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ORIGINAL RESEARCH

Threshold-Dependent Discordance Between STRATIFY JCV™ and IMMUNOWELL™ Assays in Relapsing Multiple Sclerosis

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ABSTRACT

***Introduction*:** Anti-JC virus (JCV) antibody testing is central to progressive multifocal leukoencephalopathy (PML) risk stratification in patients treated with natalizumab. While STRATIFY JCV™ is the historical reference assay, IMMUNOWELL™ is increasingly used in the context of biosimilar adoption. We aimed to determine whether inter-assay discordance reflects true biological divergence or threshold-dependent interpretative effects within clinically relevant PML risk categories.

***Methods*:** In this single-center observational study, 250 consecutive patients with relapsing multiple sclerosis (RMS) underwent same-day paired STRATIFY and IMMUNOWELL testing. Agreement was evaluated under two interpretative frameworks: (i) manufacturer-defined analytical cutoffs and (ii) clinically adopted PML risk-based thresholds. Quantitative comparability was assessed using Pearson correlation and Bland–Altman analysis.

***Results*:** Using analytical cutoffs, concordance was 78.0% ($\kappa = 0.55$), with discordance predominantly in borderline index ranges. When applying clinically adopted risk thresholds, concordance increased to 93.2% ($\kappa = 0.73$). Index values correlated strongly ($r = 0.922$; $p < 0.0001$). Bland–Altman analysis showed minimal mean bias (+0.06), with wider deviations confined largely to low-to-intermediate index values rather than high-risk profiles.

***Conclusions*:** Inter-assay discordance between STRATIFY and IMMUNOWELL is largely threshold-driven and substantially attenuated when applying clinically meaningful PML risk categories. These findings support practical interchangeability for routine monitoring in the biosimilar era, while underscoring the need for cautious interpretation of borderline results.

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Key Summary Points

Why carry out this study?

Progressive multifocal leukoencephalopathy (PML) remains a key safety concern in patients treated with natalizumab, and anti-JC virus (JCV) antibody testing is central to risk stratification in clinical practice.

As IMMUNOWELL™ is increasingly used alongside STRATIFY JCV™, understanding whether discordant results reflect analytical differences or clinically meaningful disagreement has become increasingly relevant.

This study assessed the agreement and clinical interchangeability of STRATIFY JCV™ and IMMUNOWELL™ in patients with relapsing multiple sclerosis (RMS).

What was learned from the study?

In this cohort of 250 patients with RMS, agreement between assays improved substantially when clinically adopted risk thresholds were used instead of manufacturer-defined analytical cutoffs.

STRATIFY JCV™ and IMMUNOWELL™ index values were strongly correlated, with only minimal overall bias between assays.

Most discordant classifications were observed in borderline index ranges, indicating that discordance is largely threshold-dependent.

These findings support the use of both assays for routine monitoring in patients treated with natalizumab, while reinforcing the need for cautious interpretation of borderline results.

INTRODUCTION

Natalizumab (Tysabri®; Biogen) was originally approved in 2004 as an intravenous infusion for the treatment of relapsing multiple sclerosis (RMS) and, more recently, is also available as a subcutaneous formulation [1]. In 2023, the first biosimilar of natalizumab, Tyruko® (Sandoz), received regulatory approval in both the United States and Europe [2].

Despite its high efficacy, natalizumab's use in patients with MS is tempered by the risk of progressive multifocal leukoencephalopathy (PML), a rare but often devastating central nervous system (CNS) infection caused by reactivation of the John Cunningham virus (JCV) [3–5].

To mitigate this complication, detection of anti-JCV antibodies has become a key element of risk stratification in clinical practice. Since its introduction, the STRATIFY JCV™ enzyme-linked immunosorbent assay (ELISA), developed by Biogen, has represented the reference assay for routine monitoring. This test, using JCV virus-like particles as antigen, classifies patients as seronegative or seropositive, and, in the latter case, further stratifies the risk by quantifying antibody index levels, which are directly correlated with the probability of developing PML [6, 7].

Following the approval of Tyruko®, the ImmunoWELL™ JCV IgG assay was introduced to support risk stratification in patients treated with the natalizumab biosimilar [2].

