South Korea were included. Serial serum samples (n = 95 in total) were collected from 17 patients during the first 39 days of illness or up to time of discharge from hospital. This study was approved by the Institutional Review Board of SNU. Clinical data on these patients have been previously reported [9].

Viruses

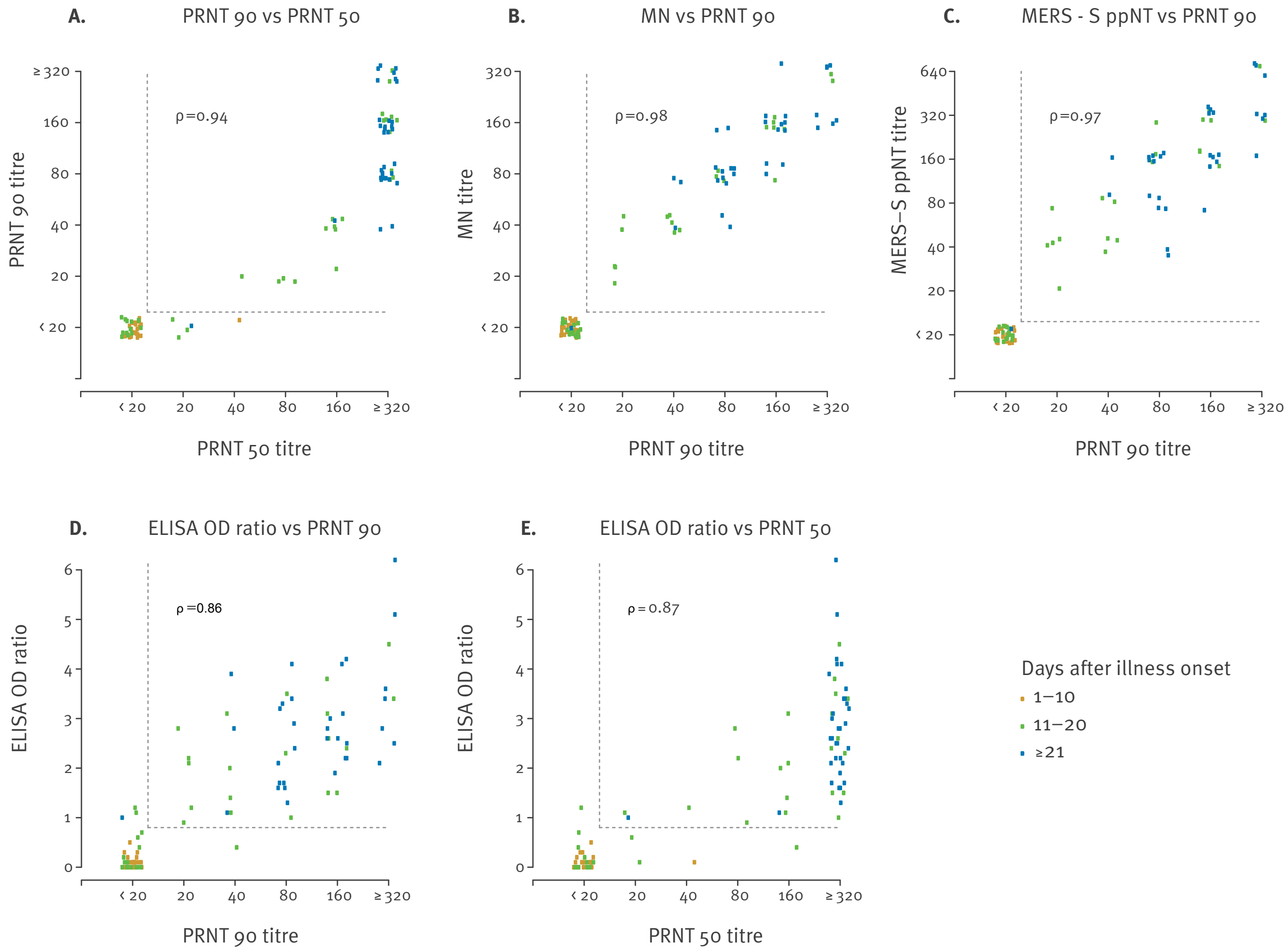
The MERS-CoV strains used in the virus neutralisation assays belonged to clade A (MERS-CoV-strain EMC), clade B (dromedary camel MERS-CoV Al-Hasa FKU-HKU13 2013) as well as a virus from a distinct non A and B clade (dromedary camel Egypt NRCE-HKU 270 2013) as previously described [10].

