

Comparison of Tissue Culture Infective Dose (TCID₅₀) and Quantitative PCR (QPCR) in the Diagnosis and Follow Up of Influenza in Hospitalized Subjects

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ABSTRACT (Revised)

BACKGROUND: RT-PCR or culture can confirm influenza diagnosis, while change (Δ) from BL in TCID₅₀ or QPCR quantifies viral dynamics. We used data from a phase 3 study of subjects hospitalized with suspected influenza treated with IV peramivir (PVR) to compare these methods.

METHODS: Subjects were hospitalized with influenza symptoms and met eligibility criteria in the 2009/10 pandemic in 5 countries. Virology was done on nasopharyngeal specimens at BL and at later timepoints. TCID₅₀/mL (BL to 48 hours) was the primary endpoint, Δ from BL in QPCR (vp/mL) a secondary endpoint. We assessed performance characteristics and the correlation between Δ from BL in titers with the two methods using descriptive statistics and Pearson’s coefficient; multivariable logistic regression with stepwise selection was used to identify predictors of Δ from BL.

RESULTS: 234 subjects were randomized (230 treated) to 5 to 10 days IV PVR 300mg BID (n = 117), or IV PVR 600mg QD (n = 117). 127 subjects had confirmed influenza (all methods); similar antiviral effect was seen in both groups. The table shows characteristics and Δ from BL for both diagnostic tests.

| Characteristic | TCID ₅₀ Log ₁₀ vp/mL n=230 | Quantitative PCR: Log ₁₀ vp/mL n=230 |
|--|---|--|
| Sensitivity | 0.35 | 0.68 |
| Specificity | 1 | 1 |
| Positive Predictive Value | 1 | 1 |
| Negative Predictive Value | 0.55 | 0.72 |
| Accuracy | 0.64 | 0.92 |
| Odds Ratio | 109.2 | 432.1 |
| Chi Square | 42.5 | 109.5 |
| BL Median (min, max) titer log ₁₀ /mL, Influenza A and B | 3.13 (0.75, 6.75) (n = 44) | 4.60 (1.60, 7.69) (n = 86) |
| 48 hour Median (min, max) titer log ₁₀ /mL, Influenza A and B | 1.27 (0.53, 5.17) (n = 44) | 2.79 (1.58, 6.26) (n = 86) |
| Time-weighted Δ in Titer from BL to 48 hours (median ± 95% CI log ₁₀ /mL) | -1.51 (-1.85, -1.04) (n = 86) | -1.04 (-1.23, -0.79) (n = 86) |
| Proportion of subjects with negative titers at 48 hours | 86% (TCID ₅₀ /mL <0.5) | 33% (vp/mL <1.58) |
| Time point at which 100% of subjects tested were negative | 96 hours | 13 days |

A direct correlation between Δ from BL to 48 hours in TCID₅₀ and QPCR (r² = 0.56, p<0.001) was seen. Similar predictors of Δ from BL in titers were identified: BL TCID₅₀ (log₁₀/mL) or BL vp (log₁₀/mL), BL G3/4 lymphopenia and vaccination status or BL steroid use.

CONCLUSIONS: TCID₅₀ and QPCR showed complementary characteristics for diagnosis and follow up of influenza in this study. Δ in titers from BL to 48 hours was similar. QPCR was more sensitive and accurate but may detect dysfunctional viruses. Their usefulness to assess response to therapy deserves further study.

INTRODUCTION

- Real-time polymerase chain reaction (RT-PCR) or tissue culture can confirm influenza diagnosis
- Change from baseline in quantitative RT-PCR (QPCR) or tissue culture infective dose (TCID₅₀) can quantify changes in viral shedding with treatment
- The suitability, performance, and predictive value of each test has not been adequately studied or compared in randomized clinical trials of influenza
- Peramivir is an intravenous neuraminidase inhibitor (NAI) approved in Japan and South Korea for treatment of influenza and in Phase 3 trials in the rest of the world
- In a large randomized multicenter Phase 3 trial of peramivir treatment (BCX1812-303),¹ both of these methods were used to measure viral dynamics
- We used data from BCX1812-303¹ to compare QPCR and TCID₅₀ for their performance characteristics and predictive value in influenza diagnostics and treatment