The ImmunoWELL™ JCV assay employs a two-step ELISA format, consisting of an initial screening followed by a confirmatory inhibition test when results fall within an indeterminate range. Results are classified as negative if the screening index ≤ 0.20, positive if >0.50, and indeterminate if >0.20 to ≤ 0.50. When indeterminate, confirmation is performed via percent inhibition (>45% indicating positivity in the confirmatory assay) [8]. For comparison, studies using the STRATIFY test have also proposed practical reference ranges for clinical risk stratification: baseline indices <0.20 tend to denote stable seronegativity, while indices >0.90 are associated with stable seropositivity and increased risk of PML; intermediate values (for example, >0.20–≤ 0.90, or ≤ 1.5 in some study schemes) are used to categorize moderate risk or to trigger closer monitoring [6].

A recent comparative study conducted by our group demonstrated a high concordance

(94.2%) between STRATIFY and IMMUNOWELL, with a strong correlation of antibody index values ($r = 0.79$), confirming that IMMUNOWELL can be considered a reliable tool for clinical monitoring [9].

Although previous studies have reported high overall concordance between STRATIFY and ImmunoWELL assays, some inconsistencies in serostatus classification have also been described, particularly in patients with low or intermediate anti-JCV antibody index values [9–12]. These discrepancies may partly reflect methodological differences between the two assays, including the use of distinct antigens, assay formats, and, most importantly, different cutoff values and decision algorithms for defining seronegativity, indeterminate results, and seropositivity. As a result, patients falling near the diagnostic thresholds may be classified differently depending on the assay employed, with potential implications for longitudinal risk stratification and clinical decision-making. Moreover, published studies have been conducted in relatively heterogeneous cohorts and with variable analytical approaches, which may contribute to the variability in reported results [9–12]. Therefore, further comparative analyses performed in well-characterized real-world populations are warranted to better understand the sources of discordance between assays and to clarify their impact on JCV-related risk assessment in patients treated with natalizumab.

The present study provides a head-to-head comparison of STRATIFY JCV™ and IMMUNOWELL™ in patients with RMS, focusing on assay concordance and clinical interchangeability. Agreement was evaluated under two interpretative scenarios: manufacturer-defined analytical cutoffs and clinically adopted risk-based thresholds used for PML stratification, complemented by quantitative comparison of index values.

METHODS

Study Design

This comparative observational study was conducted at the Multiple Sclerosis Center of the University of Catania, from January 2025 to September 2025.

The primary endpoint was the inter-assay concordance between STRATIFY JCV™ and IMMUNOWELL™ in qualitative anti-JCV serostatus classification, assessed under two interpretative frameworks: (i) manufacturer-defined analytical cutoffs (including confirmatory inhibition testing for indeterminate results) and (ii) clinically adopted risk-based thresholds used in routine practice for PML stratification. Secondary endpoints included the quantitative relationship between continuous antibody index values and the characterization of discordant classifications (with particular attention to borderline ranges).

Eligible participants were adults (≥ 18 years) with a diagnosis of RMS, defined according to the Lublin classification of multiple sclerosis phenotypes [13]. All included patients underwent both STRATIFY and ImmunoWELL testing on the same day. The study population comprised patients currently treated with natalizumab, either intravenously or subcutaneously; treatment-naïve patients who were candidates for natalizumab initiation; and patients receiving other disease-modifying therapies (DMTs) who underwent anti-JCV antibody testing as part of routine screening for potential natalizumab eligibility. Demographic and disease-related variables, including age, sex, disease duration, and treatment status at the time of testing, were collected.

Exclusion criteria included prior exposure to immunosuppressive therapies, a history of PML, treatment with high-dose corticosteroids within 1 month prior to anti-JCV antibody testing, and incomplete clinical or laboratory data precluding reliable serological evaluation.

Blood samples were collected during scheduled clinical visits, and all study procedures were conducted in accordance with institutional guidelines and international ethical standards. The study protocol was reviewed and

approved by the Ethics Committee Catania 1, Italy (PO/891/2025). The study was conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments. All participants provided written informed consent prior to any study-related procedures.

