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Short Communication

Hemodialysis acutely altered interferon-gamma release assay test result and immune cell profile

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Abstract Patients receiving hemodialysis (HD) are at risk of TB development. IGRA-positive patients showed significant decrease in quantitative IGRA result with alterations in

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Interferon gamma-release assay;
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Peripheral immune profile

$CD3^+CD4^+CD45RO^+$, NK cell, and monocyte subsets immediately upon HD procedure. Our result suggested that the timing of IGRA testing is crucial in end-stage renal disease population.

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Prompt diagnosis and treatment for latent TB infection (LTBI) is important yet challenging in patients with end-stage renal disease (ESRD) to prevent disease progression and spread of active TB disease.[1] Interferon-gamma Release Assay (IGRA) is a state-of-art modality for LTBI diagnosis, and is preferred in ESRD patients.[2] However, as it relies on interferon (IFN)-γ secretion from T cells, the result may not be readily accurate in hemodialysis (HD)-requiring ESRD patients[3—5] as alterations of immune cells and functions have been reported.[6] We aim to investigate IGRA test changes immediately upon HD, and concurrently elucidate detailed phenotypic features of peripheral immune cells in ESRD patients with presumptive LTBI. The results are expected to provide an insight into preferable strategies on the timing of the IGRA test in patients receiving maintenance HD.

Along with a contact investigation program in May 2019, forty-five HD-requiring ESRD patients visiting Taipei Municipal Wan Fang Hospital with known history of contact with pulmonary TB patients were prospectively recruited. Participants with known malignancy or autoimmune disease receiving chemotherapy, previously documented TB, or an abnormal chest radiography suggesting pulmonary TB were excluded. The study was approved by the Institutional Review Board of Taipei Medical University, Taiwan (N201811039) and informed consents were obtained from all patients. Cohort recruitment flow and characteristics are presented in Supplementary Figure 1.

All patients underwent HD three-times per week with duration 4—4.5 h under low flux HD (Fresenius polysulfone F8 or F10, Fresenius Medical Care Asia—Pacific Ltd, Hong Kong) or high flux HD (Fresenius Helixone® FX60, FX80 or FX100). Blood for IGRA was sampled through the tubing of HD. Before and immediately after a single HD session, IGRA reactivity was evaluated with QuantiFERON-TB Gold (Qiagen, United States) according to the manufacturer's instructions. A cut-off value (TB antigen-nil $\geq$0.35 IU/mL) was used to define a positive result. The peripheral blood mononuclear cells (PBMCs) profile was analyzed with flow cytometry.

IGRA tests from 28 patients (62.2%) were consistently negative and 12 (26.7%) were persistently positive. Conversions (negative to positive test) and reversions (positive to negative test) were observed in two (4.4%) and three patients (6.7%), respectively. Among all cohort, the IGRA tests showed no significant difference in TB-specific antigen response (antigen-nil) quantitatively (*p-value* = 0.06, paired-samples *t*-test) or mitogen response (mitogen-nil) (*p*-value = 0.3). However, when stratified by IGRA positivity, patients with positive IGRA pre-HD showed a noticeable quantitative decrease in antigen response after a single HD session (*p*-value = 0.04, paired-samples *t*-test) while mitogen response remained unchanged (*p*-value = 0.1, paired-samples *t*-test) (Fig. 1B; Supplementary Figure 2).

IGRA is a preferred test in diagnosing LTBI due to its superior specificity, particularly on the BCG-vaccinated population.[7] However, concerns have been raised about its sensitivity and reproducibility in ESRD-requiring individuals, as studies have revealed 8—44% IGRA variation rates in four to 12 months.[3—5] Although inter-experiment variations have been reported to account for 8% of cases of variability,[8] we proposed that dynamic changes within individuals should also be taken into consideration, particularly in regards to variations of cellular and humoral immunity in patients undergoing HD.[6]