METHODS

Study Subjects

- ≥12 years old
- Hospitalized with clinical signs/symptoms of influenza and fever
- Broad entry criteria, including those with previous antiviral treatment and >48 hours of illness

Study Design and Treatment

- Open-label, randomized trial at 59 hospitals in 5 countries between October 2009 and October 2010 during local pandemic influenza activity
- Randomly assigned to 5 days treatment with IV peramivir 300 mg BID or 600 mg QD
- Stratified for length of prior illness (≤48 hours vs >48 hours)
- Concomitant antivirals or high-dose corticosteroids prohibited
- Subjects without clinical resolution by day 5 or with detectable virus by RT-PCR on day 4 (if locally available) could continue IV 600 mg QD for 5 more days

Study Assessments

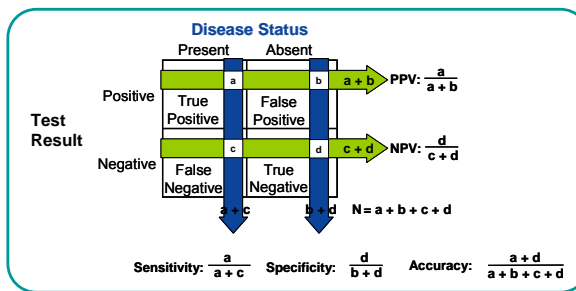
- Nasopharyngeal swabs were collected at baseline, 12, 24, 36, 48, 60, 72, 84, 96, and 108 hours post-enrollment and day 10
- Samples were transported to a central laboratory and quantitative viral burden was assessed by RT-PCR as virus particles (vp)/mL of viral transport medium and TCID₅₀/mL using MDCK cells for culture
- Confirmed influenza was defined as having a positive central laboratory test by viral culture, PCR, and/or serology, and/or a positive local test

Statistical Analysis

- The primary endpoint in BCX1812-303 was time-weighted change in TCID₅₀/mL from baseline to 48 hours
- A secondary endpoint was time-weighted change in QPCR (vp/mL) from baseline to 48 hours
- Negative titers were log₁₀ TCID₅₀/mL of 0.5 for influenza A and B and log₁₀ vp/mL of 1.58 for influenza A and 1.49 for influenza B
- The performance capabilities of a given diagnostic test can be deduced from the testing of a random sample of subjects with a determined disease status (present or absent) using the following model:

- A test’s usefulness is based on these performance characteristics
 - Sensitivity: The ability of a test to identify the subjects with the disease by a positive test
 - Specificity: The ability of a test to identify the subjects without the disease by a negative test
 - Positive predictive value (PPV): The likelihood of having the disease when having a positive test
 - Negative predictive value (NPV): The likelihood of not having the disease when having a negative test
 - Accuracy: The ability of a diagnostic test to identify the subjects with the disease by a positive test (true positives) and the subjects without the disease by a negative test (true negatives)

- The performance characteristics were calculated using the following descriptive statistics:



- We made a formal assessment comparing the 2 test methods using the Pearson’s coefficient correlating the time-weighted change from baseline in viral titers
- We used multiple logistic regression with stepwise selection to identify baseline predictors of time-weighted change from baseline

RESULTS

Study Subjects

- 234 subjects were randomized and 230 were treated for 5 or 10 days
- Demographics and baseline characteristics were similar for the 2 treatment groups