Anti-JCV Antibody Testing

Each patient underwent blood sampling, and serum was divided into two aliquots: one tested with the STRATIFY-2 JCV™ DxSelect ELISA and the other using the IMMUNOWELL™ Anti-JCV ELISA. Both assays use a two-step ELISA format for the qualitative detection of anti-JCV IgG antibodies [14].

In the STRATIFY JCV test, microplate wells are coated with the JCV capsid protein VP1, assembled into virus-like particles (VLPs) serving as the capture antigen. Serum samples are added, allowing specific anti-JCV antibodies, if present, to bind to the immobilized antigen. After washing steps to remove unbound components, bound antibodies are detected by an enzyme-conjugated anti-human IgG. Addition of substrate generates a colorimetric reaction whose intensity, measured as optical density (OD), is compared with assay calibrators and expressed as a relative index [6, 7, 14, 15]. The STRATIFY assay applies defined cutoff values to classify samples: an index ≥ 0.40 is considered as positive, < 0.20 as negative, and intermediate values from ≥ 0.20 to < 0.40 are considered indeterminate. Samples within the indeterminate range undergo a confirmatory inhibition test; if the signal is reduced by > 45% in the presence of excess antigen (binding inhibition), the sample is classified as positive. This two-step method maximizes specificity by excluding false positives from the borderline range [7, 15].

The IMMUNOWELL assay also employs an ELISA-based methodology. The initial screening step detects antibody–antigen binding using a peroxidase-conjugated goat anti-human IgG and classifies samples as positive (> 0.50), negative (≤ 0.20), or indeterminate (> 0.20–≤ 0.50) for anti-JCV antibodies. Indeterminate samples, falling between the defined thresholds, undergo a confirmatory competitive inhibition assay in which chicken hyperimmune anti-JCV VP1 IgY is used to competitively inhibit human anti-JCV antibody binding; upon confirmation, an inhibition of > 45% is interpreted as positive [16].

Clinical Risk Stratification Based on Anti-JCV Antibody Index Values

In clinical practice, the interpretation of anti-JCV antibody index values has evolved beyond the analytical cutoffs defined by the manufacturers of the STRATIFY JCV™ and IMMUNOWELL™ assays. These indices are now widely used as clinical categories to stratify the risk of PML in patients treated with natalizumab [6, 17, 18].

For the STRATIFY JCV™ DxSelect™ assay, longitudinal and real-world studies have consistently identified an index of approximately 0.9 as a meaningful boundary between persistently seronegative and seropositive profiles. Patients with index values ≤ 0.9 are considered to be at very low risk of developing PML (estimated at < 0.1 cases per 1000 patients), particularly if they have never received prior immunosuppressive therapy. Risk increases with higher index values and longer natalizumab exposure, with indices 0.9–1.5 generally regarded as intermediate-risk profiles and indices > 1.5 associated with a substantially higher risk, with published estimates ranging from approximately one to several cases per 1000 patients depending on treatment duration, particularly beyond 24 months [17, 18].

Although the analytical threshold for seropositivity in STRATIFY is technically lower (0.4) [7, 15], the 0.9 boundary is applied in daily practice as a clinically validated marker of stable antibody positivity and long-term risk. These categories are grounded in extensive longitudinal cohorts and are reflected in regulatory risk-minimization materials and consensus guidance for natalizumab therapy [4, 11, 17–19].

Importantly, anti-JCV seropositivity should not be interpreted as an absolute contraindication to natalizumab therapy, but rather as one component of a multifactorial risk assessment integrating antibody index, treatment duration, prior immunosuppressant exposure, and

ongoing clinical and radiological monitoring [11, 17, 18].

With the IMMUNOWELL™ JCV IgG assay, introduced as an alternative ELISA platform and currently used for both natalizumab and its biosimilar formulations (e.g., Tyruko®), the same principle has been adopted, with numerical adjustments reflecting analytical differences between the assays. Data from the manufacturer and from recent comparative and real-world studies [8, 9, 16] indicate that index ranges of ≤ 0.8, >0.8 to ≤ 1.4, and >1.4 broadly correspond to the low-, intermediate-, and high-risk categories used in clinical decision-making. This alignment was formalized in recent regulatory documents, including the UK Tyruko® Risk Minimisation Materials, which explicitly recommend these categories for patient monitoring and counseling [8].

