

**Refining NK cell cytotoxicity assessment in RA patients treated with JAK inhibitors:  
Methodological considerations**

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Dear Editor,

As researchers engaged in NK cell biology and the immunopathology of autoimmune diseases, we read with great interest the article by Akagi et al., “NK cell cytotoxicity is markedly reduced in younger patients with rheumatoid arthritis treated with JAK inhibitors” (*Rheumatology* 2025; doi:10.1093/rheumatology/keaf308) (1). The study addresses an important safety concern associated with JAK inhibitor therapy in RA patients, reporting decreased NK cell cytotoxicity and impaired maturation in younger individuals treated with JAK inhibitors, which correlated with increased herpes zoster (HZ) incidence. While the findings are clinically meaningful and represent a significant contribution to the field, we believe several methodological and interpretational issues warrant further scrutiny to clarify the immunological implications of JAK inhibitor therapy.

First, the use of peripheral blood mononuclear cells (PBMCs) in the cytotoxicity assays may limit the interpretability of the findings. While PBMC-based assays are commonly employed, they may not fully differentiate between the intrinsic functional changes of NK cells and indirect influences from other immune cells (NKT or CD8+T cells) affected by JAK inhibitors. Given the broad immunosuppressive effects of JAK inhibitors, it is important to consider the potential confounding effects of other immune cell types present in the PBMC fraction that might influence NK cell activity. To further strengthen the findings and provide a more precise understanding of the direct impact of JAK inhibitors on NK cells, we suggest that future studies consider using purified NK cells (CD3-CD56+ cells) for cytotoxicity assays. This approach would more precisely distinguish between direct effects on NK cells and indirect effects mediated by other cellular components within PBMCs. Additionally, examining NK cell subset distribution, expression of activating and inhibitory receptors, and intracellular signaling pathways (e.g., JAK-STAT phosphorylation) may elucidate mechanisms underlying NKCC reduction. These additional analyses would greatly enhance the understanding of how JAK inhibitors specifically modulate NK cell function.

Second, variation in NK cell frequency within the PBMC population across groups may influence assay results, making it difficult to distinguish between a functional impairment and a reduction in NK cell numbers. In <sup>51</sup>Cr-release assays, the effective effector-to-target (E:T) ratio is a critical determinant of cytotoxicity (2). Without standardization or adjustment for NK cell proportions, reductions in lytic activity may reflect differences in cell number rather than intrinsic dysfunction. Purified NK cells with defined E:T ratios would better isolate functional impairment. Alternatively, stratifying or matching patients by NK cell frequency when using PBMCs represents a feasible adjustment in PBMC-based analyses.

Third, categorizing cytotoxicity into quartiles provides a straightforward approach but may obscure continuous trends. Quartile-based grouping imposes artificial thresholds and can reduce statistical power (3). Modeling cytotoxicity as a continuous variable using regression methods (e.g., linear regression or ANCOVA) would allow for adjustment of covariates and finer resolution of associations with clinical outcomes. If the aim is to evaluate predictive capacity, ROC curve analysis could also be considered to identify clinically meaningful thresholds.

Finally, the observed link between reduced cytotoxicity and HZ is notable, but the limited number of HZ cases and the cross-sectional design limit causal interpretation. Clarifying the temporal relationship between cytotoxicity and HZ onset, ideally through longitudinal sampling, would help determine whether reduced NK function precedes or follows viral reactivation.

In summary, Akagi et al. provide valuable data on the immunological effects of JAK inhibition in RA. The suggestions above may help guide future work toward a more detailed understanding of NK cell function and infection risk in this setting.

Sincerely,  
Tsuneyasu Yoshida, MD

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#### References:

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