Supporting this hypothesis, we showed that a single HD session elicited alterations of peripheral immune cells. By flow cytometric analysis, $CD3^+CD4^+CD56^-$ (helper T (Th) cells) were significantly increased in all patients after HD; however, the increase was in-part dominated by $HLA\text{-}DR^+CD4^+CD3^+CD56^-$ (active Th) in IGRA-negative group, and $CD62L^-HLA\text{-}DR^-CD4^+CD3^+CD56^-$ (memory Th) in IGRA-positive group (Fig. 1B). Scrutinizing each Th phenotypes, $CXCR3^+CD3^+CD4^+$ (Th1) and $CCR5^+CD25^+CD3^+CD4^+$ (regulatory T (Treg) cells) were markedly upregulated upon a single HD session in both groups, with a noticeable increase of its $CD45RO^+$ (memory) subsets (Fig. 1C). This was in contrast to previous finding by Lisowska et al., reporting lower $CD3^+CD8^+$cells with no apparent difference of Th cells immediately following HD in otherwise healthy-ESRD patients.[9] The total NK cell number, dominated by $CD56^{dim}CD16^+$ ($CD56^{dim}$ NK), was overall downregulated. Moreover, IGRA-positive group exhibited attenuated $CD56^+CD3^+$ (NKT cells) subsets (Fig. 1D). The flow of blood through the dialyzer resulted in activation of complement system and immune cells, notably neutrophils and monocytes.[10] In current study, similar increase of total monocyte count was uniquely observed in the IGRA-positive group, with a noticeable increase of $CD14^+CD16^-$ (classical monocyte) subset (Fig. 1E).

Upon pathogen recognition, memory Th cells with enhanced capacity to respond upon re-exposure to mycobacteria and its components are generated. In fact, in IGRA tests, IFN-γ was mainly secreted by memory Th cells,[11] which may explain its domination in our IGRA-positive group. While pronounced low-grade inflammation and oxidative stress are commonly present in ESRD patients,[12] the quantitative IGRA result was surprisingly downregulated immediately after a single HD session. We evidenced that induced expression of