Statistical Analysis

The analysis was conducted in two stages, each including a full set of agreement and correlation metrics. In the first stage, a 2×2 contingency table (STRATIFY-positive/negative vs. IMMUNOWELL-positive/negative) was constructed using the manufacturer-defined analytical cutoffs (STRATIFY: 0.40; IMMUNOWELL: 0.50) to evaluate agreement under standard reference conditions. During this phase, the inhibition coefficient was also considered, and indeterminate samples were classified based on the outcome of the confirmatory inhibition assay. In the second stage, a corresponding table was generated applying the clinically used "real-world" risk thresholds (0.9 for STRATIFY and 0.8 for IMMUNOWELL) to distinguish low- versus intermediate- and high-risk serological profiles. This dual analytical approach enabled evaluation of concordance both under standard assay conditions and within the context of clinical risk stratification as applied in everyday practice.

For each stage, Cohen's kappa coefficient was calculated to assess inter-assay agreement beyond chance, with interpretation based on established benchmarks (e.g., $\kappa > 0.80$ indicating almost perfect agreement). Sensitivity and specificity of IMMUNOWELL relative to STRATIFY, considered as the reference, were also computed, while acknowledging the absence of a true gold standard in this context. McNemar's test was applied to discordant pairs to assess whether one assay tended to produce positive results significantly more frequently than the other one ($p < 0.05$ considered significant).

For quantitative index values, the linear correlation between the two assays was analyzed. Pearson's correlation coefficient (r) was calculated for STRATIFY versus IMMUNOWELL indices across all patients, and its significance was evaluated using a two-tailed test ($\alpha = 0.05$). To additionally examine the agreement in continuous values and detect any systematic bias, a Bland–Altman analysis was performed. For each patient, the difference between IMMUNOWELL and STRATIFY indices (IMMUNOWELL minus STRATIFY) was plotted against the mean of the two indices. The mean difference (bias) and the 95% limits of agreement (mean difference $\pm$ 1.96 standard deviations) were calculated.

All statistical analyses were performed using STATA version 18.0. Throughout the analysis, a p value < 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

The study enrolled 250 patients with RMS, including 183 women (73.2%) and 67 men (26.8%). At the time of blood sampling, 205 patients (82.0%) were receiving natalizumab (intravenous or subcutaneous), 32 (12.8%) were treatment-naïve and considered eligible to start therapy, and 13 (5.2%) were receiving other disease-modifying therapies (DMTs) and underwent anti-JCV antibody testing as part of routine screening for potential natalizumab eligibility. Demographic and clinical characteristics of the study population are summarized in Table 1.

Qualitative Concordance

In the first stage of analysis, qualitative agreement between the STRATIFY and

Table 1 Demographic and clinical characteristics of the study cohort

N = 250	
Female, *n* (%)	183 (73.2)
Male, *n* (%)	67 (26.8)
Age (years), mean ± SD	41.5 ± 11.7
Disease duration (years), mean ± SD	8.2 ± 7.4
Treatment characteristics at time of JCV testing, *n* (%)	
NTZ	205 (82.0)
Naïve to treatment	32 (12.8)
*Other DMTs**	13 (5.2)
No. of NTZ administrations**, mean ± SD	58.4 ± 33.9

NTZ natalizumab; *DMT* disease-modifying therapies; *SD* standard deviation

*Cladribine *n* = 6 (46.2% of DMT subgroup), dimethyl fumarate *n* = 3 (23.1%), interferon-beta *n* = 2 (15.4%), teriflunomide *n* = 2 (15.4%)

**Calculated for patients treated with NTZ (*n* = 205)

IMMUNOWELL assays was assessed using the manufacturer-defined analytical cutoffs (STRATIFY: 0.40; IMMUNOWELL: 0.50) (Table 2). Out of 250 patients, 195 (78.0%) showed concordant results between the two assays. Specifically, 67 patients (26.8%) were JCV-antibody-positive on both tests, while 128 (51.2%) were negative on both. Discrepancies were observed in 55 patients (22.0%), of whom 53 patients (21.2%) were STRATIFY-negative but IMMUNOWELL-positive, and only two patients (0.8%) were STRATIFY-positive but IMMUNOWELL-negative. Overall, the two assays demonstrated a concordance rate of 78.0%, with a Cohen's κ coefficient of 0.55 (95% CI 0.45–0.65), indicating moderate agreement according to standard interpretation benchmarks. McNemar's test confirmed a statistically significant imbalance between discordant pairs (53 vs. 2, $p < 0.001$), indicating that IMMUNOWELL tended to yield more positive results than STRATIFY.

