Stratify and ImmunoWELL JCV tests performances in a real-world Multiple Sclerosis cohort: are we really optimizing healthcare resources allocation using biosimilar Natalizumab?

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Introduction

In recent years, generic and biosimilar molecules of originator disease modifying treatments (DMTs) for multiple sclerosis (MS) have become available, theoretically contributing to reduce healthcare costs [1]. Natalizumab was initially marketed by Biogen Inc with brand name Tysabri™ [2]. In 2023 EMA provided a favorable opinion for the marketing authorization of a Natalizumab biosimilar product commercialized by Sandoz GmbH with brand name Tyruko™ [3], now available in Europe. During Natalizumab treatment, John Cunningham virus (JCV) index monitoring is used to assess the risk of developing progressive multifocal leukoencephalopathy (PML) [4]. Currently, two JCV serology tests are commercially available: Stratify JCV® DxSelect™ assay (Stratify, linked to Tysabri) [5] and ImmunoWELL JCV IgG test (ImmunoWELL, associated with Tyruko) [3]. The aim of the present study is to compare the accuracy (in terms of qualitative results and titer concordance) of these JCV tests, assessing the potential clinical implications of their adoption in a real-world setting.

Methods

Between May 2024 and February 2025, we enrolled at IRCCS San Raffaele Hospital (Milan, Italy) a cohort of consecutive MS patients with a clinical indication to JCV serostatus determination, since being treated with or candidate to receive Natalizumab. JCV serology was cross-sectionally assessed using both Stratify and ImmunoWELL with blood samples acquired on the same day. For each patient, any previous or subsequent available JCV serology test was also collected. Collected samples were analyzed by respective centralized laboratories (detailed Stratify and ImmunoWELL methodology is available elsewhere) [3, 5]. According with previous evidence, JCV index obtained with Stratify was classified as negative or, if positive, as low-, medium-, and high-positive for values < 0.9, 0.9–1.5, and > 1.5 respectively [6, 7]. Thresholds were corrected to < 0.8, 0.8–1.4, and > 1.4 for ImmunoWELL, according to current proposed cut-off [3]. Statistical analyses were performed using the SPSS™ software version 25.0 (IBM - Armonk, NY - USA). JCV serostatus results (i.e., negative vs. positive) obtained with Stratify and ImmunoWELL were compared using McNemar test. Wilcoxon Signed-Rank Test was used to compare JCV index distributions; McNemar test was then applied for post hoc paired comparisons. Considering ImmunoWELL-to-Stratify relative specificity value (i.e., 74%) declared in the biosimilar Natalizumab EPAR document released by EMA in 2023 [3], a pre-planned simple size of 100 patients would have allowed to detect a discrepancy in JCV serostatus results with a 95% power and 5% α-error.

Results

Simultaneous Stratify and ImmunoWELL samples were obtained in 113 MS patients (mean age 36.7 ± 10.9 years, female-to-male ratio 79/34, mean disease duration 6.4 years - range 0.0–37.9, median EDSS 1.5 - range 0–6.5). In 91/113 patients JCV serology was performed because treatment with Natalizumab was considered. The remaining 22/113 patients were already receiving Natalizumab (average therapy duration = 7.3 ± 5.9 years), all with at least one previous Stratify available (median number of tests per patient = 11, range 1–26): 16/22 patients always tested negative; 6/22 subjects had at least one previous positive result. Only 6/91 patients eligible for Natalizumab had a previous Stratify available (one measurement per patient): 3/6 with a negative JCV serostatus and 3/6 with a previous positivity. Considering Stratify test, we found 54/113 (47.8%) patients to have a negative JCV serostatus, while 59/113 (52.2%) were positive. Using ImmunoWELL we found a significantly higher proportion of JCV-positive patients: 29/113 (25.7%) had a negative serology, while 84/113 (74.3%) tested positive (p<0.001). The two tests showed discordant results in 27/113 (23.9%) patients: 26/27 with negative Stratify and positive ImmunoWELL; one patient was negative to ImmunoWELL with a positive Stratify (**Table 1**). Considering those 28 patients with previous Stratify available, we found a good concordance with Stratify assessment performed in our study: 21/28 patients had a negative in-study Stratify (19/21 also showed a negative JCV serology at every previous measurement, 2/21 patients had at least one previous low-positive result); 7/28 patients had instead a positive in-study Stratify (7/7 known for previous JCV positivity). Eight patients had a follow-up ImmunoWELL measurement (obtained 6 months after in-study test), showing poor concordance: at repetition, 4/8 patients presented a negative result (1/4 previously positive); 4/8 had instead a positive result (2/4 previously negative). We then compared JCV index distributions obtained using the proposed cut-off for the two tests: observed differences in terms of JCV-negativity frequency directly resulted in a different proportion of low-positive patients (19/113 for Stratify vs. 35/113 for ImmunoWELL, p = 0.010). The performances were instead similar considering medium positive (10/113 for Stratify vs. 14/113 for ImmunoWELL, p=0.481) and high-positive (30/113 for Stratify vs. 35/113 for ImmunoWELL, p=0.125) subjects (**Figure 1**).