Serology tests

The sera were heat-inactivated for 30 min at 56 °C before testing. The PRNT assays were performed in a 24-well format in duplicate for each serum dilution. Twofold serum dilutions were incubated with 40 to 60 plaque-forming units of virus for 1 hour at 37 °C. The virus – serum mixture was added onto a Vero cell monolayer and incubated for 1 hr at 37 °C in a 5% CO2 incubator. Then, the supernatant was removed and the cells overlaid with 1% agarose (SeaKem LE Agarose, Lonza,

Figure

Scatter plots comparing antibody titres obtained from different assays in relation to duration (days) after onset of illness due to Middle East respiratory syndrome (MERS)-coronavirus infection



ELISA: enzyme-linked immunosorbent assay; MN: microneutralisation; OD: optical density; PRNT: plaque reduction neutralisation test; ppNT: pseudoparticle neutralisation test.

Spearman correlation for each comparison is denoted in each panel. Sera collected 1–10; 11–20 and ≥ 21 days from onset of illness are denoted in yellow, green and blue, respectively. The MERS-spike ppNT, MN, PRNT50, and PRNT90 titres have been jittered for better presentation. The negative cut-off titres or OD is denoted in a dotted line.

Switzerland) in cell culture medium (Minimum Essential Medium with 2% fetal bovine serum). The plates were fixed and stained after three days incubation. Antibody titres were defined as the highest serum dilutions that resulted at ≥ 50% ($PRNT_{50}$) and ≥ 90% ($PRNT_{90}$) reduction in the number of plaques, respectively.

The ppNT assays were performed as previously described, with triplicate serum dilutions [5,11]. MN tests were carried out to determine the highest serum dilution that suppressed virus cytopathic effect in Vero cells following infection with a virus dose of 100 tissue culture infection $dose_{50}$ mixed with the respective serum dilution [5]. Serum dilutions were done in quadruplicate. Positive and negative controls and virus back-titrations were included in each assay. Antibody titres of ≥ 1:20 were regarded as positive.

The S1 ELISA EI 2604–9601G kit was purchased from EUROIMMUN Luebeck, Germany for detection of human IgG against MERS-CoV. The test was done on single serum samples in duplicate according to the manufacturer's instructions. The assay included a calibrator which defined the upper limit of the reference range in non-infected humans and this value was defined as the cut off. The assay was made semi-quantitative by calculating the ratio of the extinction of the patient sample/extinction of the calibrator. Ratios < 0.8 were considered negative, those ≥ 1.1 as positive and those ≥ 0.8 to < 1.1 regarded as borderline.

Statistical methods

Spearman correlation coefficient was calculated to assess the correlations between the different assays.

Table 1

Proportion of sera that were positive for antibodies to Middle East respiratory syndrome (MERS) in various assays at different times post-disease onset

Time periods in days from onset of illness	Number of serum samples	Number of patients	Proportion of sera with neutralising antibody titres ≥ 1:20 n/N				ELISA positive
			$PRNT_{90}$	$PRNT_{50}$	ppNT	MN	
1–5	7	7	0/7	0/7	0/6[a]	0/7	0/7
6–10	17	11	0/17	0/17	0/17	0/17	0/17
11–15	18	17	7/18	9/18	6/18	6/18	7/18
16–20	19	17	14/19	15/19	14/18[a]	14/19	15/19
≥ 21	34	9	33/34	34/34	31/33[a]	33/34	33/34

ELISA: enzyme-linked immunosorbent assay; MN: microneutralisation; PRNT: plaque reduction neutralisation test; ppNT: pseudoparticle neutralisation test.

[a] One serum in each of these groups could not be tested in the ppNT assay, thus the denominator for the ppNT differed from the others.

Table 2

Antigenic cross-reactivity of human convalescent sera with genetically diverse MERS-CoV in 90% plaque reduction neutralisation tests (PRNT90)

Patient ID	Days of illness	Reciprocal $PRNT_{90}$ antibody titre to MERS-CoV strains representing different MERS-CoV clades		
		Strain EMC (clade A)	Dromedary camel Al-Hasa KFU-HKU13 2013 (clade B)	Dromedary camel Egypt NRCE-HKU 270 2013 (clade non A/B)
B	12	320	160	160
B	39	320	320	640
G	17	40	40	80
G	35	160	80	160

ID: identity; MERS-CoV: Middle East respiratory syndrome (MERS)-coronavirus.

Results

Scatter-plots showing correlation between $PRNT_{90}$, $PRNT_{50}$, ppNT, MN and S1-ELISA assays are shown in Figure A-E. As expected, the $PRNT_{50}$ assay was more sensitive than the $PRNT_{90}$ because it uses the less stringent end-point of 50% reduction in the plaque count (Figure A). There was excellent correlation between the $PRNT_{90}$, MN and MERS-spike ppNT titres with Spearman correlations of 0.97–0.98 (Figure B,C). MERS-CoV S1 ELISA was less strongly correlated with the different neutralisation assays with Spearman correlation of 0.86–0.87 (Figure D and E).