In the second stage, qualitative agreement was reassessed using clinically adopted risk-stratification thresholds (STRATIFY: 0.90; IMMUNOWELL: 0.80) (Table 3). Out of 250 patients, 233 (93.2%) showed concordant results between the two assays. Specifically, 29 patients (11.6%) were JCV-antibody-positive on both assays, and 204 (81.6%) were negative on both. Discordant classifications were observed in 17 patients (6.8%), comprising 14 cases (5.6%) that were STRATIFY-negative but IMMUNOWELL-positive and three cases (1.2%) that were STRATIFY-positive but IMMUNOWELL-negative. Overall agreement thus reached 93.2%, with a Cohen's κ of

Table 2 Cross-tabulation of anti-JCV antibody results using manufacturer-defined analytical cutoffs

N (%)	IMMUNOWELL-positive	IMMUNOWELL-negative	Total
STRATIFY-positive	67 (26.8)	2 (0.8)	69 (27.6)
STRATIFY-negative	53 (21.2)	128 (51.2)	181 (72.4)
Total	120 (48.0)	130 (52.0)	250

Table 3 Cross-tabulation of anti-JCV antibody results using clinically adopted risk thresholds

N (%)	IMMUNOWELL-positive	IMMUNOWELL-negative	Total
STRATIFY-positive	29 (11.6)	3 (1.2)	32 (12.8)
STRATIFY-negative	14 (5.6)	204 (81.6)	218 (87.2)
Total	43 (17.2)	207 (82.8)	250

0.73 (95% CI 0.66–0.80), indicating substantial agreement according to standard benchmarks. McNemar's test confirmed a statistically significant asymmetry among discordant pairs (14 vs. 3, $p = 0.015$), indicating that IMMUNOWELL tended to yield positive classifications more frequently than STRATIFY under these clinical thresholds.

In the first analysis, among the 53 STRATIFY-negative/IMMUNOWELL-positive cases, quantitative STRATIFY values were not available, while IMMUNOWELL indices ranged from 0.21 to 1.94. Notably, only one patient exhibited a clearly high-titer result (1.94), whereas all remaining values lay near the analytical cutoff and often required confirmation through the inhibition assay. Similarly, the two STRATIFY-positive/IMMUNOWELL-negative cases showed STRATIFY values just above the threshold (0.31–0.41), with correspondingly low IMMUNOWELL results (0.13–0.16), suggesting borderline fluctuations rather than true seropositivity. In the second analysis, among the 14 STRATIFY-negative/IMMUNOWELL-positive cases, STRATIFY index values ranged from 0.26 to 0.89, whereas IMMUNOWELL indices extended from 0.82 to 1.94. Regarding the three STRATIFY-positive/IMMUNOWELL-negative cases, STRATIFY index values ranged from 0.91 to 1.37, with IMMUNOWELL values between 0.28 and 0.76.

Quantitative Correlation

A strong quantitative correlation was observed between STRATIFY and IMMUNOWELL index values. Pearson's correlation coefficient was $r = 0.922$ ($p < 0.0001$), indicating that higher antibody levels measured by STRATIFY were associated with higher IMMUNOWELL values, and conversely, low STRATIFY indices generally corresponded to low IMMUNOWELL results. The coefficient of determination ($r^2 = 0.85$) indicates that approximately 85% of the variance in IMMUNOWELL values is explained by STRATIFY. The remaining variability likely reflects methodological differences, minor biological fluctuations, and borderline index values close to the analytical cutoffs (Fig. 1).