| | 300 mg BID (n=117) | 600 mg QD (n=117) | Total (n=234) |
|---|-----------------------|----------------------|------------------|
| Age, median yr (min, max) | 48.3 (14, 93) | 48.4 (15, 92) | 48.3 (14, 93) |
| Gender, n (%) | | | |
| Male | 43 (37) | 52 (44) | 95 (41) |
| Female | 74 (63) | 65 (56) | 139 (59) |
| BMI, median kg/m ² (min, max) | 30.1 (17, 70) | 29.5 (18, 56) | 29.7 (17, 70) |
| Influenza vaccination, n (%) | 41 (35) | 36 (31) | 77 (33) |
| ICU admission at baseline, n (%) | 18 (15) | 21 (18) | 39 (17) |
| APACHE II score, mean (min, max) | 14 (4, 28) | 17 (9, 28) | 15 (4, 28) |
| Abnormal chest x-ray at baseline, n (%) | 31 (26) | 28 (24) | 59 (25) |
| Duration of illness at baseline, n (%) | | | |
| ≤ 48 h | 18 (15) | 16 (14) | 34 (15) |
| >48 h | 99 (85) | 101 (86) | 200 (85) |
| Subjects receiving antivirals at baseline, n (%) | 88 (75) | 82 (70) | 170 (73) |
| Subjects receiving corticosteroids at baseline, n (%) | 52 (44) | 68 (58) | 120 (51) |

APACHE = acute physiology and chronic health evaluation

- 127 subjects had confirmed influenza by at least one diagnostic method

| | 300 mg BID (n=57) | 600 mg QD (n=70) | Total (n=127) |
|---------------------------------------|----------------------|---------------------|------------------|
| Confirmed influenza by subtype, n (%) | | | |
| Influenza A – 2009 H1N1 | 43 (75) | 51 (73) | 95 (74) |
| Influenza A – indeterminate | 10 (17) | 16 (23) | 26 (20) |
| Influenza B | 2 (3) | 1 (1) | 3 (2) |
| Influenza A + B | 1 (2) | 1 (1) | 2 (2) |
| Influenza indeterminate subtype | 1 (2) | 1 (1) | 2 (2) |
| Confirmed influenza by test*, n | | | |
| QPCR | 43 | 54 | 97 |
| Viral culture | 22 | 26 | 48 |
| Serology | 4 | 3 | 7 |
| Local lab (QPCR or culture) | 47 | 52 | 99 |

*Could be confirmed by >1 test

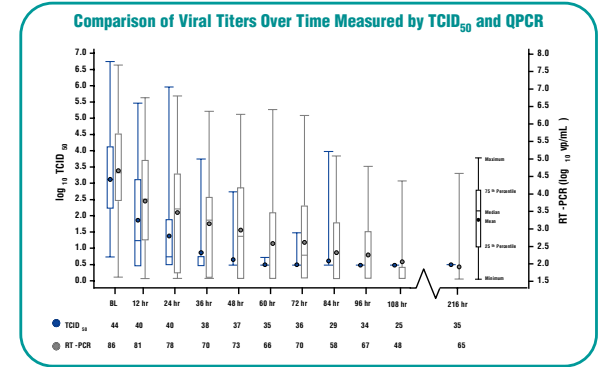
Virology

- Similar antiviral effects were seen in both the 300 mg BID and 600 mg QD treatment groups
- Over time, viral shedding in subjects with positive baseline and post-baseline viral titers was similarly reduced in both treatment groups when measured by TCID₅₀ and QPCR

| | 300 mg BID n=20 | 600 mg QD n=24 |
|---|----------------------|----------------------|
| Titers measured by viral culture (log ₁₀ TCID ₅₀ /mL) | | |
| Baseline, median (range) | 3.13 (0.75, 6.75) | 3.13 (0.75, 5.00) |
| Change to 48 h, median (95% CI) | -1.66 (-2.32, -0.61) | -1.47 (-1.89, -0.75) |
| Change to 108 h, median (95% CI) | -2.02 (-3.38, -1.08) | -2.01 (-2.66, -1.36) |
| Change to 216 h, median (95% CI) | -2.29 (-3.55, -1.67) | -2.09 (-2.85, -1.44) |
| Titers measured by RT-PCR (log ₁₀ vp/mL) | n=37 | n=49 |
| Baseline, median (range) | 4.67 (2.92, 7.69) | 4.36 (1.60, 6.97) |
| Change to 48 h, median (95% CI) | -1.00 (-1.52, -0.77) | -1.07 (-1.24, -0.67) |
| Change to 108 h, median (95% CI) | -1.65 (-1.99, -1.45) | -1.59 (-1.86, -1.24) |
| Change to 216 h, median (95% CI) | -2.15 (-2.38, -1.96) | -1.98 (-2.22, -1.76) |

