# Diagnostic accuracy of the Fujifilm/Wako (1,3)-beta-D-glucan assay in the diagnosis of Pneumocystis jirovecii pneumonia

Authors: **Tjomme van der Bruggen, Hendrik Gremmels, Pieter-Jan A. Haas**

Department of Medical Microbiology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, J.T.vanderbruggen@umcutrecht.nl

## Background

Pneumocystis jirovecii is an opportunistic fungal pathogen that can cause severe and life-threatening pneumonia (PJP), in immunocompromised patients. At present, the diagnosis of PJP pneumonia is performed on specimens obtained directly from the respiratory tract, either sputum or Broncho-Alveolar Lavage (BAL) fluid. Obtaining these samples may be invasive and difficult in patients that suffer from respiratory decompensation.

β-D-glucan (BDG) is a major constituent of fungal and yeast cell walls that is released into the blood during PJP. A plasma BDG measurement for the diagnosis of PJP could potentially reduce diagnostic delay and reduce the number of invasive BAL procedures

## Methods

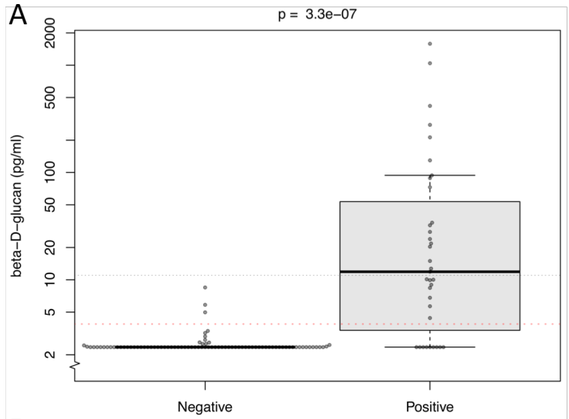
In a retrospective study, we identified 120 patients that underwent a BAL procedure following clinical suspicion of PJP from whom serum was available for BDG testing. Only serum samples obtained within ± 14 days of a BAL procedure were included. Patients with confirmed candidemia or a positive serum galactomannan were excluded from the present study. Clinical diagnosis of PJP was established by reviewing individual cases, taking into account PCR results, cytology, co-morbidities, alternative microbiological diagnoses and response to antifungal treatment. BDG in serum samples was measured using the Fujifilm/Wako β-Glucan Test, on a Toxinometer MT-6500.

Table 1: Patient Characteristics

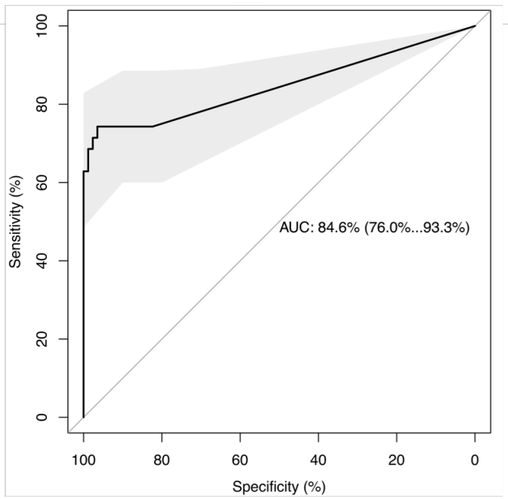
| | Negative | Positive | p-val |
|---|---|---|---|
| n | 85 | 35 | |
| PCR Material (%) | | | 0.472 |
| BAL | 42 (49.4) | 15 ( 42.9) | |
| Flush | 35 (41.2) | 14 ( 40.0) | |
| Sputum | 8 (9.4) | 6 ( 17.1) | |
| PJP PCR = Pos (%) | 16 (18.8) | 35 (100.0) | <0.001 |
| PJP CT | 40.00 [40.00, 40.00] | 30.25 [28.73, 32.41] | <0.001 |
| Time delay (days) | -0.48 (2.86) | -1.23 (5.21) | 0.316 |
| BDG (pg/ml) | 2.36 [2.36, 2.36] | 11.89 [3.38, 53.50] | <0.001 |
| Gender = F (%) | 22 (25.9) | 12 ( 34.3) | 0.480 |
| Age (Y) | 55.15 [31.15, 62.48] | 53.00 [40.83, 66.42] | 0.463 |
| Disease cat (%) | | | 0.098 |
| CID | 1 (1.2) | 1 (2.9) | |
| HIV | 11 (12.9) | 9 (25.7) | |
| Hematological | 53 (62.4) | 15 (42.9) | |
| ICU | 1 (1.2) | 0 (0.0) | |
| Oncological | 5 (5.9) | 3 (8.6) | |
| Rheumatological | 11 (12.9) | 2 (5.7) | |
| Solid organ transplant | 3 (3.5) | 5 (14.3) | |
| CD4 Count (cells/ml) | 215.50 [74.50, 476.50] | 41.00 [17.50, 189.50] | 0.015 |
| Corticosteroids = Yes (%) | 37 (43.5) | 17 (48.6) | 0.762 |
| Chemotherapy = Yes (%) | 66 (78.6) | 22 (62.9) | 0.121 |
| Fungal Disease (%) | | | 0.008 |
| Maybe | 8 (9.4) | 2 (5.7) | |
| No | 48 (56.5) | 30 (85.7) | |
| Yes | 29 (34.1) | 3 (8.6) | |
| Start antifungal = Yes (%) | 45 (52.9) | 8 (22.9) | 0.005 |
| PJP Radiology (%) | | | <0.001 |
| Maybe | 3 (3.5) | 6 (18.8) | |
| No | 72 (84.7) | 4 (12.5) | |
| Yes | 10 (11.8) | 22 (68.8) | |
| LDH (U/L) | 537.05 (1195.68) | 442.45 (234.52) | 0.659 |

*Values between parentheses () indicate the standard deviation and are preceded by the mean; values between brackets [] indicate the interquartile range and are preceded by the median. The p-values are obtained by Chi-square test, unpaired Student's t-test or Mann-Whitney test, where applicable*

A




B




C

| | Pos | Neg | | |
|---|---|---|---|---|
| BDG Pos | 25 | 3 | 29 | PPV 89% |
| BDG Neg | 10 | 82 | 91 | NPV 89% |
| | 35 | 85 | 120 | |
| | Sens 72% | Spec 96% | | |

## Results

35 PJP infections were identified by case review. Positive cases were more likely to have a diagnosis of HIV, typical CT-findings, and lower CD4+ T-cell counts compared to controls (Table 1). Interestingly, in 16/85 negative subjects, Pneumocystis jirovecii was detected by PCR.

Median BDG values were higher in cases than in controls (2.4 vs 11.9 pg/ml, $p = 3 \times 10^{-7}$, Panel A).

ROC curve analysis showed an Area-Under-Curve (AUC) of 84.6% (Panel B), with an optimal cutoff (Youden statistic) of 5 pg/ml. This is significantly lower than the cut-off value of 11 pg/ml recommended by the manufacturer. A significant number (9/35) of positive patients had no detectable BDG in serum. The sensitivity, specificity, positive and negative predicted value were 72%, 96%, 89% and 89% respectively, at the optimal cutoff of 5 pg/ml (Panel C).

## Conclusion

The Fujifilm/Wako BDG assay shows robust diagnostic accuracy in the detection of PJP, albeit with a limited sensitivity.

The test may be suitable as a rapid and minimally invasive procedure in patients suspect of having PJP. Negative test results in high-suspicion patients should however always be followed up by further diagnostic procedures