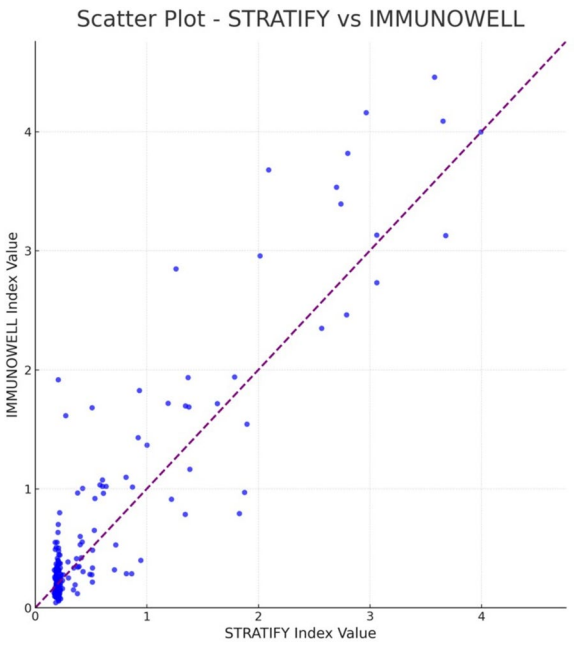

Fig. 1 Scatter plot of anti-JCV antibody index values measured by STRATIFY JCV™ vs. IMMUNOWELL™ assays. Each point represents an individual patient measurement. The dashed line corresponds to the identity line ($y = x$), indicating perfect agreement between the two assays

Bland–Altman Agreement

A Bland–Altman analysis was performed to assess absolute agreement between the quantitative indices of the two assays. The mean bias (IMMUNOWELL – STRATIFY) was +0.06, indicating that IMMUNOWELL tended, on average, to yield slightly higher index values than STRATIFY. This difference was minimal and did not reflect a systematic deviation, but rather a modest analytical shift consistent with the greater sensitivity of IMMUNOWELL in detecting low-titer responses. The 95% limits of agreement (LoA) ranged from –0.58 to +0.70, within which the vast majority of paired measurements were contained. Thus, despite isolated deviations, the inter-assay differences remained clinically acceptable in nearly all cases. Although a mild proportional trend was observed, its magnitude was limited, suggesting that agreement between assays was