		ImmunoWELL		
		Neg	Pos	
Stratify	Neg	28 (24.8)	26 (0.23)	54 (47.8)
	Pos	1 (00.9)	58 (0.51)	59 (52.2)
		29 (25.7)	84 (74.3)	113 (100)

Table 1. Stratify and ImmunoWELL serostatus contingency table. Reported values in each cell represent the absolute number of patients with the corresponding percentage in brackets.

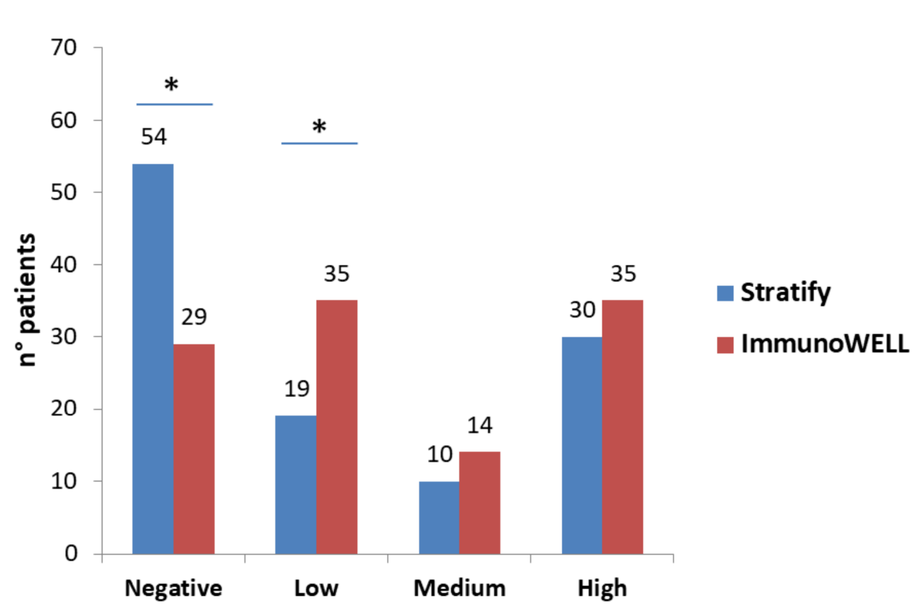

Figure 1. JCV index distributions obtained with Stratify and ImmunoWELL. *Indicates significant comparisons.

Discussion and Conclusions

To the best of our knowledge this is among the first reports directly comparing Stratify and ImmunoWELL performance in a real-world MS cohort. Gelissen and colleagues recently reported their experience on 94 Natalizumab-treated MS patients tested with both assays, describing a higher incidence of JCV positivity (55.4% vs. 22.3%) and the possibility of global higher index values with ImmunoWELL [8]. Consistently, Varley et al. found 119 out 250 (47.6%) patients negative on Stratify to be subsequently positive on ImmunoWELL after switching from originator to biosimilar Natalizumab [9]. Accordingly, our findings point out a ***significantly different ability of the two tests to discriminate between a negative and a low-positive JCV status: when ImmunoWELL is used, a prominent reduction in the number of JCV-negative patients is observed, counterbalanced by an increase in the proportion of low-positive subjects***. Long-standing clinical experience indicates PML risk is extremely low in Natalizumab-treated patients with negative Stratify results [10, 11]. Therefore, in our opinion, the ***discrepant performances could be attributed to a lower specificity of ImmunoWELL*** rather than to a higher sensitivity of this method. Supporting this hypothesis, the proportion of ImmunoWELL-positive/Stratify-negative patients identified in our cohort (23.0%) roughly corresponds to ImmunoWELL-to-Stratify relative specificity (74%), declared in the biosimilar Natalizumab EPAR document [3]. Clinical implications related to ImmunoWELL lower accuracy may be particularly significant. In our cohort, 24/54 (44.4%) Stratify-negative patients would have been incorrectly classified as JCV positive: of those 24 subjects, 8 patients already receiving Natalizumab would have been probably directed toward a therapy switch or a more frequent neuroradiological monitoring, and the remaining 16 subjects, eligible for Natalizumab, would have been likely advised to start another DMT. Therefore, ImmunoWELL use not only could determine an unmotivated denial of an effective and safe therapy such as Natalizumab but, potentially, could also negatively balance (due to alternative therapeutic strategies costs and more frequent MRIs) the economic advantages offered by biosimilar products, although specific studies are warranted to properly assess pharmacoeconomic implications. Longitudinal studies involving the repeated use of both tests are necessary to more accurately describe the extent of these negative implications. At present, ***unless ImmunoWELL optimization, we suggest to consider biosimilar Natalizumab use only in patients already proved to be JCV positive with Stratify***.

Bibliography and Disclosures

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