Reassessing PML Risk in Multiple Sclerosis: Impact and Limitations of Anti-JCV Antibody Assay Selection

JC Polovina, D. Landi, G. Mataluni, C. Nicoletti, C. Dionisi, V. Di Gianvito, A. Miscioscia, M. Campobasso, G. Marfia [1]

[1] *Multiple Sclerosis Research Unit, Department of Systems Medicine - Tor Vergata University of Rome - Roma*

INTRODUCTION

Natalizumab (NTZ) therapy has been associated with an increased risk of developing progressive multifocal leukoencephalopathy (PML) caused by reactivation of John Cunningham poliomavirus (JCV) in people with Multiple Sclerosis (pwMS) [1].

To minimize this risk, a reference enzyme-linked immunosorbent assay for the detection of JCV antibodies (Stratify JCV - ST) has been adopted to **stratify patients' PML risk** [2].

Recently, a new assay (Immunowell JCV - IT) has entered clinical use alongside the approval of biosimilar NTZ.

AIMS

- To reassess PML risk using the IT in comparison with the established reference assay ST,
- To evaluate whether either test correlates with peripheral viral replication.

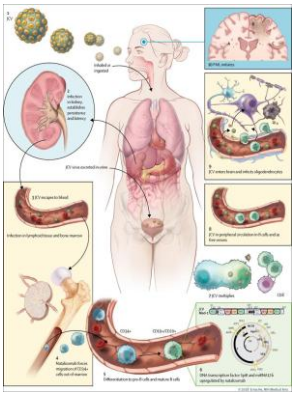

Fig. 1 Stages of PML pathogenesis in patients treated with natalizumab [3]

METHODS

- We enrolled patient with a diagnosis of Multiple Sclerosis, treated with NTZ and followed up at the MS Center of Tor Vergata University Hospital, Rome;
- Two serum samples were collected the same day from each patient to perform both Stratify JCV and ImmunoWELL JCV tests;
- Based on established cut-off values, we classified patients into null, low, intermediate, or high PML risk (0, 1, 2, 3) according to the results of each test;
- We compared the distribution frequencies across risk categories for the two tests with McNemar-Bowker test;
- In a subgroup of patients, urine samples were collected the same day for JCV DNA PCR testing.

RESULTS

We enrolled 147 pwMS:

- 118 ♀ 29 ♂
- Mean age 40.67 ±9.78 years
- Median infusions 67, range 1–167
- Mean and median values for ImmunoWELL test: 0.69 and 0.31 ±0.86
- Mean and median Stratify test values: 0.46 and 0.00 ±0.82

Risk Classes		ImmunoWELL				Total
		0	1	2	3	
Stratify	0	40	47	2	0	89
	1	1	19	6	2	28
	2	0	1	9	6	16
	3	0	0	0	14	14
Total		41	67	17	22	147

Fig. 2 Contingency Table Showing Test Concordance.

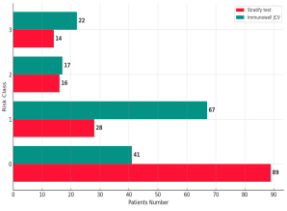

Fig 3. Distribution of patients by risk class according to Stratify test and Immunowell JCV.

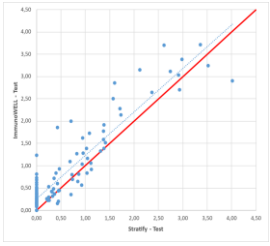

Fig. 4 Correlation of the ImmunoWELL JCV test to the Stratify JCV test.

- **Index values** differed significantly between the two tests: **ImmunoWELL** showed consistently **higher values** (Wilcoxon signed-rank test, **p < 0.001**).
- **45%** of patients (n = 65) were classified into **discordant risk classes**
- **Risk class frequency distribution** comparison via McNemar-Bowker test highlighted a statistically significant difference (**p<0.001**), with the largest discrepancies at the **lower and upper risk extremes** of the risk spectrum.
- Both Wilcoxon and Sign tests confirmed that ImmunoWELL assigned significantly **higher risk classes** than Stratify (**p < 0.001**).
- In the urine PCR subgroup (n = 44; 13 ST+, 31 ST–; 23 IT+, 21 IT–), **only one patient** tested **positive** for JCV DNA, but had no detectable viremia, indicating replication was limited to the renal reservoir.

CONCLUSIONS

Our data show that the ImmunoWELL assay tends to assign **higher PML risk categories** (though mainly within lower-risk categories), which could impact clinical decision-making.

However, the higher number of seropositive cases, not accompanied by evidence of viral replication, may reflect increased sensitivity at the expense of specificity, possibly capturing **broader immune activation** rather than active infection, highlighting the need for more specific **biomarkers of viral replication**.

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