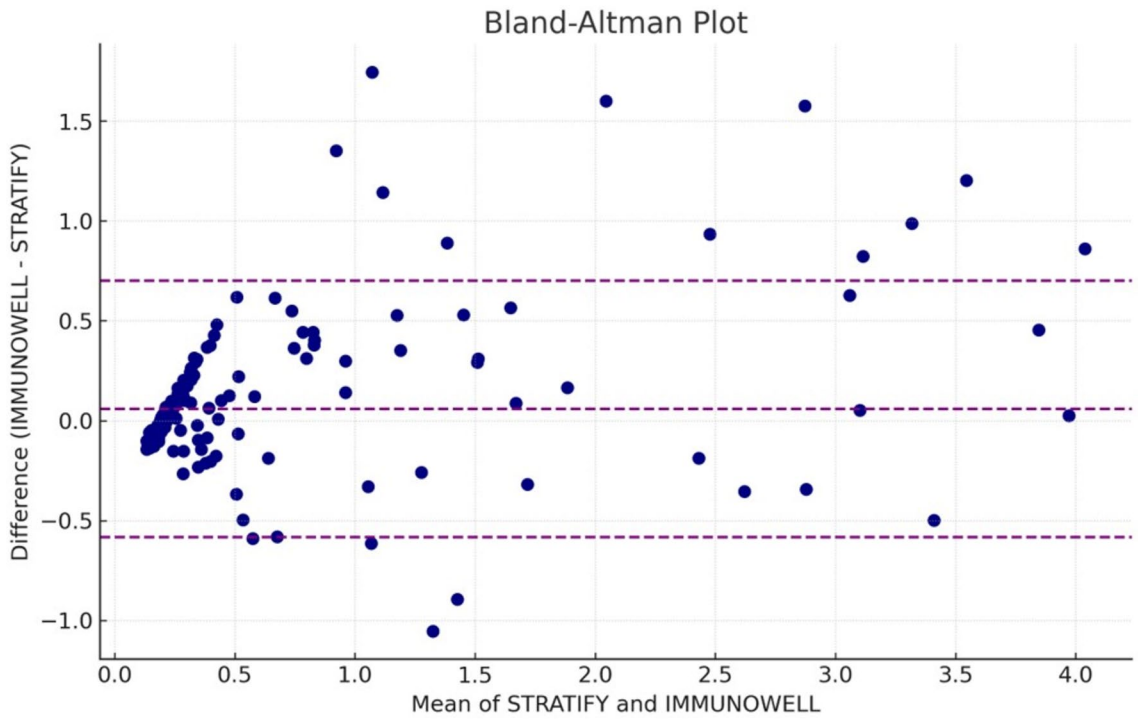

Fig. 2 Bland–Altman plot comparing the IMMUNOWELL and STRATIFY assays for anti-JCV index values. The central dashed line represents the mean bias (+0.06), while the upper and lower dashed lines indicate the 95% limits of agreement (−0.58 to +0.70)

maintained consistently across most of the analytical range.

As illustrated in the Bland–Altman plot (Fig. 2), most data points clustered densely around the zero line, confirming stable agreement. The few wider discrepancies occurred almost exclusively in borderline profiles, where one assay reported a low-positive index while the other remained just below the cutoff. For example, positive deviations approaching +1.0 were observed in cases with a STRATIFY index near 0.4 and an IMMUNOWELL index around 1.2. Conversely, negative deviations close to –0.8 corresponded to samples in which STRATIFY yielded an index above 1.0 while IMMUNOWELL remained in the low-positive range.

DISCUSSION

In this real-world head-to-head comparison, we demonstrate that concordance between STRATIFY JCV™ and IMMUNOWELL™ is strongly influenced by the interpretative framework applied. Agreement was moderate when manufacturer-defined analytical cutoffs were used, but increased substantially when clinically adopted PML risk-based thresholds were applied. Most discordant classifications clustered within low-index ranges, indicating that apparent inter-assay discrepancies largely reflect differences in decision boundaries rather than true biological divergence.

Although the 95% limits of agreement observed in the Bland–Altman analysis may appear relatively wide from a purely analytical perspective, these differences should be interpreted within clinically meaningful index ranges. Deviations exceeding ±0.5 occurred predominantly in low-to-intermediate index values, where small numerical shifts may modify categorical classification without substantially altering PML risk category. Notably, high-risk values (>1.5), which are most relevant for therapeutic decision-making, demonstrated stable

concordance across assays. These findings suggest that quantitative dispersion does not translate into clinically meaningful disagreement in the majority of cases.

A recent comparative study reported an overall agreement of 85.5%, with a Cohen's κ of 0.62, and noted a tendency for IMMUNOWELL to classify more patients within intermediate- or high-risk categories [20]. Differences across studies may reflect variability in cohort composition, sample handling, laboratory workflow, and, importantly, the interpretative cutoffs applied. More recent analyses have demonstrated agreement rates exceeding 90%, with higher κ coefficients, findings that align closely with the concordance observed in our cohort when clinically adopted thresholds were applied [9].

A detailed evaluation of discordant cases further supports the interpretation that most discrepancies reflect low-level antibody reactivity rather than stable seropositivity [19, 21]. Under analytical cutoffs, the majority of discordant cases were STRATIFY−/IMMUNOWELL+, with IMMUNOWELL indices clustered near the lower positivity boundary and only a single patient exceeding 1.5. Conversely, STRATIFY+/IMMUNOWELL− cases were rare and involved low-index values close to the analytical threshold. When clinical risk thresholds were applied, discordance decreased markedly, reinforcing the notion that most discrepancies arise from differences in decision thresholds.

These findings are consistent with longitudinal studies demonstrating that patients with low or intermediate anti-JCV index values frequently exhibit oscillations around the cutoff without progressing to sustained seropositivity [22]. Minor fluctuations near assay thresholds may reflect biological variability, analytical sensitivity differences, or transient immune responses rather than true changes in viral activity [6, 19, 21]. In this context, IMMUNOWELL-positive results in the absence of STRATIFY positivity should not automatically be interpreted as false positives, but rather as detection of low-level antibody reactivity whose clinical significance remains uncertain [19, 21].

From a clinical perspective, these findings suggest that isolated low-positive results, particularly when not confirmed by an alternative assay or not sustained over time, should not prompt immediate changes in risk classification or treatment strategy, but rather closer monitoring and longitudinal reassessment.