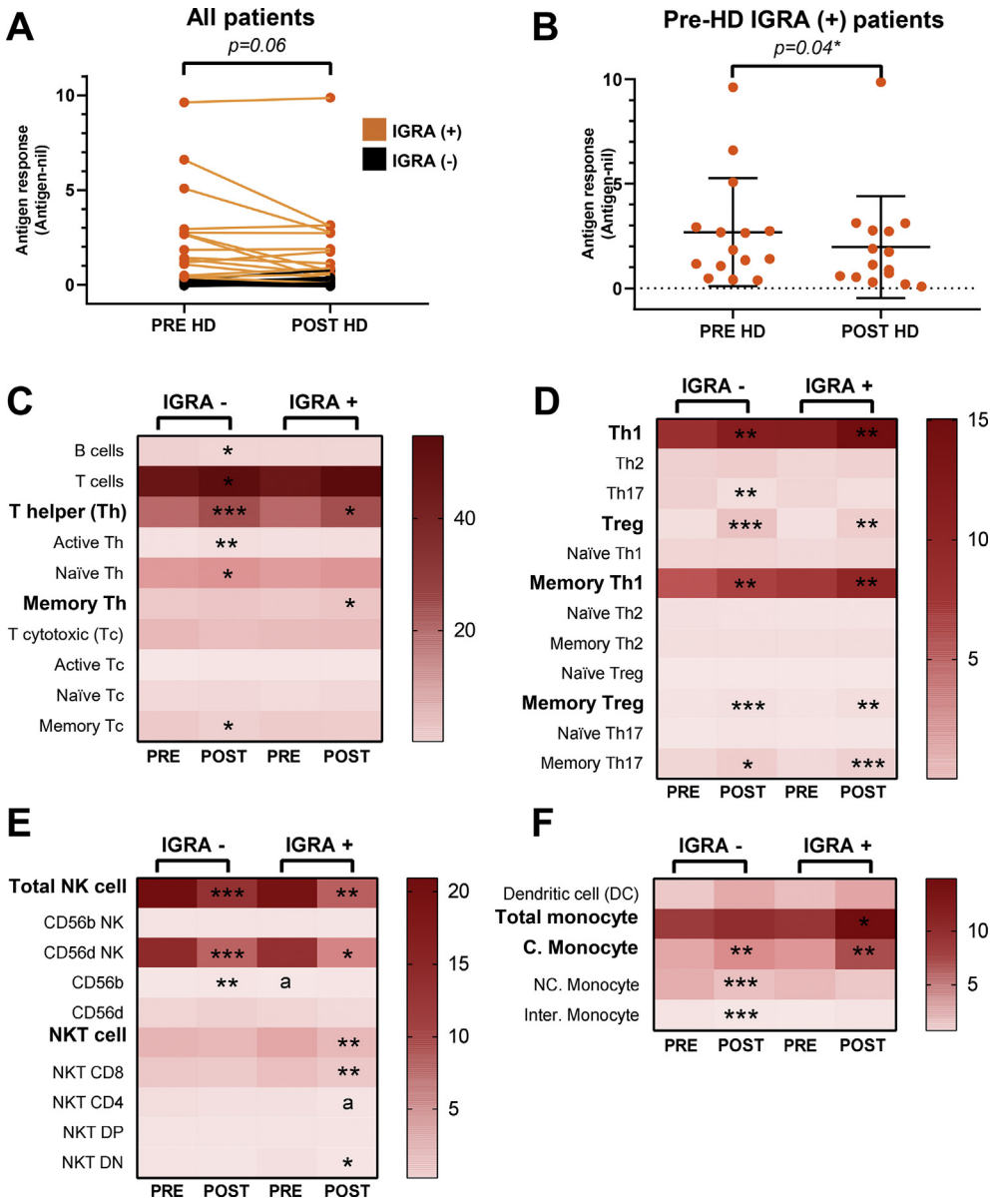

Figure 1. **A**: Antigen response (antigen-nil) of interferon-gamma release assay (IGRA) results from all cohort (left) and B: pre-HD IGRA (+) patients (right) before and immediately after hemodialysis (HD); (**C**–**F**) Heatmap of relative immune cell numbers based on expression of certain markers by using flowcytometry assay. **C**: Lymphocytes; **D**: T helper subsets; **E**: NK cells; **F**: Dendritic cell and monocyte. Scales are % of all immune cell population. POST vs PRE-HD (*) *p value* < 0.05; (**) *p value* < 0.01; (***) *p value* < 0.001. IGRA (+) vs IGRA (−) (ª) *p value* < 0.05.

pro-inflammatory Th1 and Th17 phenotypes were also followed by increased Treg cell expression likely as a homeostatic measure, implying an attenuated inflammatory response at this time-point. Moreover, $CD56^{dim}$ NK cells, potent cytotoxic cells in circulation, as well as NKT cells exhibited beneficial pro-inflammatory responses in anti-TB immunity, and low level of NKT cells is correlated with failure of mycobacterial containment and active disease.[13] The IGRA-positive group also showed a marked increase of classical-monocyte subset, which retains a high anti-microbial capacity as a scavenger (reviewed in[14]), but without a noticeable increase of $CD16^{+}$ monocytes which generally possess a pro-inflammatory attribute.

The present study is limited with the small case number in a relatively homogenous population. A larger-scale validation and immune cell profiling warrant further investigations. In addition, the causal-relationship of the immune cell changes to the IGRA variability cannot be readily defined. Altogether, we presented a cohort of HD-requiring ESRD patients with a history of TB contact. In the IGRA-positive group, quantitatively, the IGRA test showed a prominent decrease immediately after HD. Comprehensive mapping of the peripheral immune cells indicated that a measure to limit the triggered inflammation upon HD procedure occurred through the higher expression of Treg cells. Supporting this finding, we also pointed out attenuated anti-TB immunity, as evidenced by

lower NK and NKT levels in the IGRA-positive group. Conflicting result to previous studies may arose from difference in cohort characteristic, blood sampling timing, as well as the type of dialyzer membrane used. Despite the high specificity of IGRA-testing for LTBI diagnosis, our study should raise concerns on deciding the appropriate timing for its application in the immunocompromised population, specifically on ESRD patients requiring HD. We propose that IGRA-testing preferably performed before a single HD session, to avoid a reduction of sensitivity. Alternatively, an extensive study on threshold adjustment for IGRA-testing, as well as development of novel diagnosis approaches for immunocompromised LTBI patients, are deemed necessary.

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Declaration of competing interest

The results presented in this paper have not been published previously in whole or part. None of the authors have any conflicts of interest to declare in relation to this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.03.022.