Comparison of Diagnostic Tests

- Measurement of viral titers by TCID₅₀ at baseline (BL) and post-baseline timepoints in all confirmed influenza subjects with paired samples was similar to that measured by QPCR



- Using the previously described model and the data from BCX1812-303, the usefulness of the TCID₅₀ and QPCR diagnostic tests are shown:

| TCID ₅₀ Result | Confirmed Influenza | | Total |
|---------------------------|---------------------|-----|-------|
| | Yes | No | |
| Positive | 44 | 1* | 45 |
| Negative | 83 | 103 | 186 |
| | | 127 | 230 |

*This should be 0 but is set at 1 to calculate values

- When the performance of the two tests for diagnosis of influenza and post-treatment assessment of viral shedding was compared, QPCR demonstrated greater sensitivity, negative predictive value, and accuracy
- However, when measuring viral titers after therapy, the proportion of patients testing positive was higher, and the duration of positivity was longer with QPCR, compared to TCID₅₀; some of these results may reflect detection of non-viable viruses

| | TCID ₅₀ Log ₁₀ vp/mL n=230 | Quantitative PCR: Log ₁₀ vp/mL n=230 |
|--|---|--|
| Characteristic | | |
| Sensitivity | 0.35 | 0.68 |
| Specificity | 1 | 1 |
| Positive Predictive Value | 1 | 1 |
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| Accuracy | 0.64 | 0.82 |
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- There was a direct correlation between time-weighted change from baseline to 48 hours between results of the 2 diagnostic tests (r² = 0.56, p<0.001)
- The predictors of time-weighted change from baseline were similar for the 2 tests:

| Predictor | Time weighted change from baseline measured with | |
|---|--|---------|
| | TCID ₅₀ | QPCR |
| Baseline viral titer | P<0.001 | P=0.06 |
| Presence of Grade 3/4 lymphocytes at baseline | P=0.001 | P<0.001 |
| Influenza vaccination status | P=0.008 | - |
| Baseline corticosteroid use | - | P=0.020 |

DISCUSSION

- Clinicians and regulatory authorities are interested in rapid and accurate quantitative methods for the diagnosis and follow up of influenza
- The comparison of culture and QPCR using data from a randomized clinical trial of influenza treatment with peramivir and a simple statistical model suggests that the 2 tests have complementary characteristics for diagnosis and follow up of influenza
- Although QPCR yielded more positive results at baseline and over time suggesting it may be a more robust virologic endpoint for clinical studies, QPCR may detect non-viable viruses²
- Since the assays are correlated, QPCR positive but culture negative results may represent replication below culture’s limit of detection

CONCLUSIONS

- TCID₅₀ and QPCR were found to be complementary methods for diagnosis and follow up of influenza in a large, randomized clinical trial of IV peramivir treatment
- For the diagnosis of influenza in this study, QPCR showed better sensitivity and accuracy
- The 2 tests demonstrated similar results for time-weighted change from baseline in viral titers and were directly correlated
- Baseline predictors of change from baseline in viral shedding were similar for the 2 tests

ACKNOWLEDGMENTS

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REFERENCES

- Ison M, O’Neil B, Elder J, et al. Safety and antiviral effect of multi-day therapy with IV peramivir 300mg BID or 600mg QD in hospitalized influenza subjects. Presented at ICAAC, Chicago, September 2011
- Ward, C.L., Dempsey, M.H., Ring, C.J., Kempson, R.E., Zhang, L., Gor, D., Snowden, B.W. and Tisdale, M. Design and performance testing of quantitative real time PCR assays for influenza A and B viral load measurement. J Clin Virol 2004; 29:179-88.