From a practical perspective, the marked improvement in agreement observed when applying PML risk-based categories has practical implications. In the current era of natalizumab biosimilar adoption, switching assay platforms without contextual interpretation may generate apparent serostatus shifts if strictly analytical cutoffs are applied [6, 17]. Our findings provide reassurance that, when results are interpreted within clinically validated risk categories, both assays yield highly concordant classifications suitable for routine monitoring. Importantly, anti-JCV antibody status alone should not be considered sufficient for PML monitoring in clinical practice. In patients treated with natalizumab, particularly those who are JCV seropositive, risk assessment relies on a combination of serological markers and periodic MRI surveillance, which remains essential for the early detection of asymptomatic PML lesions and for guiding clinical decision-making [4, 11, 17].

In this context, the decision to initiate or continue natalizumab treatment remains first and foremost a clinical one, in which anti-JCV antibody index values should be interpreted as supportive rather than determinant information. The observed variability of index values and the potential for inter-assay discordance further underscore the need to interpret serological results within the broader clinical context.

Nevertheless, some degree of caution remains warranted. Results near analytical or clinical decision thresholds should prompt continued surveillance rather than immediate risk reclassification. While the overall quantitative correlation between assays is strong, perfect analytical interchangeability at the individual level cannot be assumed, particularly in low-index ranges where classification boundaries are inherently sensitive to minor numerical variation [12].

This study has several limitations. First, it was conducted at a single tertiary center, and laboratory procedures were standardized within a single institutional workflow. While this reduces internal variability, it may limit generalizability

across different laboratory settings. Multicenter validation studies would strengthen external validity. Second, discordant samples were not systematically retested, which limits our ability to distinguish transient biological fluctuation from analytical variability. Indeed, the cross-sectional design of the study does not allow assessment of longitudinal variability of anti-JCV antibody indices over time, which may be particularly relevant in patients with borderline values. Furthermore, we did not perform stratified analyses according to treatment status, which could influence both antibody dynamics and clinical interpretation. Future studies incorporating longitudinal follow-up and treatment-specific stratification are warranted to further refine the clinical interpretation of inter-assay variability. Third, no confirmed cases of PML were present in our cohort, precluding direct evaluation of predictive performance. Finally, an additional limitation is the absence of a true gold standard for anti-JCV antibody detection. In this context, STRATIFY was used as a reference comparator rather than a true gold standard. Therefore, our conclusions pertain to concordance and interpretative alignment rather than outcome-based validation.

In the broader context of JCV risk stratification, current clinical practice relies on a limited number of validated serological platforms. While both STRATIFY and IMMUNOWELL have demonstrated robustness in routine use, further refinement of complementary biomarkers or integrated risk modeling strategies may eventually help address the grey zone between analytical thresholds and clinical decision-making.

CONCLUSIONS

In conclusion, inter-assay differences between STRATIFY JCV™ and IMMUNOWELL™ are predominantly threshold-dependent and largely confined to borderline index ranges. When interpreted within clinically validated PML risk categories, both assays demonstrate high concordance suitable for routine monitoring in patients treated with natalizumab, including those transitioning to biosimilars. Borderline fluctuations should prompt continued surveillance rather than immediate risk reclassification.

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Author Contributions. Clara Grazia Chisari and Pina Crimì contributed to the study design, collected and managed the database, and drafted the manuscript. Giulio Spampinato, Erika Ferraro, Andrea Alecci, Salvatore Lo Fermo, Vincenzo Cimino, and Mario Zappia contributed to data collection, data analysis, data interpretation, and critical revision of the manuscript. Francesco Patti contributed to the study design, interpreted the data, discussed the results, approved the final manuscript, and takes responsibility for the submission. All authors meet the ICMJE criteria for authorship.

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Data Availability. The datasets generated during and/or analyzed during the current study are not publicly available due to institutional and privacy restrictions but are available from the corresponding author on reasonable request.

Declarations

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Ethical Approval. The study protocol was reviewed and approved by the Ethics Committee Catania 1, Italy (PO/891/2025). The study was conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments. All participants provided written informed consent prior to any study-related procedures.

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