Table 1 shows the proportion of sera that were positive in neutralising tests at titres ≥ 1:20 or in ELISA. None of the patients were seropositive in the first 10 days of illness. At 11–15 days of illness, 50% of sera were positive in $PRNT_{50}$ assays, 39% in $PRNT_{90}$ and S1-ELISA assays and 33% positive in ppNT and MN assays. After 21 days of illness, the majority of patients were seropositive. However, even at day 32 of illness, one patient remained seronegative in $PRNT_{90}$, ppNT and MN assays, borderline positive in the S1 ELISA and was only positive in the $PRNT_{50}$ test at a titre of 1:20. She was aged in her mid-fifties with no underlying diseases, and presented with a relatively mild pneumonic illness (reported more fully in [9]).

Twelve patients seroconverted (fourfold increase in antibody titre) by all five assays and one had static high titres (first serum sample of this patient was at day 13 of illness). The woman in her mid-fifties noted above failed to seroconvert by S1 ELISA, MN and $PRNT_{90}$, and only reached $PRNT_{50}$ antibody titre of 1:20 up to day 32 of illness. Three other patients did not seroconvert in any of the assays, but the latest available sera from them was at day 8, 9 and 16, respectively, too early to conclude whether sera of these patients at a later stage of illness would have shown seroconversion.

In order to compare antigenic similarity of genetically diverse MERS-CoV, we selected four sera from two patients. These sera had been sampled early (day 12, 17) and later (day 35, 39) during the course of illness. The antibody titres of each serum to clade A, clade B and the genetically divergent Egyptian camel viruses were within twofold (Table 2).

Discussion

The different virus neutralisation assays (MN; ppNT $PRNT_{50}$; $PRNT_{90}$) all had excellent correlation among them (Spearman correlation ≥ 0.94) (Figure). The $PRNT_{50}$ antibody test was more sensitive in detecting early antibody responses and had higher antibody titres throughout, as would be expected, given the less stringent end point of ≥ 50% reduction of plaque numbers, in contrast to the ≥ 90% reduction of plaques needed for the $PRNT_{90}$ antibody titre endpoint (Figure A) (Table 1). In studies of household transmission of MERS-CoV, it was shown that $PRNT_{50}$ can detect some infections undetected by $PRNT_{90}$ tests [12].

Unlike other neutralisation tests that require handling live MERS-CoV in biosafety level (BSL)-3 containment, the MERS-spike ppNT assay does not require BSL-3 containment. It gave good correlation with $PRNT_{90}$ and MN tests. We have previously shown that sera from healthy humans sera from Hong Kong (n = 115), Egypt (n = 100) and Saudi Arabia (n = 237) were negative in the ppNT test [5,13], confirming the specificity of this assay. Although the ppNT assay had been extensively used and validated for seroepidemiology in animals and livestock with good correlation between MERS ppNT and MN assays, [5,11,13], this is the first extensive demonstration of its performance in humans with confirmed MERS-CoV infection and during the first six weeks of infection. ppNTs have proved to be reliable surrogates for neutralisation tests in other infections including avian influenza A(H5N1) [14]. Thus, the MERS-spike ppNT may be usable for large scale seroepidemiology studies to assess extent of MERS-CoV infection in the general population, to assess risk factors of infection in high-risk groups, or when selecting patient sera for plasmapheresis for preparation of convalescent plasma where quantification of neutralising antibody may be important.

The semi-quantitative optical density (OD) ratios of the MERS S1 ELISA had acceptable but lower Spearman correlations (0.86–0.87) with the different neutralisation tests, in terms of the time to becoming positive in patients with MERS (Figure D,E). The S1-ELISA assay was a binding assay detecting IgG alone, rather than a functional neutralising assay and thus the lower correlation with this type of assay was not surprising.

In contrast to viruses such as avian influenza A(H5N1) where there is great antigenic diversity, genetically diverse MERS-CoV remain antigenically homogenous. Similar results had been previously reported using dromedary camel sera [11], and also clade B viruses and MERS-CoV EMC (clade A) were antigenically indistinguishable with human sera [15].

The Korean outbreak was caused by a clade B virus. Limitations of this study are that all the sera tested were from one outbreak and from one ethnic background and that a MERS-CoV isolate from these patients was not available for use in the serology tests. However, we have demonstrated in this, and previous studies, that antibody titres are not affected by the clade of virus used.

In conclusion, the different types of neutralisation or ppNT assays can be used in MERS-CoV diagnosis and seroepidemiology. $PRNT_{50}$ was more sensitive than other assay formats and may be the only assay that can be positive early in the course of infection and in a few patients with poor serologic responses. Genetically diverse MERS-CoV are antigenically homogenous suggesting that future vaccines generated by any MERS-CoV strain will cross-protect against genetically and geographically diverse viruses.

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Conflict of interest

None declared.

Authors' contributions

MP and MDO conceived, planned and coordinated the study, the study; RAPMP carried out the serology assays, RAPMP and LLMP developed the serology assays, WBP, PGC, SJC, JYC, HSO, KHS, JHB, ESK, HBK, SWP, NJK coordinated the clinical studies, EYHL carried out the statistical analysis, and all authors critically reviewed the manuscript.

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